DRFZ BERLIN

Deutsches Rheuma-Forschungszentrum
Ein Institut der Leibniz-Gemeinschaft

Annual Report 2011|2012






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Reflection of the DRFZ Lobby during the Avrion Mitchison Award ceremony

Deutsches Rheuma-Forschungszentrum Berlin

a Leibniz Institute

This Annual Report covers the research activities of the Deutsches Rheuma-Forschungszentrum Berlin (DRFZ) during the years 2011 and 2012.

The research activities of the DRFZ are made possible through financial support from the Senate Administration for Economy, Technology and Research of the Land Berlin, the German Research Foundation (DFG), the Federal Ministry of Education and Research (BMBF), the European Commission and through various other third parties, as mentioned in the text. Thanks are due to all of them.

Please visit our website: www.drfz.de

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Word of welcome

Traudl Herrhausen
President of Board of Trustees

Dear Friends of the DRFZ,

as President of the Board of Trustees of the German Rheumatism Research Centre (Deutsches Rheuma-Forschungszentrum Berlin, DRFZ), it is a pleasure and honour for me to welcome and to invite you to study this annual report.

As you may be aware, the DRFZ is a non-profit foundation of the Civil Law. Its founders are the State of Berlin and the Immanuel Hospital Ltd. The DRFZ in 2009 became a full member of the Leibniz Association. The responsibilities of the permanent and elected members of the DRFZ´s Board of Trustees are similar to those of a supervisory board of a commercial enterprise. The main responsibility is laid down in Paragraph 10 Section 1 of the Statutes: "... The Board of Trustees will secure the continuous quality of the research".

In particular, the Board´s responsibilities include:
- appointment (and dismission) of the directors,
- approvement of tenure for staff,
- appointment of members of the Scientific Advisory Committee.

This means that, when it comes to staff-related decisions, each Board member will have to ask him/herself: will my decision secure the DRFZ´s quality of research, will my decision improve the creative environment, will my decision increase the attraction of the DRFZ, will my decision preserve its independence and, am I really open to venture into new territories?

This report will show that, again, the DRFZ was able to attract or keep exceptional scientific talents, who at the DRFZ can follow their passion by working towards the big goal: to cure rheumatic diseases. I warmly recommend this report to you.

However, as Board members, we are also urged to get sponsors, friends, donors, in brief: true Maecenats interested in the DRFZ and the research done there. It is with much pleasure that the Board of Trustees welcomes the decision of the Willy Robert Pitzer Foundation to finance, for a period of several years, a new research group that will study Osteoarthritis, and to speed up the translation of findings to the clinic. Eventually, this commitment is to result in an endowed professorship. It is a very encouraging signal by a private foundation in times of continuing federal budget cuts that also affect scientific research. It is also an invitation to other institutions hopefully to follow suit.

Traudl Herrhausen

Word of welcome

Photo: Charité

Gerd R. Burmester, MD
Professor of Medicine
Director, Department of Rheumatology and Clinical Immunology
Charité - University Medicine Berlin

Dear friends of the German Rheumatism Research Centre,

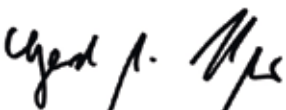
As a clinical and scientific partner of the German Rheumatism Research Centre, I would like to express a warm welcome to all the readers of the Annual Report.

Rheumatic and musculoskeletal diseases (RMDs) including systemic autoimmune diseases lead to a major burden for both the patients affected and the society as a whole, with early invalidity, work loss and – if uncontrolled – a lower life expectancy. They also represent one of the three leading causes why a patient consults a general practitioner or a specialist. Despite major progress in the diagnosis and treatment of RMDs, such as cytokine directed approaches and cell depleting/modulating therapies, there is still a high and unmet need to explore further treatment modalities. The Department of Rheumatology and Clinical Immunology of the Charité, in conjunction with the Rheumatology Unit of the Campus Benjamin Franklin, closely cooperate with the German Rheumatism Research Centre (DRFZ) to meet these challenges. This is documented by a very fruitful interaction both in the clinic and the research laboratory.

What do we know about the current situation of people with arthritis? Much knowledge has been gathered by the epidemiology unit of the DRFZ that conducts novel and comprehensive studies and has established the national data bank (Core Documentation) and the Biologic Registry RABBITT which belong to the leading international data banks. These are supplied with clinical data, but they also provide major stimuli for future clinical research including investigator initiated studies. Thus, important results were generated especially in the field of rheumatoid arthritis and the spondyloarthritides.

What do people with arthritis wish for? Of course, pain relief and prevention of loss of function are very important. Still, when engaging in a longer discussion with the patient, cure is the ultimate goal. This, however, as in most internal diseases, is difficult to achieve. But is it impossible? Here, scientists of the DRFZ and the Charité work hard to find new avenues to reset the disturbed immune system into a fresh state so that it does not react against self-structures with a regained tolerance. Here, novel cell directed therapeutic approaches are carried out ranging from rather mild procedures to the very complex autologous stem cell therapy.

What do we need to reach our goals in the future? Of course, a tight interaction between basic and clinical research leading to the translation of findings from the bench to the clinic is paramount. Here, the close cooperation between basic scientists and physician scientists from all areas of rheumatology research at the Charité and the DRFZ may be an excellent example. Luckily, these units are also geographically close which promotes a setting where excellent research may pave the way for the best possible care of our patients.



Yours, Gerd-Rüdiger Burmester

Preface

Andreas Radbruch, PhD
Scientific Director

Petra Starke
Adminstrative Director

Photos: G.Rottmann

Dear friends of the DRFZ,

The past two years were dominated by one major event: the first evaluation of the DRFZ by the review board of the Leibniz Association, since the DRFZ became a full member in 2009. In 2011 we prepared an extensive report and in November 2011 we hosted an evaluation board appointed by the Leibniz Association, for a 2-day site visit. We received the final evaluation report in autumn 2012. The DRFZ was rated as a prime research institute with a convincing research strategy, very good and excellent research groups and a remarkable integration into the biomedical academic environment of the Charité, the Medical Faculty of the Humboldt University and the Free University of Berlin. The evaluation board particularly highlighted the concept of the liaison groups, most of them with the Charité. The integration of these joint groups creates a unique environment for translational rheumatological research and stimulates both basic and clinical research.

In 2012, two new research groups joined the DRFZ. The group Osteoimmunology is headed by Koji Tokoyoda, who came from Chiba University. Koji Tokoyoda also coordinates the new International Leibniz Research Network “ImmunoMemory”. A new liaison group Cellular Immunology was established with the Charité, headed by Alexander Scheffold, who formerly worked with Miltenyi Biotech. Alexander Scheffold was appointed professor at the Charité, succeeding Jochen Hühn. Another new liaison group with the Charité is the research group “Intravital Microscopy”, headed by Anja Hauser, formerly group leader at the DRFZ, who was appointed professor at the Charité in 2012. Finally, together with the Charité, the DRFZ succeeded in winning a competition for a chair in “Health Services Research in Rheumatology”, an endowed professorship of the “Rheumastiftung”, a joint enterprise of the German Society for Rheumatology and the Rheumaliga, Germany´s largest patient organisation.

Most of the research at the DRFZ is financed by research grants. In particular, research groups at the DRFZ are integrated into national and international research networks. DRFZ scientists contributed to 6 Collaborative Research Programmes of the German Research Council (DFG) and 9 of the Federal Ministry of Research (BMBF), and to 5 research networks of the European Commission (EC). In 2011 an ERC Advanced Grant was awarded to the Scientific Director.

As an intimate partner of the Charité, the DRFZ views itself also as a technology-hub, in particular in the fields of molecular biology and single cell analysis. The Regine-von-Ramin Labor offers all options for the global analysis of gene expression in cells of interest, while the Flow Cytometry Core Facility (FCCF) offers top technololgy for the cytometric analysis of cells and the isolation of cells-of-interest by magnetic or fluorescence-activated cell sorting. In 2011, the Core Facility for Innovative Imaging and Microscopy Approaches (CINIMA) was newly established. This facility allows to monitor and track individual cells in living animals by intravital microscopy. In 2012, the DRFZ obtained the first CyTOF cytometric massspectometer in Germany. In the new CyTOF core facility, single cells can now be analysed for expression of up to 100 proteins simultaneously. For the research on rheumatic diseases, these technologies provide unique tools to understand which cells are promoting the disease, and how they do it?

Let me now leave you with the lecture of this report, enjoy it!

Andreas Radbruch

Petra Starke

Science at the DRFZ

Our aim is simple - yet challenging: we want to cure rheumatic diseases.

The aim of research at the German Rheumatism Research Centre (DRFZ) is challenging: we want to develop curative therapies for rheumatic diseases, or in medical terminology, develop therapeutic strategies to induce "therapy-free remission" for diseases for which today at best remission-maintaining therapies exist. Inflammatory rheumatic diseases, like Rheumatoid Arthritis, Systemic Lupus Erythematosus and Ankylosing Spondylitis affect about 2% of the German population.

Since 2009, the DRFZ is a Leibniz institute, receiving basic funding from both the federal government and the state of Berlin. In addition, research is funded by grants from the German Research Council, the European Research Council, the German government, the European Commission and other funding partners. Our strength is the tight liaison with the Charité - University Medicine Berlin. Thus research at the DRFZ is oriented at the clinical frontline of rheumatology, contributing advanced tools and technologies and innovative basic concepts to it. At the DRFZ, clinicians, clinical scientists, biologists, biochemists, bioinformaticians, engineers and social scientists all work together to achieve a profound understanding of rheumatic diseases and to discover effective ways to treat them.

24 research groups are working at the DRFZ in the two program areas "Epidemiology of Rheumatic Diseases" and "Pathophysiology of Rheumatic Inflammation". Most of these groups are rather small, with an average size of about 10 members, most of them PhD students. Groups frequently team up for projects, combining their expertise.

Pathophysiology of Rheumatic Inflammation

The experimental research strategy of this program area, combining 19 research groups, 10 of which are liaison groups of Charité and DRFZ, is based on a simple clinical observation: patients with chronic inflammatory rheumatic diseases can be cured by ablation of their entire immune system, followed by it's regeneration from autologous stem cells. However, this treatment implies a period of several weeks of immunodeficiency and thus has some risk of infection-related mortality. At present, it is not an option for the majority of patients. But those patients treated have taught us a valuable lesson: First, the immune system does not only initiate inflammatory rheumatic diseases, it also drives them in the chronic phase. Second, the immunological driver of rheumatic inflammation is refractory to conventional therapies, but is eliminated by a reset of the immune system. We speculate that this essential driver of rheumatic inflammation is an autoreactive pathogenic immunological memory.

Thus, research at the DRFZ aims at a fundamental understanding of immunological memory, its generation, regulation and maintenance. Which cells are involved? Only T and B lymphocytes, or also other cells, innate lymphocytes like NK cells, and antibody-secreting plasma cells? Do the pathogenic memory cells have special features that distinguish them from their protective counterparts? Can we use these differences to develop new therapeutic strategies to selectively eliminate the pathogenic memory cells while maintaining the protective ones? Scientists at the DRFZ have originally identified longlived "memory" plasma cells secreting autoantibodies, and have shown that they are resistant to conventional therapies. They reside in inflamed tissues and in the bone marrow. In the recent past, research at the DRFZ has been able to show that also protective memory T lymphocytes reside and rest in the bone marrow. Apparently dedicated mesenchymal stromal cells organize survival niches for memory cells and regulate the volume of immunological memory. Memory T lymphocytes showing the hallmarks of repeated restimulations are found in inflamed tissues, and apparently they are

Postersession in the lobby of the DRFZ

DRFZ at the Campus Charité - University Medicine Berlin

also resistant to conventional therapies. They express genes and microRNAs which adapt them to chronic inflammation, are proinflammatory and qualify as prime suspect pathogenic memory cells. Overall research at the DRFZ has generated a new view on immunological memory and from these new basic concepts, a number of innovative new targets have been identified. Initial attempts to address memory plasma cells selectively have been started at the Charité by Falk Hiepe, in collaboration with the group of Reinhard Voll from Freiburg University. In patients with Systemic Lupus Erythrematosus (SLE) plasma cells are eliminated by the proteasom-inhibitor Bortezomib (Velcade). Initial results are encouraging. But this is only the beginning. Scientists at the DRFZ look beyond, and develop strategies to selectively deplete only those plasma cells, secreting antibodies to defined autoantigens, sparing those plasma cells secreting protective antibodies.

How does a pathogenic immunological memory for chronic rheumatic inflammation develop? Apparently, those pathogenic cells have escaped physiological immunoregulation, which prevents autoimmune reactions, terminates expansion of reactive cells, contracts the population of effector cells generated, limits immunopathology, and controls the generation of immunological memory. Restoration of physiological immunoregulation, based on a thourough understanding of its mechanisms, has to complement ablative therapeutic strategies. Research at the DRFZ has centerstaged B lymphocytes as cells regulating inflammatory and allergic immune responses, and shown that and how vitamin D induces this regulation. Currently, this knowledge is translated into an experimental therapy using provitamin D for the enhancement of desensitisation to allergens. It also has shown how B and T lymphocytes interact to generate memory cells, highlighting the role of the inducible-costimulator (ICOS) and its ligand. Scientists at the DRFZ have identified a unique epigenetic mark of professional regulatory T cells, allowing to track them in rheumatic inflammation and beyond. A concept developed at the DRFZ postulates that regulatory T cells from patients with Systemic Lupus Erythematosus are not activated in vivo, because too little interleukin-2 is produced in the chronic Lupus immune reaction. Experimental therapy of an SLE patient with interleukin-2 has shown impressive efficacy, and more patients will be treated soon. Apart from dedicated regulatory lymphocytes, also effector lymphocytes regulate immune reactions, by expression of cytokines like interleukin-10, and genes and microRNAs regulating the activity, expansion and survival of the effector cells themselves. The molecular adaptations of proinflammatory T cells to chronic rheumatic inflammation, as described above, are such regulators, in particular the gene twist1, which limits the potential of the Th1 cells to induce immunopathology but promotes their persistence.

Finally, it is known that inflammation in various rheumatic diseases does have shared principles, but that there is also drastic differences between the diseases, and even between individual patients having the “same” disease. Tailoring the therapy to each patient individually requires the identification of biomarkers and “biosignatures” which predict the response to therapy. Here, the DRFZ explores the potential of cytometry, a technology allowing to analyse multiple parameters of single cells from blood or inflamed tissue. Using blood monocytes as “biosensors” indicating even subtle changes of the inflammatory milieu, initial results are encouraging and suggest that the response to therapy with particular biologicals may be predictable in the near future. This research is accompanied with in depth basic research on the mechanisms-of-action of the most popular biologicals, those blocking TNF-alpha and those eliminating B lymphocytes. In the end, this research may allow us to finetune therapies for the individual needs of a patient.

Epidemiology of Rheumatic Diseases

Research in the 5 working groups of this program area aims to identify predictors of long-term outcome of inflammatory rheumatic diseases in children and adults, to investigate the quality of health care as well as the safety and efficacy of new treatments.

Large cohorts that have been established with the help of rheumatologists and patients all over Germany are providing the basis. Separate databases are maintained for monitoring adults and children. The long-term observational studies require sophisticated statistical analysis which is provided by the biostatistics group.

The National Database of the German Collaborative Arthritis Centres provides data on treatment, clinical outcome and burden of disease of more than 17.000 adult patents per year. This series was started in 1993 and demonstrates how health care and clinical outcomes has improved in the past 20 years.

Scientist at work, Johanna Callhoff, Biostatistic, Epidemiology

In addition, the DRFZ has established the world´s largest paediatric rheumatology database. More than 10.000 children with juvenile idiopathic arthritis (JIA) and other chronic rheumatic illnesses are annually monitored by this study. It shows that nowadays children with JIA are first seen by a rheumatologist 6 months earlier than in the year 2000, allowing for earlier and thus more effective intervention. It furthermore demonstrates that novel treatment strategies have led to an improved mobility and fewer secondary health problems in the long term.

Another large national cohort study led by the DRFZ is the German biologics register "RABBIT (Rheumatoid Arthritis: oBservation of Biologics Therapy)" – a study that focuses on the safety and effectiveness of biologics in real life. Based on these data, our scientists recently developed and evaluated a risk-score for serious infection to support rheumatologists in their treatment decisions for high-risk patients.

Since 2010 the multicentric inception cohort "Course And Prognosis of Early Arthritis (CAPEA)" investigates how newly diseased patients can derive sustained benefit from early intervention and to what extent. ■

The Leibniz Association

The Leibniz Association connects 86 independent research institutions that range in focus from the natural, engineering and environmental sciences via economics, spatial and social sciences to the humanities. Leibniz institutes address issues of social, economic and ecological relevance. They conduct knowledge-driven and applied basic research, maintain scientific infrastructure and provide research-based services.

The Leibniz Association identifies focus areas for knowledge transfer to policy-makers, academia, business and the public. Leibniz institutions collaborate intensively with universities – in the form of "WissenschaftsCampi" (thematic partnerships between university and non-university research institutes), for example – as well as with industry and other partners at home and abroad. They are subject to an independent evaluation procedure that is unparalleled in its transparency. Due to the importance of the institutions for the country as a whole, they are funded jointly by the Federation and the Länder, employing some 16,500 individuals, including 7,700 researchers. The entire budget of all the institutes is approximately 1.4 billion EUR.

not all... but the majority of DRFZ staff

New faces at the DRFZ

Group leader and coordinators

Alexander Scheffold
Cellular Immunology, W2 Professor at the Charité - University Medicine Berlin. Liaison group at the DRFZ since January 2012

Katrin Moser
Scientific Coordinator with focus at technology transfer, since April 2011

Koji Tokoyoda
Osteoimmunology
New DRFZ group since August 2012

Eva-Juliane Kreiß
Scientific Coordinator with focus at EU Projects, since May 2011

Administration and Technical Staff

The Scientific Director's Office
Christine Raulfs
Tanja Durez

Assistant to the Business Director
Holger Schnurre

Travel Costs/ Secretary
Marion Nowak

Personnel Department
Angela Hahn

Finance Department
Norbert Adametz
Katrin Peter
Brigitte Neuse (till 11/2011)
Elli Buchholz (till 06/2012)

Third Party Money
Corinna Krüger
Katharina Horn

Purchasing Department
Catja Wendt
Alexander Hinz (since 3/2013)

Science Manager
Dr. Elke Luger, Leibniz Officer

Scientific Coordination
Dr. Eva-Juliane Kreiß, EU Projects
Dr. Katrin Moser, technology transfer

Library
Beate Löhr

Veterinarians
Dr. med. vet. Anja Schulz
Astrid Puppe (since 11/12)
Dr. med. vet. Kristina Ullmann (till 10/12)

Animal Facilities
Maria Dobberstein
Sabine Gruczek
Helmut Schäfer
Manuela Ohde
Laura Prüfer
Trainee: Vivien Theißig

Job Safety
Gudrun Steinhauser

Lab Kitchen
Birgit Füßel
Angela Lindner
Regina Schuck

IT Services
Falk Neumann
Hilmar Frank
Hilmar Fünning

Public Relations
Jacqueline Hirscher

Store
Carsten Tressin

Reception Desk
Lutz Hildebrandt

„Long night of science" - the DRFZ and the Max Planck Institute for Infection Biology receive 1.293 visitors

DRFZ - Facts and Figures

The DRFZ is a foundation of the civil law and was established in 1988. Since 2009 it ihas been a member of the Leibniz Association.

The DRFZ is holding four endowed professorships together with the Charité:

- Andreas Radbruch - Experimental Rheumatology
- Angela Zink - Rheumatism Epidemiology
- Falk Hiepe - Rheumatology
- Anja Hauser - Intravital Microscopy and Immunodynamics

Together with the Department of Rheumatology and Clinical Immunology, the DRFZ is one of 25 „Center of Excellence in Rheumatology“ of the European League Against Rheumatism (EULAR).

It is also a certified training center for expert immunologists of the German Society for Immunology (DGfI).

In 2010, the DRFZ received the TOTAL E-QUALITY award for best personnel policy, a model based on equal opportunity, for a period of 3 years. In 2013, the DRFZ applied for extension of the certificate.

The DRFZ has a total workforce of 193, 54 of which on established posts (effective date 12/2012, liaison groups not included). 38 employees are from abroad from 20 different countries. Female workforce stands at 130.

Currently the DRFZ is training one animal caretaker. Another trainee completed its training in 2012 and was taken on as an employee.

The DRFZ is participating in 5 graduate colleges: ZIBI-GRK 1121 and ZIBI-IMPRS-IDI, BSRT, IMMUCO (SFB 633) and the Robert Koch graduate school (RoKoDoKo).

The research activities of the DRFZ are made possible through financial support from the Senate Administration for Economy, Technology and Research of the Land Berlin, the German Research Council (DFG), the Federal Ministry of Education and Research (BMBF), the European Commission and through various other third parties, as mentioned in the text.

Thanks are due to all of them.

Once a year the Scientific Advisory Committee evaluates the development and the progress of the research groups. Based on the results of this evaluation, it notifies the Board of Trustees.

Third party funding

DRFZ groups	2011	2012
DFG	1.671,1	1.864,7
Government	1.152,0	1.093,7
State of Berlin	68,0	2,8
Leibniz Competition		146,1
EU	204,2	643,8
Industry	1.022,2	1.109,2
Foundations	89,8	113,9
Others	20,3	50,50
Total	**4.227,7**	5.024,5

As per July 23, 2013 in Mio Euro

Liaison groups	2011	2012
DFG	1.536,3	1197,9
Government	906,2	686,2
State of Berlin	0,0	0
	-	-
EU	325,6	2,2
Industry	2.940,7	2.184,4
Foundations	517,8	386,2
Others	60,0	70,0
Total	**6.286,6**	**4.526,9**

As per July 23, 2013 in Mio Euro

Map: Overview of the cities and countries where DRFZ scientists presented their current research at conferences, symposia or workshops in 2012. World map: www.unicommunication.de

Publications, Präsentations, Qualification

Publikationen im Detail	2011	2012
Peer reviewed publications1)	**140**	**120**
First author papers DRFZ	34	26
Last author papers DRFZ	52	27
Co author 2) papers DRFZ	79	77
Impact factor ›3	98	94
Impact factor ‹3	40	25
Reviews [3]	**29**	**37**
First author papers DRFZ	15	13
Last author papers DRFZ	16	27
Co author [2] papers DRFZ	7	7
Impact factor ›3	12	23
Impact factor ‹3	17	14
Books [4]	**0**	**1**
Book chapters	**5**	**3**
Other publications [5]	**25**	**22**

[1] Peer reviewed incl. „Letters“ with original data.
[2] Co-authors: DRFZ scientists and liaison scientists (no first or last authors).
[3] Peer reviewed and non peer reviewed.
[4] (Guest) Editorships.
[5] Incl.: editorials, comments, letters, flyers, meeting reports.

Qualifications	2011	2012
Habilitations	0	0
PhD theses	11	6
MD theses	5	3
Bachelor/Master/Diploma theses	12	14

Kooperationsverteilung bei Publikationen	2011	2012
DRFZ research groups	6	7
DRFZ research groups & Charité	33	41
DRFZ research groups & Charité & external collaborations	118	98
DRFZ research groups & one external collaboration	13	7
DRFZ research groups & several external collaborations	27	26

Invited talks	2011	2012
national	121	111
international	61	47
Total	**182**	**158**

Day of Rheumatology - Angela Zink, „Health care in rheumatology“, DRFZ lobby

DRFZ Bodies

Board of Trustees

The Board of Trustees supervises the management of the DRFZ and ensures the quality of the DRFZ's research. It defines the framework concerning the realisation of the foundation's purpose. In November 2011, the Board of Trustees saw off the current representative of the Berlin Senate for Science, Karola Hladky, as well as Klaus Eichmann from the Board Committee. The new president is Traudl Herrhausen from Bad Homburg

Permanent members
Immanuel-Krankenhaus GmbH, Berlin,
represented by Prof. Dr. Andreas Krause

Chairman of Charité – Universitätsmedizin Berlin
represented by Prof. Dr. Karl-Max Einhäupl

Federal Ministery of Education and Research (BMBF)
represented by Dr. Ute Rehwald

Senator for Education, Science and Research, Berlin
represented by Dr. Björn Maul

Elected members
Traudl Herrhausen, Bad Homburg,
President of the Board of Trustees

Prof. Dr. Josef Smolen, Academic Hospital for Internal Medicine III, Dep. of Rheumatology, Vienna, Austria

Prof. Dr. Max M. Burger, Novartis International AG, Basel, Switzerland

Prof. Dr. Elisabeth Märker-Hermann, Klinikdirektorin der Klinik Innere Medizin IV (Schwerpunkt Rheumatologie, klin. Immunologie und Nephrologie), der HSK Dr. Horst Schmidt Kliniken GmbH Wiesbaden

Prof. Dr. Erika Gromnica-Ihle, President of the „Deutsche Rheuma-Liga"

Prof. Dr. Jörg Hacker, President of the Leopoldina

Scientific Advisory Committee

The principal task of the Scientific Advisory Committee (SAC) is to advise the Board of Trustees on scientific matters concerning research at the DRFZ. The Scientific Advisory Committee is composed of internationally renowned scientists in the field of rheumatology and in related basic research areas.

Prof. Günter Hämmerling, Abteilung Molekulare Immunologie, Deutsches Krebsforschungszentrum (DKFZ), Heidelberg

Prof. Iain McInnes, Institute of Infection, Immunity and Inflammation, University of Glasgow, UK

Prof. Lars Klareskog, Dept. of Medicine, Karolinska Institute, Rheumatology Unit, Stockholm, Sweden

Prof. Michael S. Neuberger, Laboratory of Molecular Biology, Medical Research Center (MRC), Cambridge, UK

Prof. Brigitta Stockinger, Division of Molecular Immunology, MRC National Institute for Medical Research, London, UK

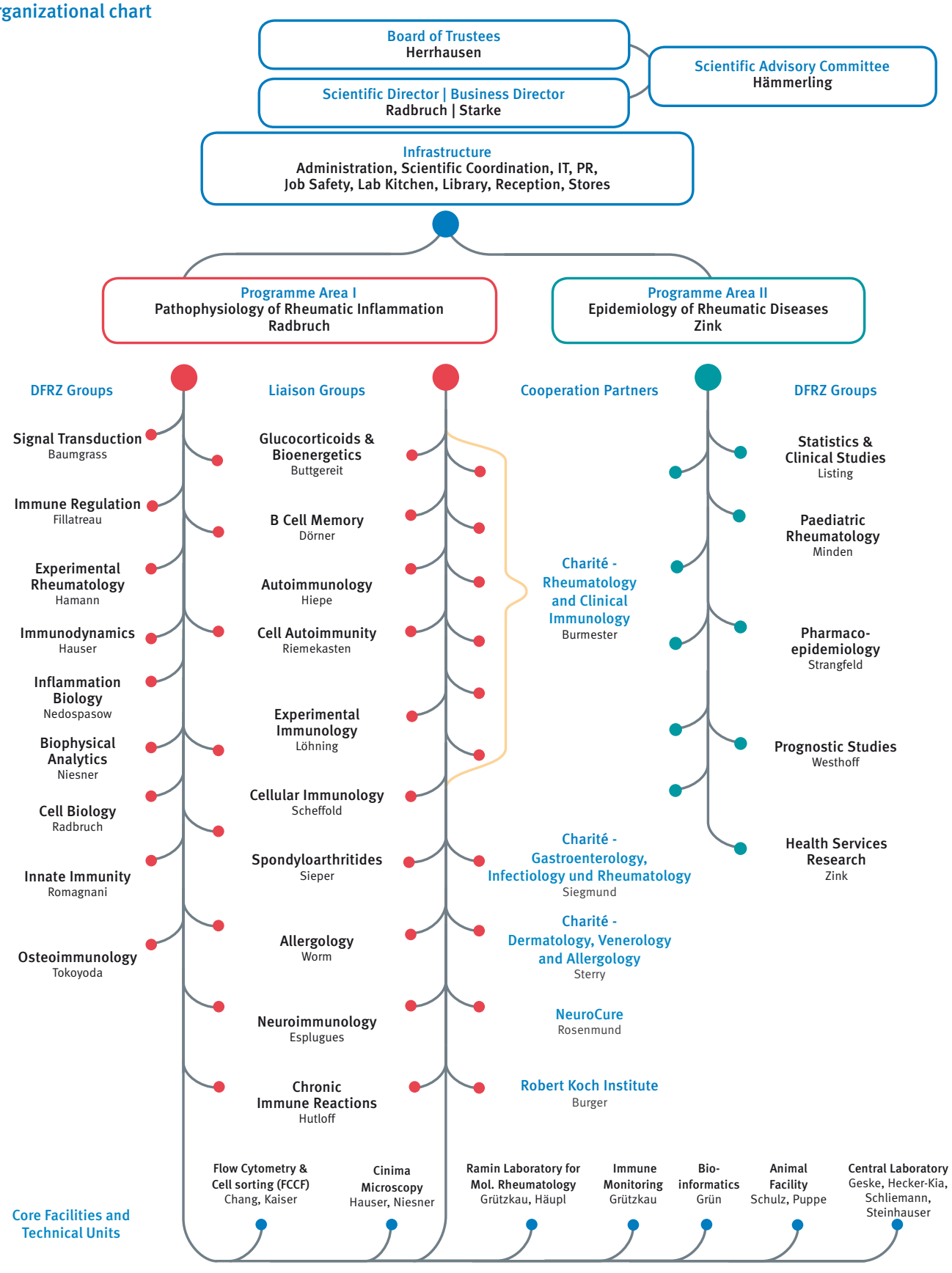
Prof. Deborah Symmons, Professor of Rheumatology & Musculoskeletal Epidemiology, arc Epidemiology Unit, School of Translational Medicine, University of Manchester, UK

Prof. Andrea Vortkamp, Dept. of Developmental Biology I, ZMB, Universität Duisburg-Essen

Scientific Advisory Committee, from left: G. Hämmerling, A. Vortkamp, B. Stockinger, I. McInnes, M. Neuberger, D. Symmons, L. Klareskog

Board of Trustees, from left: A.Radbruch, A. Zink, P. Starke, J. Hacker, G.-R. Burmester, A. Krause, U. Rehwald, E.Märker-Herrmann, E.Gromnica-Ihle, T. Herrhausen, B. Maul, K.-M.Einhäupl

Organizational chart



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Ria Baumgrass

Signal Transduction

Targeted manipulation of T lymphocyte functions

KEYWORDS

T cell activation
T cell differentiation
Transcription factors
Ser/Thr phosphatase calcineurin
Cytokines

GROUP MEMBERS

Group leader:
PD Dr. habil. Ria Baumgrass

Postdocs

Dr. Astrid Menning,
Dr. Tobias Scheel

PhD Students
Anna Abajyan,
Hanna Bendfeldt,
Claudia Brandt,
Stefan Frischbutter,
Christian Gabriel,
Manja Jargosch,
Stefan Kröger,
Melanie Krüger,

Diploma/Master Students

Lina Burbat,
Anett Köhler,
Yü-Hien Lee,
Claudia Schlundt,
Katherine Sturm

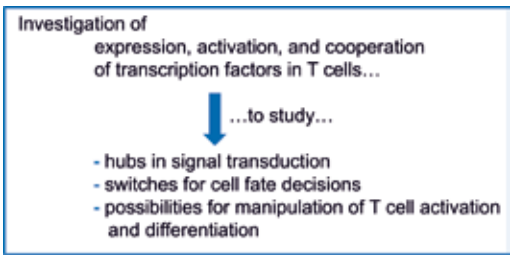
Students/Bachelor Students
Maria Jäpel,
Ivan Keel,
Diana Szabo

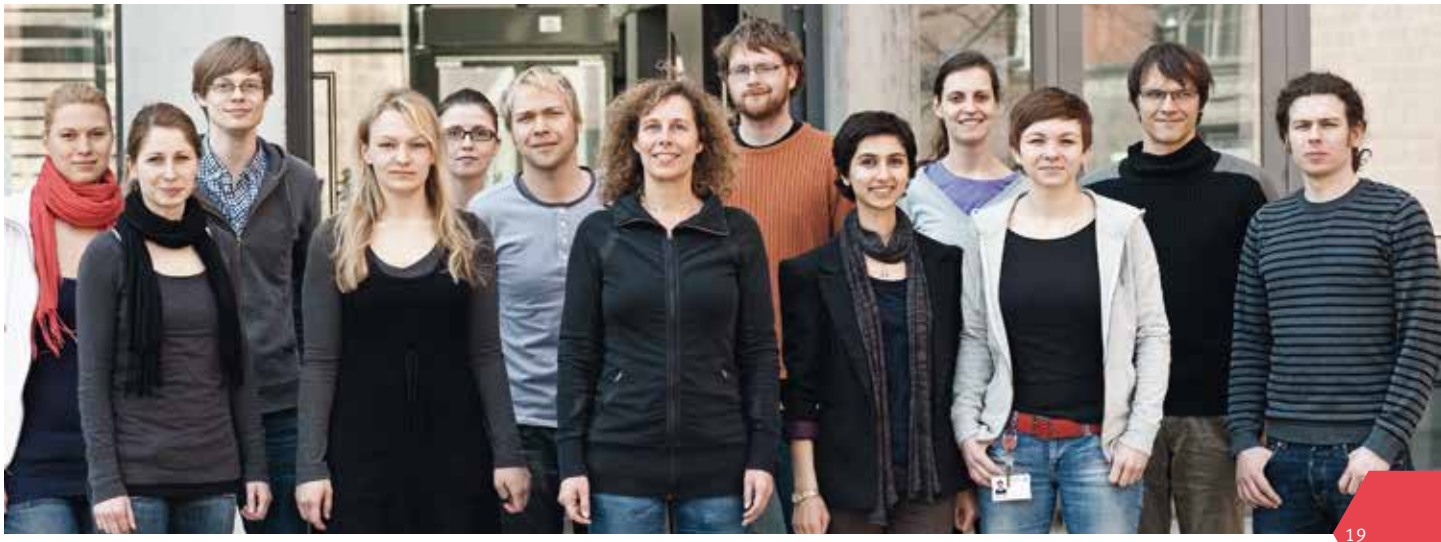
The immune response is a complex network of processes which are controlled by a bulk of regulating factors. T lymphocytes are key players within this system. Their malfunction is related to various disorders, either in the form of immune deficiencies or of hyperreactive responses, such as rheumatoid arthritis.

Our group investigates molecular mechanisms of T lymphocyte activation and activation-induced cell fate decisions in order to understand the role of T cells in allergy, autoimmune diseases and chronic inflammation. Our focus is on transcription factor networks, digital decision making and the regulation of cytokine expression.

We make use of a broad spectrum of original technologies in order to identify, isolate and analyse T cells according to the cytokines they express and to allocate transcription factors cytometrically in cells and isolated nuclei of Th cells. We have special experience in investigating signal transduction on single cell level and in manipulating signal transduction by low-molecular weight compounds. We are increasingly integrating mathematical models and systems biology approaches into our work.

Our findings will enable us to carry out targeted manipulations of T cell differentiation. Our main aim is to create new therapeutic strategies for the treatment of autoimmune disorders, especially rheumatoid arthritis and atopic dermatitis.





COOPERATION PARTNERS

Christian Becker, Dermatologie, Johannes-Gutenberg-Universität, Mainz

Tobias Bopp, Institut für Immunologie, Johannes-Gutenberg-Universität, Mainz

Wei Chen, Andreas Gogol-Döring; Max-Delbrück-Centrum für molekulare Medizin, Berlin

Gunter Fischer; Max-Planck-Forschungsstelle für Enzymologie der Proteinfaltung, Halle

Hanspeter Herzel, Manuela Benary; Humboldt-Universität, Institut für Theoretische Biologie, Berlin

Ulf Leser; Humboldt-Universität, Institut für Informatik, Berlin

Burkhard Micheel, Universität Potsdam, Biotechnologie and Immunologie, Potsdam

Michael Pätzel, Humboldt-Universität, Strukturanalysis, Berlin

Edgar Serfling, Universität Würzburg, Pathologie, Würzburg

Johannes Schuchard, Holger Klein, Microdiscovery, Berlin

Kerstin Steinbrink, Gutenberg-Universität Mainz, Hautklinik der Universität Mainz; Mainz

Margitta Worm, Gabriela Riemekasten, Charité Berlin

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2. Schroeter, M. F., B. A. Ratsch, J. Lehmann, R. Baumgrass, A. Hamann, and U. Syrbe. 2012. Differential regulation and impact of fucosyltransferase VII and core 2 (beta) 1,6-N-acetyl-glycosaminyltransferase for generation of E- and P-selectin ligands in murine CD4(+) T cells. Immunology. 137:294-304.

3. Smiljanovic, B., J. R. Grun, R. Biesen, U. Schulte-Wrede, R. Baumgrass, B. Stuhlmuller, W. Maslinski, F. Hiepe, G. R. Burmester, A. Radbruch, T. Haupl, and A. Grutzkau. 2012. The multifaceted balance of TNF-alpha and type I/II interferon responses in SLE and RA: how monocytes manage the impact of cytokines. J Mol Med 90: 1295-1309.

4. Bendfeldt, H., M. Benary, T. Scheel, K. Steinbrink, A. Radbruch, H. Herzel, and R. Baumgrass. 2012. IL-2 Expression in Activated Human Memory FOXP3(+) Cells Critically Depends on the Cellular Levels of FOXP3 as Well as of Four Transcription Factors of T Cell Activation. Front Immunol 3: 264.

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Determination of critical transcription factors limiting IL-2 expression in FOXP3+ regulatory T cells

SCIENTISTS

T. Scheel, H. Bendfeldt, A. Abajyan, R. Baumgrass

COOPERATION PARTNERS

M. Benary, H. Herzel, Institut für Theoretische Biologie, Humboldt University, Berlin

A. Radbruch, DRFZ, Berlin

K. Steinbrink, Department of Dermatology, Johannes Gutenberg-University, Mainz

PUBLICATIONS

Bendfeldt, H., M. Benary, T. Scheel, K. Steinbrink, A. Radbruch, H. Herzel, and R. Baumgrass. 2012a. IL-2 Expression in Activated Human Memory FOXP3(+) Cells Critically Depends on the Cellular Levels of FOXP3 as Well as of Four Transcription Factors of T Cell Activation. Front Immunol 3:264.

Bendfeldt, H., M. Benary, T. Scheel, S. Frischbutter, A. Abajyan, A. Radbruch, H. Herzel, and R. Baumgrass. 2012b. Stable IL-2 decision making by endogenous c-Fos amounts in peripheral memory T-helper cells. J Biol Chem 287:18386-18397.

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FUNDING

DFG, Transregio52
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BMBF, FORSYS-Partner Berlin

The human FOXP3⁺ regulatory T (Treg) cell population is very heterogeneous and consists of different subpopulations that are not known in great detail so far. For a better discrimination and characterization of such subpopulations we compared IL-2-producing with IL-2-nonproducing cells within the memory FOXP3⁺ Th cell population. Using transcription factor (TF) analysis on single cell level by flow cytometry, mathematical analysis and mathematical modelling we demonstrated that high levels of NFATc2, c-jun, c-fos, and NF-κBp65 lead to potential IL-2 expression in those FOXP3⁺ cells with low levels of FOXP3. We conclude that not only the level of FOXP3 expression but also the amounts of the main transcription factors represent determining factors for the anergic phenotype of FOXP3⁺ Treg cells.

Background

A detailed knowledge of human FOXP3⁺ cell subsets is essential for a better understanding of the regulation of human FOXP3 expression, the study of abnormalities among the FOXP3⁺ subpopulations in autoimmune diseases and allergies, and the selection and purification of the most promising subpopulation(s) of Treg cells for *in vitro* expansion and adoptive transfer.

Goal

In this study we aimed at a better characterization of human memory FOXP3-expressing T cell subpopulations (CD4⁺CD45RO⁺FOXP3⁺) concerning anergy defined by the inability to produce proinflammatory cytokines upon TCR/CD28 stimulation. Anergy and suppression are two main functional characteristics of FOXP3⁺ Treg cells. However, in a previous study (Bendfeldt et al., 2012a) we discovered that there are always 8 to 20% IL-2-expressing FOXP3⁺ Th cells. Therefore, we compared IL-2-expressing and IL-2-nonexpressing FOXP3 memory T cells using Treg cell markers and the expression levels of five transcription factors. Performing a combination of flow cytometric measurements with statistical analysis (Bendfeldt et al., 2012b) we studied the impact of low, medium low, medium high, and high levels of the transcription factors FOXP3, NFATc2, c-Jun, c-Fos, or NF-κBp65 on the decision whether IL-2 is expressed or not in activated memory FOXP3⁺ cells.

Results

An approach combining flow cytometric measurements with statistical interpretation for quantitative transcription factor analysis enabled us to reveal that the physiological expression levels not only of FOXP3 but also of NFATc2, c-jun, c-fos, and NF-κBp65 are limiting for the decision whether IL-2 is expressed or not in activated peripheral human memory FOXP3⁺ cells (Fig.1). In particular we found out that:

- IL-2-expressing FOXP3⁺ cells express a lower level of phenotypic markers of Treg cells and more NFATc2 and AP-1 but less FOXP3 than IL-2-nonexpressing FOXP3⁺ cells,
- low levels of FOXP3 and high levels of NFATc2, c-Jun, c-Fos, and NF-κBp65 are important for IL-2 decision making in FOXP3⁺ cells,
- a minimal heuristic model estimates the balance of transcription factors regulating IL-2 production in FOXP3⁺ cells, and
- manipulation of c-Fos expression confirms the causal relationship between the level of c-Fos and the probability of IL-2 production per cell in the FOXP3⁺ population.

Conclusion and perspective

Altogether, our results indicate that human IL-2-expressing compared to IL-2-nonexpressing FOXP3+ cells exhibit a lower level of phenotypical properties of Treg cells, a lower concentration of FOXP3, and higher concentrations of NFATc2, c-Fos, c-Jun, and NF-κBp65. We suggest that these two FOXP3⁺ cell subsets behave also differently *in vitro* and *in vivo*. Therefore, it is important to further characterize these two subpopulations concerning their proliferative behavior and suppressive capacity as well as in the context of autoimmune diseases, if it is possible to separate distinct phenotypic subpopulations within the FOXP3-low cell population in sufficient amounts.

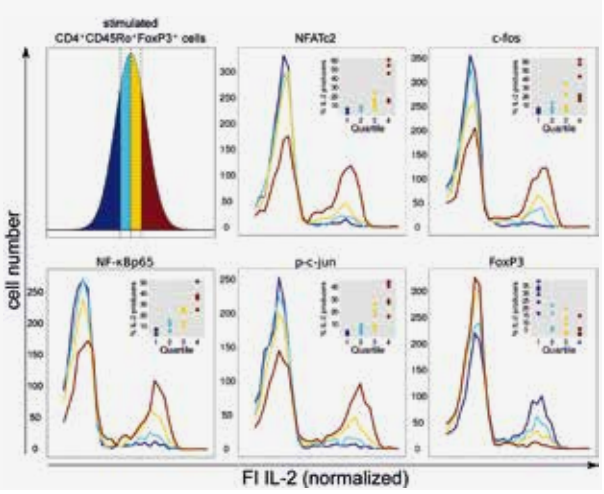

Figure 1: Segmentation of the amounts of each transcription factor of memory Treg cell populations (n=6) into quartiles revealed that the expression levels of NFATc2, c-fos, NF-κBp65, and c-jun correlate positively and of FOXP3 negatively with the probability of IL-2 production per cell (Bendfeldt et al., 2012a).

Evaluation of calcineurin/NFAT inhibitor selectivity using a semi-high-throughput flow-cytometric method

Small molecular inhibitors are excellent tools for manipulating cell reactions. They are widely used in scientific research to study molecular mechanisms of cells under physiological and pathophysiological conditions as well as in clinical applications to treat patients. However, their selectivity is often not well known. Moreover, it can vary according to cell types and the analysis methods used. Here we analyzed the selectivity of five chosen inhibitors of calcineurin/NFAT activation under the same conditions.

Our results not only show the applicability of a semi-high-throughput inhibitor test system but also that BTP1 is the most selective inhibitor of calcineurin/NFAT activation among the studied inhibitors under the used conditions.

Approach

In order to study and compare the pathway selectivity of inhibitors we used an approach combining fluorescent cell barcoding (Fig. 1) and phospho-specific flow cytometry in primary human Th cells. This allowed the simultaneous measurement of multiple samples with reduced measurement time and antibody consumption. We studied the inhibition of activation of NF-κBp65 and MAPK pathways in stimulated primary human Th cells.

Results

We analyzed the pathway selectivity of calcineurin/NFAT inhibitors by monitoring other main signaling pathways during Th cell activation. To this end, activation-induced phosphorylation of NF-κBp65 (Ser529), p38 (Thr108/Tyr182), and ERK1/2 (Thr202/Tyr204) was measured in the presence of different concentrations of the selected inhibitors, namely, CsA, AM404, BTP1, INCA6, and NCI3. We studied just these five inhibitors among the published 42 low molecular weight inhibitors and 13 protein and peptide inhibitors of calcineurin/NFAT activation (Sieber and Baumgrass, 2009). So far, very few data are available concerning the selectivity of these inhibitors and almost all of them were tested exclusively in cell lines such as Jurkat cells rather than in primary cells. Here we used primary human Th cells magnetically sorted from blood of healthy donors. .

The used semi-high-throughput approach enabled us to demonstrate that

(i) CsA and NCI3 are around 5 to 10- and 20-fold less potent, respectively, at inhibiting phosphorylation of NF-κBp65 and p38 than activation of NFAT,

(ii) AM404 is at least 15-fold selective for NFAT but already toxic at concentrations above 40 µM,

(iii) INCA6 is not selective at all, and

(iv) BTP1 is at least 100-fold selective for inhibition of NFAT activation relative to NF-κBp65, p38 and ERK1/2 phosphorylation.

Conclusion

On one hand, we demonstrated the usefulness of the simultaneous monitoring of early phosphorylation events in primary human Th cells.

On the other hand, our data provide an overview about the selectivity of certain inhibitors of the calcineurin/NFAT signaling pathway and will help to select the appropriate inhibitor as well as the correct concentration for different scientific purposes (Tab. 1).

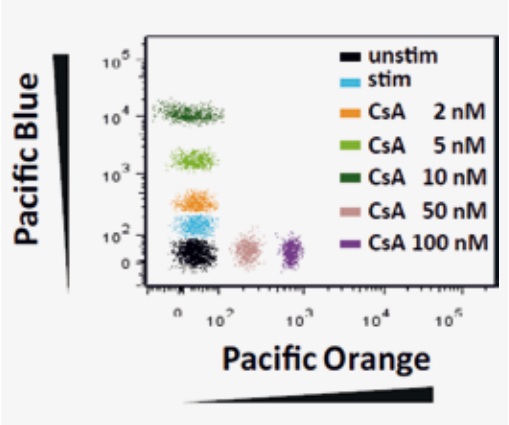

Fig. 1: Barcoding of T cells pretreated with different CsA concentrations is shown as an example.

Inhibitor	NFAT IC_{50}	% inhibition at NFAT IC_{50} concentrations			% inhibition at 10 x NFAT IC_{50} concentrations		
		p-p65	p-p38	p-ERK1/2	p-p65	p-p38	p-ERK1/2
AM404	2.4 µM	0	0	0	0	0	13
BTP1	4 nM	0	0	0	0	0	12
CsA	1 nM	5	0	0	57	57	0
INCA6	5 µM	78	43	51	toxic	toxic	toxic
NCI3	1.4 µM	0	5	0	33	0	0

Colored fields indicate three threshold levels of inhibition: green = no or low (≤ 10 %), yellow = medium (10-50 %), red = high (≥ 50 %)

Table 1: Inhibition of p65, p38, and ERK1/2 phosphorylation by different inhibitors of calcineurin/NFAT activation at NFAT IC50 concentrations and at 10-fold NFAT IC50 concentrations (Frischbutter et al., 2012).

SCIENTISTS

S. Frischbutter, K. Schultheis, R. Baumgrass

COOPERATION PARTNERS

M. Pätzel, Department of Chemistry, Humboldt University, Berlin

A. Radbruch, DRFZ, Berlin

PUBLICATIONS

Frischbutter, S., K. Schultheis, M. Patzel, A. Radbruch, and R. Baumgrass. 2012. Evaluation of calcineurin/NFAT inhibitor selectivity in primary human Th cells using bar-coding and phospho-flow cytometry. Cytometry A 81:1005-1011.

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DFG, Transregio52
DFG, SFB650

Simon Fillatreau

Immune Regulation

B lymphocytes - linkage between microbial recognition and protection against autoimmune disorders

KEYWORDS

B cells, autoimmunity, vaccination, inflammation, cytokines

GROUP MEMBERS

Group leader:
Simon Fillatreau, PhD

Scientists:
Vicky Lampropoulou, PhD
Yarúa Jaimes, PhD
Imme Sakwa, PhD

PhD students:
Ping Shen
Stefanie Ries
Ellen Hilgenberg
Duc Van Dang

Student:
Siska Wilantri

B lymphocytes have a unique role in the immune system being the only cell type capable of antibody production. In addition, B cells can contribute to immunity through the secretion of various cytokines with either pro- or anti-inflammatory activities. These antibody-independent functions of B cells played crucial roles in autoimmune diseases, acting as drivers or as regulators of the pathology. For instance, production of interleukin (IL)-10 by B cells was critical for remission from paralysis in experimental autoimmune encephalomyelitis (EAE), a T cell-mediated autoimmune pathology of the central nervous system (CNS) that is widely used as an animal model for relapsing-remitting multiple sclerosis (RR-MS). Noteworthy, natural CD4+Foxp3+ T regulatory (Treg) cells are also crucial for remission from EAE flares, and provide protection from disease at a later stage than B cells. The fact that B cells are required at an earlier time than Treg cells for the resolution of EAE led us to examine whether the beneficial functions of B cells consisted in the promotion of Treg cell activity, that is, whether B cells facilitated remission from EAE by increasing the suppressive function of Treg cells. Such instructive effect of B cells on Treg cells would be of considerable importance because B cell-depletion therapy (BCDT) is a possible treatment for RR-MS patients, raising the possibility that it might lead to a reduction of Treg cell activity in these patients. However, we found that Treg cell activation proceeded normally in absence of B cells in EAE. It therefore seems unlikely that BCDT would result in an impairment of Treg cell function. The BCDT approaches currently used to treat RR-MS patients and other autoimmune diseases might nonetheless reduce some immune protective mechanisms, because they certainly deplete both the protective and pathogenic functions of B cells. Identifying the mechanisms of B cell-mediated pathogenesis in autoimmune diseases might open the way to better therapeutic targeting of B cells for these pathologies, for instance by allowing a selective reduction of their pathogenic functions, while maintaining their protective activities. Thus, we sought to identify the mechanisms of B cell-mediated pathogenesis, and the mode of action of BCDT therapy in EAE. We found that B cells from mice with EAE expressed elevated levels of interleukin (IL)-6 compared to B cells from naïve controls. Moreover mice with a B cell-specific IL-6 deficiency showed a markedly less severe disease course than mice with wild-type B cells. BCDT reduced EAE progression only in mice with IL-6-sufficient B cells, but not in mice with IL-6-deficient B cells. From these data, we concluded that IL-6 production was the primary mechanism of B cell-mediated pathogenesis in this disease, and inferred that BCDT alleviated autoimmune symptoms through ablation of IL-6-secreting pathogenic B cells. It is tempting to speculate that a similar mechanism is taking place in RR-MS because B cells from RR-MS patients produced markedly higher levels of IL-6 than B cells from healthy individuals. Furthermore, this abnormality was corrected after BCDT because the B cell returning one year after rituximab treatment in RR-MS patients showed a normal IL-6 production, alike B cells from healthy controls. Collectively, these data indicated that IL-6 secretion by B cells represented a major mechanism of pathogenicity in autoimmune diseases. A more comprehensive understanding of the antibody-independent functions of B cells might lead to the generation of novel therapeutic approaches to selectively target their deleterious effects in autoimmune diseases.

COOPERATION PARTNERS

Prof. S.M. Anderton, Institute of immunology and Infection research, Edinburgh, Scotland.

Dr. T Barr, The University of Edinburgh, Scotland, UK.

Prof. D. Gray, The University of Edinburgh, Scotland, UK.

Prof. S. H. E. Kaufmann, Max Planck Institute for Infection Biology, Berlin, Germany.

Dr. A. A. Kühl, Charité – Universitätsmedizin Berlin, Germany.

Prof. T. Dörner, Charité – Universitätsmedizin Berlin, Germany.

Dr. P. Boudinot, Virologie et Immunologie Moléculaires, INRA, Jouy-en-Josas, France.

Prof. S. A. Nedospasow, Inflammation Biology, DRFZ Berlin.

Dr. O. Neyrolles, Institute of Pharmacology and Structural Biology, CNRS UMR5089, Department of Molecular mechanisms of Mycobacterial Infections, Toulouse, France.

Prof. A Bar-Or, Neuroimmunology Unit, Montreal Neurological Institute and Hospital, McGill University, Montreal, Quebec, Canada.

Prof. E. Meinl, Max Planck Institute of Neurobiology, Martinsried, Germany.

SELECTED PUBLICATIONS

1. Barr, T.A., P. Shen, S. Brown, V. Lampropoulou, T. Roch, S. Lawrie, B. Fan, R. O'Connor, S.M. Anderton, A. Bar-Or, S. Fillatreau*, and D. Gray*. B cell derived interleukin-6 drives pathogenesis in experimental autoimmune encephalomyelitis and multiple sclerosis. J Exp Med. In press. *these two authors contributed equally.

2. Hoehlig, K., P. Shen, V. Lampropoulou, T. Roch, B. Malissen, R. O'Connor, S. Ries, E. Hilgenberg, S.M. Anderton, and S. Fillatreau. Activation of CD4+Foxp3+ regulatory T cells occurs normally in absence of B cells during experimental autoimmune encephalomyelitis. Eur J Immunol. In press.

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P. Neves, P. Shen, U. Stervbo, P. Boudinot, S.M. Anderton, and S. Fillatreau. 2010. Suppressive functions of activated B cells in autoimmune diseases reveal the dual roles of Toll-like receptors in immunity. Immunol Rev 233:146-161.

Activation of CD4⁺Foxp3⁺ regulatory T cells proceeds normally in absence of B cells during EAE

SCIENTISTS

K. Hoehlig, P. Shen, V. Lampropoulou

COOPERATION PARTNERS

Bernard Malissen,

Centre d'Immunologie de Marseille-Luminy Universite de la Mediterranee, Marseille, France

Richard O' Connor,

University of Edinburgh, Edinburgh, EH9 3JT. Scotland, UK.

Stephen M. Anderton,

University of Edinburgh, Edinburgh, EH9 3JT. Scotland, UK.

CD4⁺Foxp3⁺ regulatory T cells play a critical role in maintenance of immune homeostasis and limitation of autoimmune disease flares[1]. In EAE remission from disease involves both Treg and B cells[2,3]. The protective mechanisms afforded by these two cell types might be reduced in RR-MS because Treg cells from RR-MS patients were less suppressive, and B cells produced less IL-10, than the corresponding cells from healthy individuals[4,5]. Nonetheless, the suppressive functions of Treg and B cells can be enhanced by standard RR-MS treatments (IFN-β and glatiramer acetate), or by chronic infection with helminth parasites, which have been found to reduce the rate of disease relapses[5-9]. The specific functions of B cells and Treg cells are still poorly understood. B cells and Treg cells are required during different phases of EAE for control of pathogenesis, with an early involvement of B cells, and a later action of Treg cells[10]. It has been proposed that the beneficial functions of B cells involve a positive effect on Treg cells i.e. B cells might facilitate remission from EAE by promoting the suppressive function of Treg cells. In line with this, expression of Foxp3 mRNA was transiently reduced in central nervous system (CNS) of B cell-deficient mice, compared with control animals, in a model of EAE induced by adoptive transfer of encephalitogenic T cells[11]. The notion that B cells instruct the protective function of Treg cells during autoimmune disease is of considerable importance, given that BCDT is a possible treatment for RR-MS patients[12]. We therefore sought to test whether B cells contributed to the function of Treg cells during autoimmune diseases of the CNS. To this end, we analyzed whether B cell deficiency affected homeostasis and activation of Treg cells during EAE.

B cell deficiency does not affect the activation of Treg cells in draining lymph nodes after EAE induction

To assess the role of B cells for the activation of Treg cells, we measured the proliferation of the CD103⁻ and CD103⁺ Treg cell subsets that compose the Treg cell compartment[13] in naïve mice as well as after EAE induction using bromodeoxyuridin (BrdU) incorporation as an indicator of cell division (Figure 1a). In naïve mice, CD103⁺ Treg cells displayed a stronger proliferation than CD103⁻ Treg cells, indicating a higher level of basal activation (Figure 1b, c).

After EAE induction the proliferation of CD103⁻ Treg cell markedly increased, while the division rate of CD103⁺ Treg cells remained unchanged (Figure 2b, c). B cell deficiency did not alter CD103⁻ or CD103⁺ Treg cell proliferation at any time point tested. In addition, Treg cells isolated from draining lymph nodes of wild-type or B cell-deficient mice on day 8 after immunization were similarly suppressive *in vitro* (Figure 1d). Thus, Treg cell activation proceeded normally in B cell-deficient mice after EAE induction.

Normal accumulation of Treg cells in CNS of B cell-deficient mice during EAE

During EAE, Treg cells enter the inflamed CNS where they might locally regulate pathogenic inflammation[3]. It has been suggested, based on a different EAE model, that B cells regulate the accumulation of Treg cells in CNS during EAE, and that this accounts for their protective role in this disease[11]. Accumulation of Treg cells was detectable in both wild-type and B cell-deficient mice by day 8 after immunization, before mice showed any sign of disability, and increased until day 21, when mice reached peak of disease severity (Figure 2a, b). A detailed kinetic analysis revealed comparable absolute numbers of CD103⁻ and CD103⁺ Treg cells in CNS of B cell-deficient and wild-type mice throughout the disease course (Figure 2a, b). The CNS Treg cells compartment progressively became enriched in CD103⁺ cells, and B cell-deficient mice transiently displayed a slightly higher frequency of CD103⁺ Treg cells among CD4⁺Foxp3⁺ T cells than wild-type mice on day 21 after EAE induction (Figure 2c). Considering the frequencies of Treg cells (defined as Foxp3⁺, or CD103⁻ Foxp3⁺, or CD103⁺Foxp3⁺ cells) among CD4⁺ T cells in CNS, we found no difference between wild-type and B cell-deficient mice, except on days 21 and 35, when B cell-deficient mice had modestly higher percentages of CD103⁺ Treg cells than control mice (Figure 2 d-f). From these data, we concluded that Treg cells normally accumulated in CNS of B cell-deficient mice during EAE.

As our analyses did not provide any evidence for reduced Treg cell accumulation or adverse alteration in the composition of the Treg cell infiltrate in the CNS of B cell-deficient mice, we next asked if a lack of B cell influenced Treg cell activity in CNS during EAE. First, we analyzed the proliferation of Treg cells in CNS on day 21 after EAE induction using BrdU. CD103⁻ Treg cells had a slightly higher cycling rate in B cell-deficient mice, while CD103⁺ Treg cells behaved similarly in the two types of mice (Figure 2g). To investigate the suppressive capacity of CNS Treg cells, B cell-deficient mice were backcrossed with a reporter Foxp3-eGFP gene-targeted mouse, which allowed isolation of CNS Treg cells in an unambiguous manner[14]. Foxp3-

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eGFP⁺CD4⁺ Treg cells isolated from CNS on day 21 after immunization were markedly more suppressive than Treg cells from secondary lymphoid organs of naïve mice (Figure 2h). This enhanced regulatory activity was, however, similar for Treg cells from B cell-sufficient and B cell-deficient Foxp3-eGFP mice, showing that Treg cells gained higher suppressive capacity independently of B cells (Figure 2h). Taken together, our data show that Treg cells accumulated in CNS and acquired enhanced suppressive functions normally in the absence of B cells.

In conclusion, our data show that Treg cell-activation can proceed normally in absence of B cells. This conclusion is supported by clinical studies, which showed that patients treated with BCDT did not experience reduction of Treg cells during the period of B cell-depletion. In fact some patients even showed increased Treg cell-numbers and frequencies in peripheral blood[15, 16]. Thus, it seems unlikely that BCDT might result in an impairment of Treg cell function in treated patients.

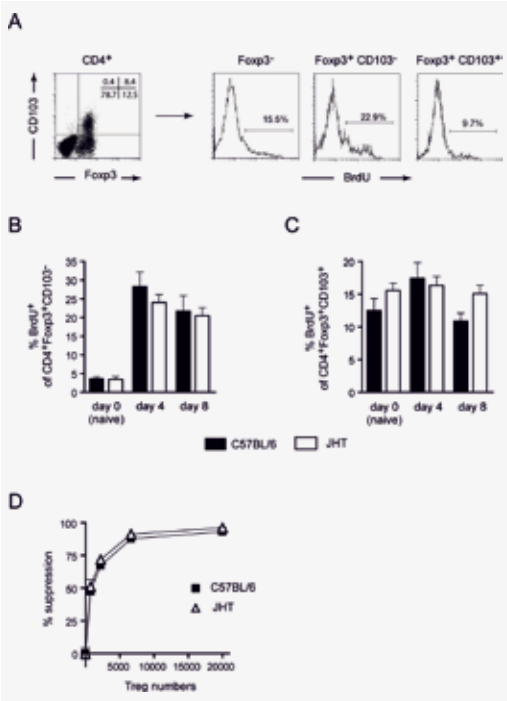

Figure 1: Activation of Treg in vivo upon induction of EAE. C57BL/6 (black bars) and JHT (white bars) mice were treated with 1mg BrdU via the intraperitoneal route on days 0, 4, or 8 after EAE induction. dLN were analysed 5 hours after BrdU injection. (A) Representative staining for BrdU incorporation by T cells in dLN of C57BL/6 mice at day 8. (B, C) BrdU incorporation by $CD4^+Foxp3^+CD103^-$ (B), and $CD4^+Foxp3^+CD103^+$ (C) cells. Data combine 2 independent experiments with 3 mice per group per experiment. (D) Suppressive capacity of $CD4^+CD25^+$ Treg from dLN of C57BL/6 (black squares) and JHT (open triangles) mice on day 8 after EAE induction. $2x10^4$ $CD4^+CD25^-$ T cells from naive C57BL/6 mice were stimulated with 0.1µg/ml anti-CD3 in presence of $1x10^5$ irradiated splenocytes and increasing numbers of Treg. Proliferation was measured by incorporation of 3H-thymidine after 64h of culture. Data show a representative example from 3 individual experiments. Graphs show mean±s.e.m. Statistical analyses were performed using an unpaired t test.

Figure 2. Infiltration of Treg in CNS during EAE. C57BL/6 and JHT mice are shown by black and white bars, respectively. (A-B) Absolute numbers of $CD45^{high}CD4^+Foxp3^+CD103^-$ (A) and $CD45^{high}CD4^+Foxp3^+CD103^+$ (B) Treg in CNS of C57BL/6 (black bars) and JHT (white bars) mice. (C) Frequencies of $CD103^+$ Treg among $CD45^{high}CD4^+Foxp3^+$ Treg in CNS. (D-F) Frequencies of $Foxp3^+$ (D), $Foxp3^+CD103^-$ (E) and $Foxp3^+CD103^+$ (F) Treg among $CD45^{high}CD4^+$ cells. Data compile 3 independent experiments with 3 mice per experiment for each time point. (G) Proliferation of $Foxp3^+CD103^-$ and $Foxp3^+CD103^+$ Treg in CNS was analysed on day 21 after EAE induction 5 hours after BrdU injection. (H) Suppressive capacity of $CD45^{high}CD4^+GFP^+$ Treg from CNS of Foxp3-eGFP (black squares) and B cell-deficient JHTxFoxp3.eGFP (open triangles) mice on day 21 after EAE induction, using $CD4^+CD25^+$ Treg from spleen and LN of naive C57BL/6 mice as controls (black circles). $2x10^4$ $CD4^+CD25^-$ T cells from naive C57BL/6 mice were stimulated with 0.1µg/ml anti-CD3 in presence of $1x10^5$ irradiated splenocytes, and indicated numbers of Treg. Proliferation was measured by incorporation of 3H-thymidine after 64h. Graph shows a representative example from 3 individual experiments. Graphs show mean±s.e.m. Statistical analyses were performed using an unpaired t test (* p<0.05).

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Depletion of IL-6 producing B cells as a therapy to alleviate pathogenic inflammation in CNS autoimmune diseases

SCIENTISTS

P. Shen, V. Lampropoulou, T. Roch

COOPERATION PARTNERS

Tom A. Barr,

University of Edinburgh, Edinburgh, EH9 3JT. Scotland, UK.

Sheila Brown,

University of Edinburgh, Edinburgh, EH9 3JT. Scotland, UK.

Sarah Lawrie,

McGill University, Montreal, Quebec, Canada.

Boli Fan,

McGill University, Montreal, Quebec, Canada

Richard O'Connor,

University of Edinburgh, Edinburgh, EH16 4TJ, Scotland, UK.

Stephen Anderton,

University of Edinburgh, Edinburgh, EH16 4TJ, Scotland, UK.

Amit Bar-Or,

McGill University, Montreal, Quebec, Canada.

David Gray,

University of Edinburgh, Edinburgh, EH9 3JT. Scotland, UK.

B cells in autoimmunity

B cells have paradoxical roles in autoimmune diseases as they can exert both pathogenic and protective functions. B cell depletion therapies could halt progression of diseases such as relapsing-remitting multiple sclerosis (RR-MS) and experimental autoimmune encephalomyelitis (EAE). B cells might promote tissue destruction in an autoantibody-dependent manner, as suggested by the presence of myelin-reactive autoantibodies in serum and central nervous system (CNS) of RR-MS patients[1]. Moreover, transfusion of autoantibody-containing serum in rats with EAE exacerbated demyelination and axonal loss[2]. However, clinical improvement in patients treated with rituximab often preceded reduction in autoantibody levels[3], and treatment with atacicept, a drug that reduced numbers of short and long-lived plasma cells[4], led to an aggravation, rather than an improvement, of RR-MS[5]. These observations concur to indicate that B cells can also propagate this autoimmune disease via antibody-independent mechanisms. Since rituximab treatment resulted in a noticeable decline of T cell numbers in CNS of treated patients[6], we hypothesized that B cells could facilitate RR-MS progression by sustaining pathogenic T cell responses, possibly through secretion of cytokines because (1) cytokines can increase T cell immunity (2) B cells can produce large amounts of cytokines[7] (3) cytokine blockade can be an effective treatment for autoimmune diseases. Among various cytokines, interleukin-6 (IL-6) was a possible candidate because it is essential for the development of EAE[8], and B cell-derived IL-6 can enhance T cell proliferation *in vitro*[9] as well as Th17 responses *in vivo*[10]. We therefore studied whether IL-6-secreting B cells could contribute to pathogenesis of EAE and RR-MS.

IL-6-secreting B cells are located in the marginal zone B cell compartment

To identify the B cell subpopulations capable of IL-6 production, we isolated splenic marginal zone (MZ) and follicular (FO) B cells by flow cytometry according to their expression of CD19, CD21, CD23, and CD1d, and compared their IL-6 production after stimulation. MZ B cells ($CD19^{+}CD21^{hi}CD23^{lo}$ or $CD19^{+}CD1d^{hi}$) showed the strongest production of IL-6 in response to TLR4 stimulation with LPS, and those levels were even higher when the cells were co-stimulated with an agonistic anti-CD40 antibody and LPS (Figure 1a). Interestingly, the frequency of MZ B cells increased in spleen during EAE from day 14 onwards (Figure 1b). These data confirm that B cells can produce IL-6 upon activation, and show that the most effective IL-6-producing B cell subset accumulates during EAE.

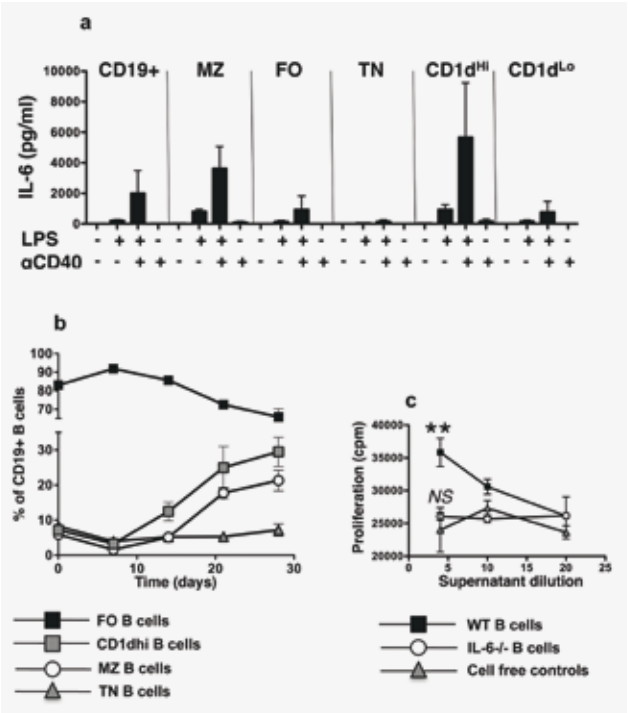

Figure 1: B cells from naïve and EAE mice constitute a major source of IL-6 that stimulates T cells *in vitro*. (a) B cell subsets were isolated from spleens of naive mice. After stimulation with LPS and/or anti-CD40, IL-6 levels were quantified by Bioplex. (b) Kinetics of B cell subset expansion during EAE were assessed by flow cytometry. (c) Proliferation of $CD4+$ T cells stimulated with anti-$CD3$, anti-CD8 and supernatants from either WT B cells, IL-6-/- B cells or cell-free controls plus LPS and anti-CD40.

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B cells modulate T cell responses via IL-6 production

Given that B cells might promote RR-MS progression by stimulating pathogenic T cells[6], we investigated the effects of IL-6 produced by B cells on T cell responses. For this, we tested how supernatants from B cells co-activated via TLR4 and CD40, which contained high amounts of IL-6, influenced T cell proliferation *in vitro*. Remarkably, T cell proliferation was significantly enhanced by supernatants from wild-type B cells (even though they were diluted at least 5-fold) but not with supernatants from IL-6-deficient B cells (Figure 1c). These observations suggested that IL-6 from B cells might contribute to T cell-mediated pathogenesis in EAE.

Mice with a B cell-specific IL-6 deficiency develop an attenuated form of EAE

We then studied the role of IL-6 production by B cells in disease pathogenesis during EAE. EAE was induced in mice with an IL-6 deficiency restricted to B cells (B-IL6-/- mice) and in control mice with wild-type B cells (B-WT mice). A lack of IL-6 production by B cells did not impact on the disease onset, yet B-IL-6-/- mice developed a markedly less severe disease than B-WT mice, demonstrating that B cells contributed to disease exacerbation through production of IL-6 (Figure 2a).

To assess whether this deleterious process might be relevant in RR-MS, we compared IL-6 production by B cells from RR-MS patients, and healthy controls. B cells from RR-MS patients secreted 4-5-fold higher amounts of IL-6 than B cells from healthy individuals, suggesting that this disease might be associated with an increased production of pro-inflammatory mediator by B cells (Figure 2b). Interestingly, this abnormality was corrected after BCDT, as B cells returning one year after BCDT displayed a normal IL-6 production, alike B cells from healthy individuals (Figure 2c).

Taking these data together, we conclude that IL-6 secretion is a major mechanism of B cell-driven pathogenesis in T cell-mediated autoimmune diseases of the CNS. More generally, our findings suggest that novel approaches to selectively modulate cytokine production by activated B cells might provide interesting approaches to control pathogenic inflammation in autoimmune diseases.

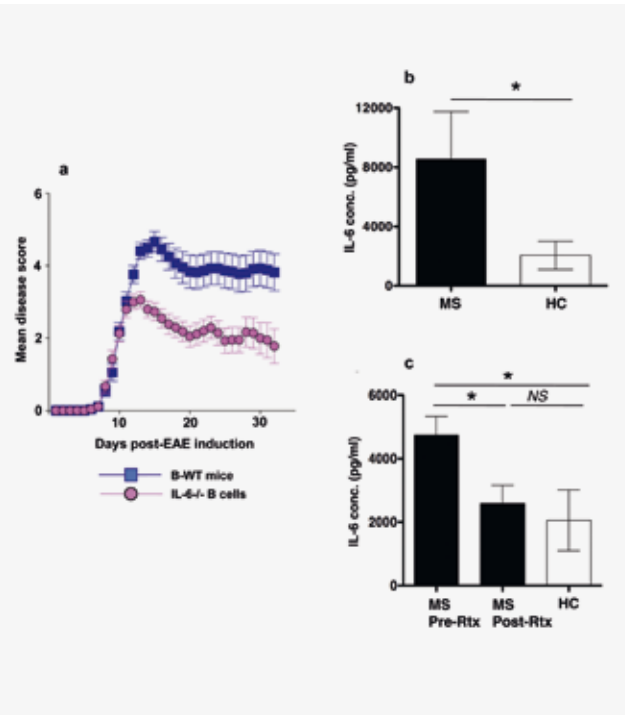

Figure 2: IL-6 producing B cells participate in EAE and MS progression. (a) EAE progression was monitored for 32 days after immunization with MOG in B-WT and B-IL6-/- chimeric mice. (b) IL-6 production by B cells isolated from MS patients and healthy controls. (c) Longitudinal study showing IL-6 production from B cells from MS patients pre- and post-Rituximab treatment. B cells were isolated ex vivo and stimulated by engagement of BCR and CD40 with or without addition of TLR9 ligand (CpG DNA) cytokine secretion was analyzed by ELISA.

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Alf Hamann

Seniorgroup Experimental Rheumatology
and Charité - University Medicine Berlin (CCM)

Experimental Rheumatology

A serious goal - the re-establishment of (immune) tolerance

KEYWORDS

Immune regulation, Therapy, Regulatory T-cells, Epigenetics, Homing

GROUP MEMBERS

Group leader
Prof. Alf Hamann

Scientists
Dr. Dirk Engelbert, Dr. Stefan Frischbutter, Dr. Ute Hoffmann, Dr. Julia Polansky-Biskup, Dr. Anne Rigby, Dr. Uta Syrbe*

*Med. Klinik m. S. Gastroenterologie, Infektiologie und Rheumatologie Charité – CBF Berlin

Ph.D. students
Dipl. Biol. Elisabeth Kenngott, Dipl. Biol. Matthias Kröger, M. Sci. Biochem. Francesca D. Liu (PhD: Dec 2012), M. Sci. Mol. Biol. Maybritt Mardahl, Dipl. Biol. Jennifer Pfeil, Dipl. Biol. Matthias Pink, Dipl. Biochem. Micha Schröter (PhD: June 2012), Dipl. Biochem. Julia Triebus

Bachelor/Diploma/Master stud.
Miriam Exner

Technical assistance
Uta Lauer, René Maier, Kerstin Schlawe

Students
Christina Poulsen

Others
Kirsten Kindler, Esra Özacar, Sabrina Schüngel

Until March 2012, the Experimental Rheumatology was at the Charité and hosted as liaison group at the DRFZ. With retirement of A.H. from the Charité, the group is now a seniorgroup in the DRFZ. Research of our group focuses on basic and translational aspects of immunoregulation and T cell biology:

1) *Immune regulation, tolerance and immunotherapy*. Induction and maintenance of immune tolerance is a critical prerequisite for health; its failure results in chronic inflammatory diseases such as autoimmunity and allergies. In the past, we studied properties of regulatory T cells (Tregs) that help to control unwanted immune reactions: how they differentiate, where they act, and how their master transcription factor Foxp3 is regulated by epigenetic modification. At present we search for ways to induce Tregs *in vivo* for the therapy of such diseases. For this, we "engineer" antigenic peptides so that they predominantly activate a tolerogenic response. Moreover, we screen substance libraries for compounds inducing Tregs or IL-10-producing cells. The goal is to develop drugs and antigen formulations that can be used as tolerogenic vaccines for clinical application. In addition, we became interested in "novel" immunosuppressive cytokines, notably IL-27, when we learned from work in an influenza model that this cytokine has an important role in limiting overt inflammation and preventing fatal immunopathology.

2) *T cells and epigenetics: How contributes epigenetic regulation to the imprinting of functional properties and memory.* By means of adhesion molecules and chemokines, T cells can migrate into specific compartments of the body. Cells memorise their initial site of antigen encounter and develop a topographical memory that consists of an imprinted homing receptor expression pattern in effector/memory cells. A challenging idea is that epigenetic mechanisms are involved in the imprinting of a stable homing phenotype as well as other functional properties of T memory cells. Transcriptional and epigenetic regulation of homing or memory-related genes in protective and pathological memory T cells is therefore one of our current fields of interest.

In these studies we use a variety of *in vitro* and *in vivo* assays, several mouse disease models, technologies in molecular biology and epigenetics, a proprietary high-throughput assay for substance screening, and others.

COOPERATION PARTNERS

Dr. Stefan Bereswill, Dr. Markus Heimesaat, Charité – University Medicine Berlin, Dept. f. Microbiology, Berlin, Germany

Celares GmbH, Berlin, Germany

Prof. Gudrun Debes and Prof. Chris Hunter, University of Pennsylvania, Dept. of Pathobiology, Philadelphia, U.S.A

Prof. Rainer Haag, Free University, Dept. f. Chemistry and Biochemistry, Berlin, Germany

Prof. Susanne Hartmann, Dr. Svenja Steinfelder, Dept. f. Immunology, Veterinary Medicine, FU Berlin, Germany

Prof. Richard Lucius, Humboldt University, Dept. f. Molecular Parasitology, HU Berlin, Germany

Prof. Jochen Hühn, Dr. Stefan Flöß, Helmholtz-Centre for Infection Research, Braunschweig, Germany

Dr. Anja Kühl, Dr. Ulrike Erben, Charité – University Medicine Berlin, RCIS, Berlin, Germany

Prof. Max Löhning, Prof. Alexander Scheffold, Charite University Medicine Berlin, Medical Clinic Rheumatology, Berlin, Germany

Dr. Sven Olek, Dr. Udo Baron, Epiontis, Berlin, Germany

Dr. Antony Pernthaner, Massey University, New Zealand

Prof. Andreas Radbruch, PD Dr. Ria Baumgrass, Dr. S. Fillatreau, DRFZ, Berlin, Germany

Prof. Peter Robinson, Charité, University Medicine Berlin, BCRT, Berlin, Germany

Prof. Tim Sparwasser, Twin Core, Inst. f. Infection Immunology, MH Hannover, Germany

Prof. Jan Schwab, Prof. Andreas Meisel, Charité, Medical Clinic f. Neurology, Berlin; Germany

Prof. Hans-Dieter Volk, Charité, University Medicine Berlin, Inst. f. Medical Immunology and BCRT, Berlin, Germany

Dr. Rudolf Volkmer, Charité , University Medicine Berlin, Inst. f. Medical Immunology and RCIS, Berlin, Germany

Prof. J. Walter, University of Saarland, FB Genetik, Saarbrücken, Germany

Prof. Martin Witzenrath , Charité, University Medicine Berlin, Medical Clinic f. Infectiology and Pneumology, Berlin, Germany

PD Dr. Thorsten Wolff, RKI, Berlin, Germany

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Topographical memory and epigenetics of T cells.

SCIENTISTS

D. Engelbert, M. Exner, R. Maier, M. Mardahl, M. Pink, J. Polansky-Biskup, J. Triebus, A. Hamann, U. Syrbe

COOPERATION PARTNERS

A. Radbruch, R. Baumgrass - DRFZ, Berlin

E. Serfling, S. Klein-Heßling – Universität Würzburg, Inst. f. Pathologie, Würzburg

S. Olek, U. Baron - Fa. Epiontis, Berlin

J. Hühn, S. Flöss - Helmholtz-Zentrum f. Infektionsforschung, Braunschweig

J. Hecht - MPI for Molecular Genetics Research und BCRT, Berlin

P. Robinson – BCRT, Berlin

J. Walter – Universität des Saarlandes, Saarbrücken

S. Steinfelder – Molekulare Parasitologie, Humboldt-Universität Berlin

J. Geginat – INGM, Mailand, Italien

M. Sperandio – LMU München

H.-R. Chung - Max Planck Institute for Molecular Genetics, Berlin

W. Chen - Max Delbrück Center for Molecular Medicine, Berlin

T effector/memory cells preferentially recirculate through the tissue, where they had primary contact to their cognate antigen. Specific homing is based on the expression of differential homing receptor patterns. E- and P-selectin ligands are important for the immigration of T cells into the skin and into inflamed tissues, whereas integrins, in particular $\alpha4\beta7$ are important for immigration into mucosal tissues. These homing receptors are induced during differentiation of naive T cells into effector/memory T cells by the action of tissue specific dendritic and stromal cells. Permanent T cell specialization is determined by the activation of transcriptional programs and the repression of competing differentiation events. This so-called transcriptional memory is reflected by a distinct combination of histone modifications and DNA methylation thereby controlling the chromatin state of the genes involved in cell function. Aberrant epigenetic signatures influence the transcriptional outcome which might lead to the development of diseases. The goal of this project is now to characterize the transcriptional and epigenetic regulation, i.e. the induction and stabilization, of skin- and gut-homing molecules on a locus-wide level as well as on a genome-wide level the characterization of epigenetic signatures of physiological and pathological memory in T cells, including those from patients suffering from chronic inflammatory diseases.

Regulation of skin- and inflammation-specific homing receptors

The critical binding epitopes of E- and P-selectin ligands are specific oligosaccharides. In T cells, their generation is controlled by the expression of glycosyltransferases, such as core-2-glycosaminyltrasferase-1 encoded by the gene *Gcnt1*. Using ChIP-on-chip to detect the histone modification pattern across the *Gcnt1* locus we identified a putative distal regulatory region within the *Gcnt1* locus (Figure 1). Cloning of this region and reporter assays confirmed the enhancer function of this region. The distinct changes in histone modifications in this region suggest that epigenetic modifications contribute to transcriptional control of *Gcnt1* expression. Further studies are required to determine whether such modifications might control long-term expression of *Gcnt1* and, by that, E- and P-selectin ligand expression in effector/memory T cells.

Regulation of $\alpha4\beta7$ as a gut-homing molecule on $CD4^+$ T cells

The functional homing receptor $\alpha4\beta7$ occurs as an integrin dimer consisting of an α- and β-chain. Transcription of both integrin chains is regulated independently. Whereas transcription of integrin $\alpha4$ is dependent on stimulation with retinoic acid, the $\beta7$-chain is expressed constitutionally. We found that $\alpha4\beta7$ is stably expressed *in vivo* on memory T cells and that this stability needs repetitive stimulation with retinoic acid. We performed Nimblegen ChIP-on-chip experiments to characterize the locus-wide distribution of histone modifications as well as DNA methylation (Figure 2). With this technique we identified two differentially regulated regions within the Itga4 gene and luciferase reporter gene assays confirmed their enhancer function. One regulatory element is demethylated upon treatment of retinoic acid and the second one is marked by a gain of the repressive H3K27me3-modification in response to Interleukin 4. Unexpectedly we also found a retinoic acid-induced DNA methylation i.e. silencing of the promoter region suggesting an alternative promoter usage. In line with this, preliminary results measuring different transcripts favor a model in which retinoic acid regulates *Itga4* expression by promoter switching thereby inducing a possibly functionally different integrin α–chain.

Epigenetic modification of *cis*-regulatory regions might affect interaction of enhancer elements with native and alternative promoters thereby providing a mechanism for stability of the gut-homing phenotype in CD4+ T cells. Identification of this postulated enhancer and functional characterization of its transcripts will shed more light into how retinoic acid regulates homing molecule expression.

Genome-wide epigenetics in T cells

In response to the rapid methodological advances concerning epigenetic profiling, we want to widen our work scope on epigenetic gene regulation from our traditional genes of interest to the genome-wide level. Screens on such a global scale will highlight new candidate genes which might serve important functions in T cell development and differentiation or maintenance.

For this, we started to characterize different murine CD4+ memory T cells subsets for their epigenetic profile concerning DNA methylation and distribution of H3K4me2 and H3K27me3, both well-established histone marks which contribute fundamentally to the activity state of epigenetically regulated genes. A first preliminary screen using the ChIP-on-Chip technology yielded in a list of candidate genes, which we are now confirming and further analyzing for their role in memory T cell development.

Furthermore, we successfully established a project on the epigenetic characterization of human CD4$^+$ T cell subsets of patients suffering from chronic inflammatory diseases. The project is part of the newly BMBF-funded German Epigenome Project ("Deutsches Epigenom Programm – DEEP"), which started off in September 2012 and is a partner of the International Human Epigenome Consortium (IHEC).

Perspectives:

Epigenetic signatures are expected to highlight differentiation pathways that result in a pathological and presumably irreversible stage of chronically activated cells that differ from normal, protective memory cells by impaired susceptibility to internal control mechanisms as well as to current therapies. The data will allow to understand the involved pathophysiological mechanisms better and thereby serve as a starting point for the development of diagnostic tools as well as therapeutic strategies.

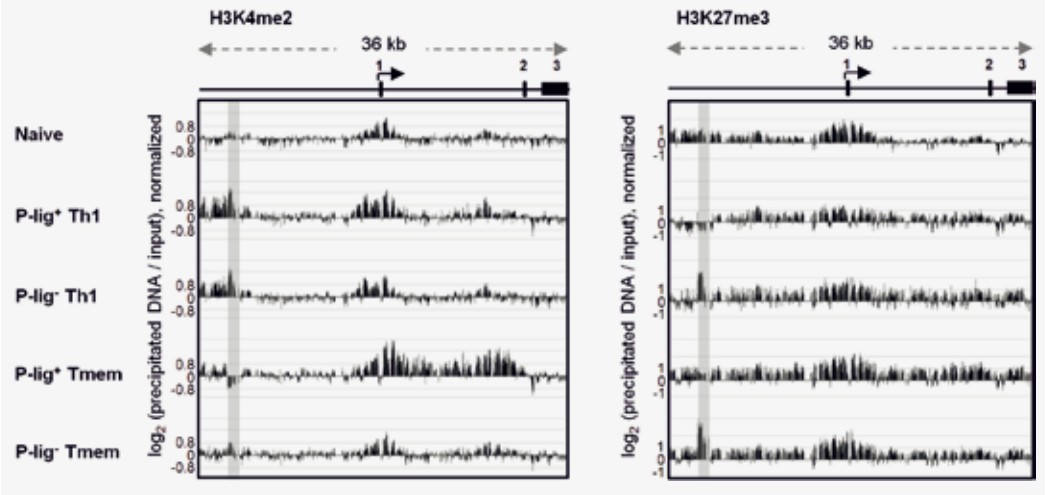

Figure 1: Identification of a distal, putative regulatory region upstream of exon 1 showing differential pattern of histone modifications in P-lig+ and P-lig- cells. DNA of naive T cells, P-lig$^+$ and P-lig$^-$ T cells sorted from Th1 cultures, or P-lig$^+$a4b7$^-$CD44highCD4$^+$ T cells (P-lig$^+$ Tmem) or P-lig$^-$a4b7$^+$CD44^{high}CD4$^+$ T cells (P-lig$^-$ Tmem) was precipitated with antibodies against H3K4me2 and H3K27me3. Enrichment across the *Gcnt1* locus and its vicinity was determined by hybridization to a custom-designed tiling array. Normalized signal intensities are depicted across the *Gcnt1* locus depicted in reverse orientation. In grey the region of the enhancer is shown.

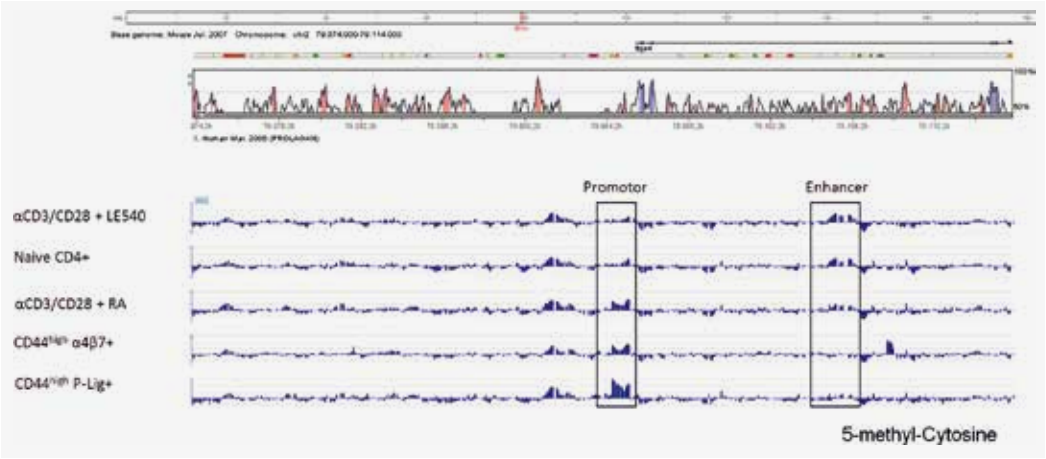

Figure 2: DNA methylation profile of the murine Itga4 locus in different T cell subsets. The locus is shown relative to a VISTA conservation plot between mouse and man. In vitro activated T cells were cultured in the presence of retinoic acid or the retinoc acid inhibitor LE540. CD44^{high} memory cells were sorted ex vivo according to their homing receptor expression. Cells were analysed by MeDIP-chip and differentially methylated regions are marked by boxes.

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Immune regulation, tolerance and immunotherapy.

SCIENTISTS

A. Hamann, S. Frischbutter, U. Hoffmann, E. Kenngott, M. Kröger, U. Lauer, F.D. Liu, J. Pfeil, C. Poulsen, A. Rigby

COOPERATION PARTNERS

S. Bereswill, M. Heimesaat, Charité University Medicine Berlin, Dept. f. Microbiology

Celares GmbH, Berlin, Germany

G. Debes C. Hunter, University of Pennsylvania, Dept. of Pathobiology, Philadelphia, U.S.A

R. Haag, Free University, Dept. f. Chemistry and Biochemistry

S. Hartmann, S. Steinfelder, Dept. f. Immunology, Veterinary Medicine, FU Berlin

R. Lucius, Humboldt University, Dept. Molecular Parasitology, HU Berlin

J. Hühn, S. Flöß, Helmholtz-Centre for Infection Research, Braunschweig

A. Kühl, U. Erben, Charité University Medicine Berlin, RCIS

M. Löhning, A. Scheffold, Charité University Medicine Berlin , Medical Clinic Rheumatology

A. Pernthaner, Massey University, New Zealand

A. Radbruch, R. Baumgrass, S. Fillatreau, DRFZ

T. Sparwasser, Twin Core, Inst. f. Infection Immunology, MH Hannover

H.-D. Volk, Charité University Medicine Berlin, Inst. f. Medical Immunology and BCRT

R. Volkmer, Charité University Medicine Berlin, Inst. f. Medical Immunology and RCIS

M. Witzenrath, Charité University Medicine Berlin, Medical Clinic f. Infektiology and Pneumology

T. Wolff, RKI

Current treatments for chronic inflammatory disorders caused by hyper- or autoreactivity of the immune system rely on non-specific immunosuppressive drugs, which show a variable degree of efficiency and are often accompanied by severe side effects. Regulatory T cells (Tregs) and additional immunosuppressive factors play an important role in the control of immune reactions. It seems attractive to exploit these regulatory mechanisms for new therapeutic strategies against unwanted immune reactions. So, we identified the suppressive cytokine IL-27 being essential for limiting lung tissue damage during murine influenza infection; indeed, treatment of mice with IL-27 alleviated lung immunopathology.

To discover natural or synthetic substances that enhance self-control mechanisms of the immune system (induction of Foxp3⁺ Tregs or IL-10 producers) we established a high-throughput–screening-platform based on flow-cytometric cell analysis. Promising hits were already found in cooperation with S. Fillatreau.

Furthermore we want to improve tolerogenic vaccination by auto-antigen peptides with three different strategies: (1) Coupling peptides to synthetic macromolecular carriers to solve disadvantages of soluble peptide treatment, (2) conjugating peptides with mucosa-targeting peptides (MUC-peptides) to enhance uptake in the gut and induction of oral tolerance, or (3) genetical fusion of peptides to ligands binding to tolerogenic receptors on antigen-presenting cells (APCs). These approaches are promising strategies to treat autoimmunity or inflammatory diseases.

Immunoregulation of influenza infection

In the murine model of influenza infection we investigate the regulatory mechanisms which keep the balance between efficient virus clearance and prevention of immunopathology causing strong tissue damage. In line with previous reports that IL-27 is a key regulator of inflammatory responses (affecting both innate and adaptive cells), treatment of infected mice with IL-27 after the peak of infection was able to improve lung immunopathology and fasten recovery (Fig.1). Basis of this protective effect is the ability of IL-27 to decrease IL-17, IFNγ and chemokine production in the infected respiratory tract. Additionally, IL-27 significantly increases the regulatory cytokine IL-10. CD11b+ or CD11c+ cells are targets of IL-27–dependent chemokine regulation. We are currently determining the IL-27-induced downstream factors regulating the chemokine production by these cells.

ImmuDrug high-throughput-screening-platform

Our aim is to discover novel drug candidates or immunomodulators that would strengthen the endogenous mechanisms of self-control in the immune system, which are based on the action of specialized suppressor cells (e.g. regulatory T cells) or on cytokines limiting the immune response (e.g. IL-10, IL-27, IL-35).

Therefore we established a high-throughput screening platform based on a miniaturized cellular assay (384-well plates) that allows the detection of cellular responses such as induction of regulatory T cells or IL-10-producing cells (using Foxp3-GFP or IL-10 reporter cells) by flow cytometry. More than 5000 samples of various substance libraries or biological materials (e.g. from commensal microflora or from parasites) are measured per week. Promising hits were already detected by screening a library of small-molecular-weight-compounds provided by the Forschungsinstitut f. Molekulare Pharmakologie, Berlin-Buch, which will be further validated in functional and biochemical assays.

We think that this approach will deliver not only insights in molecular mechanisms of immunoregulation, but also discover novel candidates for future therapies of immune-mediated diseases.

Conjugation of T cell epitopes to inert carrier molecules

Although very successful in experimental models, peptide-based tolerogenic vaccines have largely failed to show efficacy in clinical trials. We therefore investigate whether conjugation of relevant T cell epitopes to synthetic macromolecular carriers can increase their tolerogenic potential. We compared different carriers such as polyethylene glycol (PEG) or polyglycerols of different structure and size. Most strikingly, PEGylated peptides of 20 kd or larger size had a highly extended bioavailability compared to free peptide. In a mouse model of multiple sclerosis (EAE) preventive treatment with PEG-conjugated auto-antigens partially

protected mice from the development of the disease by induction/expansion of Foxp3$^+$ Tregs and reduction of antigen-specific T-effector cells. Also conjugation of polyglycerols to peptides has an impact on the bioavailability depending on the size and branching of the polyglycerol, whereby highly branched structures were most effective. They also induced strongest reduction of effector cell/regulatory T cell ratio. It is planned to test their tolerogenic capacity in our model of multiple sclerosis.

Mucosa targeting peptides

In vivo screening of peptide libraries led our cooperation partner T. Pernthaner discover mucosa-targeting peptides (MUC-peptides). He showed in sheep that these short peptides target the mucosal surfaces and are taken up into the lymphatic system. Hence, MUC-peptides are attractive carrier candidates to increase the delivery of small molecules or biologics via the mucosa. Using laser-scanning microscopy we could show, that MUC-coupled model proteins are taken up by a subset of intestinal epithelial cells. Furthermore the constructs could be detected in CD11c$^+$ dendritic cells in Peyer´s patches of the small intestine, indicating that these cells might play an important role in the transport mechanism of the construct. By coupling MUC-peptides to $OVA_{323-339}$-antigen we could reduce the dosage required for T cell activation 8 fold, suggesting an enhanced mucosal uptake. We plan to transfer this concept to molecules of larger size e.g. antibodies or hormones.

Induction of tolerance by targeting antigen to tolerogenic pathways

Presentation of self-antigens derived from apoptotic cells by phagocytes is known to favor a tolerogenic response of T cells to these antigens. To take advantage of this mechanism, we coupled $OVA_{323-339}$-antigen (pOVA) to a molecule bridging apoptotic cells to APC. We could show in an *in vitro* proliferation assay that an increased number of antigen specific Foxp3$^+$ Tregs was induced/expanded compared to free pOVA control. The *in vivo* tolerogenic effect will be investigated soon.

Perspectives:

With the above approaches, we aim to develop novel approaches for the treatment of chronic inflammatory and autoimmune diseases. Based on the modern knowledge of cellular functions in the immune system, the old vision of induction antigen-specific tolerance to treat autoimmunity is re-vitalized and other ways to exploit immunoregulatory mechanisms for therapy explored. *In vitro* work to decipher the mechanisms and animal disease models to prove efficacy will pave the way to an application in the human.

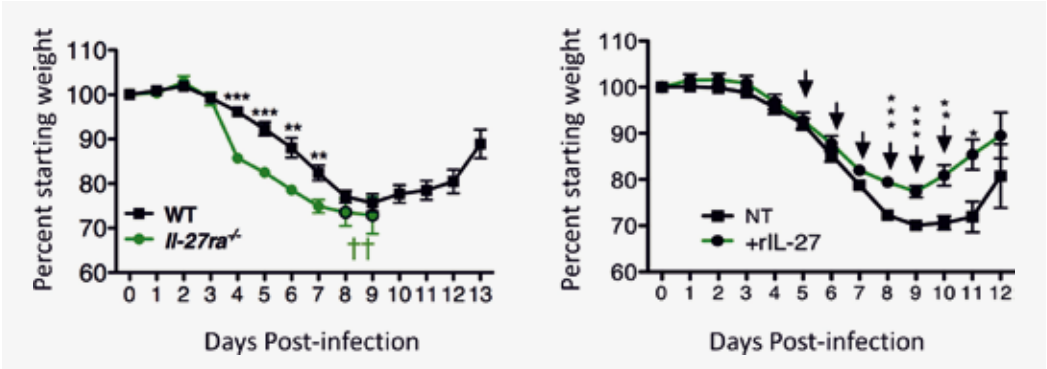

Figure 1:
Left: Increased mortality and immunopathology during influenza in mice lacking the IL-27 receptor. Weight loss and survival of infected Il-27 receptor k.o. or wild-type (WT) mice after infection with influenza virus.
Right: Delayed rIL-27 treatment supports recovery from disease. C57BL/6 mice were infected with influenza and treated daily with rIL-27 from day 5-10 p.i. Weight loss of IL-27-treated or non-treated (NT) mice. Arrows (↓) indicate points of treatment. In cooperation with C. Hunter/G. Debes, University of Pennsylvania.

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Anja Erika Hauser

Immunodynamics

Cells on the move

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GROUP MEMBERS

Group leader:
Prof. Dr. Anja E. Hauser

PhD students:
Anja Lemke,
Katrin Roth,
Laura Oehme,
Jannike Bayat Sarmadi,
Magdalena Kraft,
Jöran Keßler

Bachelor students:
Sabrina Schüngel

Technicians:
Robert Günther,
Katharina Horn

The analysis of single cells *in vivo* has received a boost in the past years by new technical developments in the field of intravital microscopy. Multiphoton microscopes now allow us to analyze dynamic processes like cellular interactions and migration in living organisms in time lapse movies. This method is ideally suited to analyze how the immune system works in health and disease because dynamic processes are essential for immune function: Cells of the immune system migrate throughout the whole body to in surveillance of invaded pathogens and get in touch with each other in secondary lymphoid organs.We are using this technology to analyze the dynamics of B cells in secondary lymphoid organs, especially in germinal centers, which are crucial stuctures for affinity maturation of the humoral immune response. After activation, B cells can differentiate into antibody secreting plasma blasts and migrate into the bone marrow, where some of them can survive for extended time periods in a special microenvironment. We are interested in analyzing the cellular and molecular components as well as the dynamics of this survival niche. We are also investigating the plasma cells generated in mucosal immune responses with respect to their life span and relation to the bone marrow plasma cell compartment. We have developed a method for intravital imaging of the gut which allows us to analyze immune cell dynamics in the lamina propria. In close collaboration with Dr. R. Niesner (Biophysical Analysis lab at the DRFZ) we are working on new methods to improve imaging depth and resolution in intravital microscopy and we are developing novel techniques for functional immunoimaging.

COOPERATION PARTNERS

Dr. Z .Cseresnyes and Dr. A. Sporbert, MDC Berlin, Germany

Dr. E. Esplugues, DRFZ and NeuroCure Cluster of Excellence Berlin, Germany

Prof. M.-T. Figge, Applied Systems Biology, HKI Jena, Germany

Prof. M. Gunzer, University of Duisburg-Essen, Germany

PD Dr. U. Höpken, MDC Berlin, Germany

Dr. R. Niesner, DRFZ Berlin, Germany

Prof. Dr. A. Radbruch, DRFZ Berlin, Germany

PD Dr. H. Schulze, Laboratory of Pediatric Molecular Biology, Charité Berlin, Germany

Prof. T. Watts, Department of Immunology, University of Toronto, Canada

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*equally contributing senior authors

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Dynamics of plasma cell survival niches in the bone marrow

SCIENTISTS

Anja Hauser,
Katrin Roth,
Katharina Horn

COOPERATION PARTNERS

Sandra Zehentmeier,
Özen Sercan,
Hyun-Dong Chang,
Andreas Radbruch,
Cell Biology, DRFZ Berlin

Raluca Niesner,
Biophysical Analysis, DRFZ Berlin

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The bone marrow plays an important role in the maintenance of immunologic memory. Long-lived plasma cells are located in the bone marrow, where they encounter a special microenvironment termed survival niche. The cells which form this niche are thought to regulate maintenance and homeostasis of the plasma cell pool by supplying the plasma cells with survival factors. Antibodies produced by long lived plasma cells protect against pathogens through the production of antibodies over extended time periods. On the other hand, long-lived plasma cells can contribute to autoimmune pathology by secreting autoreactive antibodies. It is therefore important to elucidate the cellular and molecular composition of the survival niches. Although several factors that promote the survival of plasma cells have been identified *in vitro*, the information concerning plasma cell dynamics *in vivo* is scarce. Eosinophils in the bone marrow have been shown to produce plasma cell survival factors such as APRIL and IL-6. Plasma cells were found to be located in the vicinity of eosinophil clusters in the bone marrow. However, eosinophils are generally thought to be short-lived cells, raising the question how they are able to support long-lived plasma cells over extended time periods. In this project, we determine the lifespan of the different survival niche components, investigate the organization of the survival niche and analyze bone marrow plasma cell dynamics by intravital microscopy.This project is a joint project of the DRFZ groups Immunodynamics and Cell Biology.

Bone marrow plasma cells are sessile

To analyze plasma cells in the bone marrow niches *in vivo*, we performed multiphoton microscopy in the bone marrow of live mice with green fluorescent stromal cells and red fluorescent plasma cells. The majority of the plasma cells was sessile (Fig. 1). and appeared to be in close contact with stromal cells, a finding we could confirm by confocal microscopy (Fig. 2)

Bone marrow plasma cells are in contact with stationary reticular stromal cells

Next, we analyzed bone marrow sections in order to investigate whether long-lived plasma cells interact with stromal cells. We performed pulse-chase experiments using the thymidine analog Ethinyldeoxyuridine (EdU) to label antigen-specific plasma cells generated during a systemic immune respons (Fig. 3). The frequency of EdU+ plasma cells in contact to stromal cells increased between day 12 and day 30 after boost, indicating that long-lived, resident bone marrow plasma cells preferentially contact stromal cells

Eosinophils, accessory cells of the plasma cell niche, become exchanged frequently

Eosinophils have recently been shown to constitute an important part of the plasma cell survival niche as they produce high amounts of APRIL, which is known to promote plasma cell longevity. Eosinophils are generated in the bone marrow and migrate to their effector sites in the periphery when they are mature. We asked whether a continuous presence of a subpopulation of long-lived eosinophils is required to provide APRIL within a certain plasma cell survival niche or whether

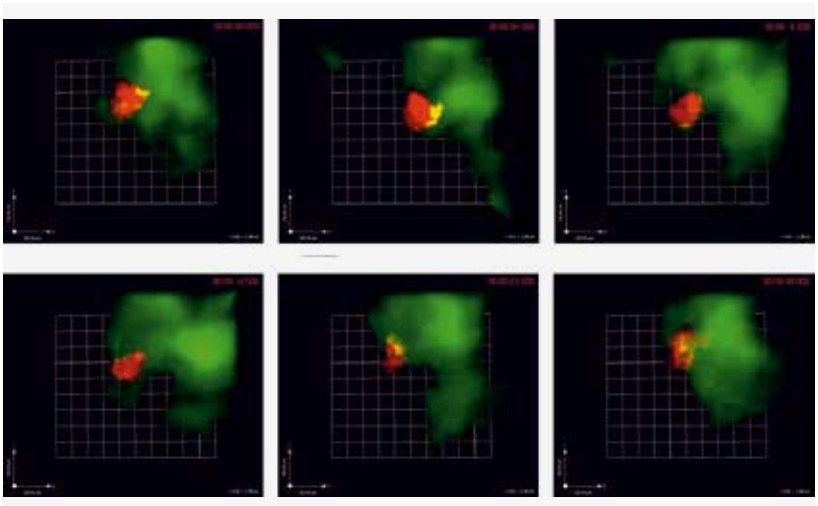


Figure 1: PC (red) form prolonged contacts with bone marrow stroma cells (green) *in vivo*, contact area is shown in yellow. Stills taken from an intravital movie of the bone marrow in the tibia at indicated time points.

the eosinophils are dynamically exchanged. To address this question, we followed the EdU-uptake in the bone marrow eosinophil population by flow cytometry. Almost the whole eosinophil compartment took up EdU during the 12 day feeding period, this stands in strong contrast to the EdU-uptake in stromal cells which show no proliferative activity.No EdU+ eosinophils were present near long-lived bone marrow plasma cells on day 30 after the boost immunization (Fig. 4). This implies a role for the stroma cells as stationary organizers of the plasma cell niche.

Perspectives:

Understanding factors influencing plasma cell survival will have consequences for the therapy of antibody-mediated autoimmune diseases as well as the development of vaccines.

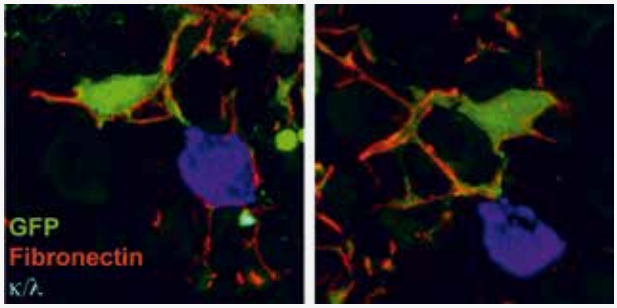
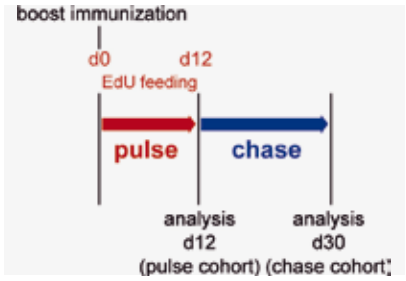


Figure 2A: Bone marrow plasma cells directly contact reticular stroma cells on day 30 after secondary immunization with NP-CGG. Femoral sections of Ubq:GFP - C57BL/6 chimeras were stained for fibronectin, intracellular κ and λ light chain. Plasma cells of day 30 are in direct contact with processes of reticular stroma cells coated with the extracellular matrix protein fibronectin. The image is generated as a 2-dimensional projection of a z-stack acquired on a Zeiss LSM710 confocal microscope

Figure 2B: *In vivo* EdU pulse chase analysis to investigate the turnover of different cellular components of the bone marrow plasma cell survival niche. Mice were fed with EdU for 12 days starting with the day of the boost immunization and analyzed right after the pulse on day 12 (pulse cohort) or after a chase period of 18 days without EdU feeding (chase cohort).



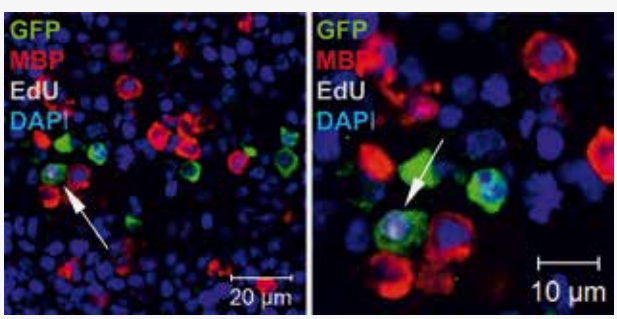


Figure 2C: Long-lived bone marrow plasma cells are surrounded by short-lived eosinophils on day 30 after the boost immunization. We analyzed femoral sections of mice with GFP⁺ plasma cells (Blimp1:GFP) after the chase perion (day 30 after the boost) that were stained for GFP, EdU, DAPI and major basic protein (MBP) to visualize eosinophils. While the plasma cell stays EdU⁺ during the chase period, eosinophils next to the long-lived plasma cell are EdU⁻, i.e. they have proliferated and/or have been exchanged.

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FUNDING

DFG HA5354/4-1, JIMI-joint intravital microscopy platform

Lifetime and in vivo migration of mucosal plasma cells

SCIENTISTS

Anja Hauser,
Anja Lemke,
Magdalena Kraft

COOPERATION PARTNERS

Raluca Niesner,
Biophysical Analysis, DRFZ Berlin

The majority (>80%) of all plasma cells in the body is located in the intestinal lamina propria and most of them produce antibodies of IgA-isotype. It is known that in the course of systemic immune responses antibody-secreting cells are able to survive for extended time periods in survival niches within the bone marrow. After the B cell activatoin which occurs in the Peyer´s patches and isolated lymphoid follicles in the intestine, IgA+ plasma blasts migrate to the mesenteric lymph nodes via lymph, then they enter the blood circulation through the thoracic duct and home back to the lamina propria to secrete antibodies. In contrast to plasma cells within the secondary lymphoid organs and the bone marrow, little is known about the lifetime of the mucosal plasma cells. In this project we focus on the analysis of migration behavior and life span of plasma cells induced in mucosal immune responses. We are investigating whether circulating mucosal plasma cells can migrate to the bone marrow and contribute to the long-lived plasma cell pool residing in the local survival niches. Moreover, we are analyzing the lifetime of plasma cells in the lamina propria in order to investigate whether plasma cell survival niches within the intestinal mucosa exist, similar to the bone marrow.

Antigen-specific plasma cells are detected up to one year after oral immunization

By ELISPOT we could detect antigen-specific antibody secreting cells one year after oral immunization with cholera toxin (CT). These plasma cells were present in the lamina propria as well as in the bone marrow (Fig. 1).

EdU labeling confirms longevity of plasma cells induced in mucosal immune responses

To obtain information about the lifespan of plasma cells generated in mucosal immue responses we fed mice with the thymidine analogon EdU for 12 days after oral immunization. EdU gets incorporated into the DNA of dividing cells (EdU-pulse) and thus labels all cells generated during the feeding period. This makes it possible to track them a later time points (chase) by

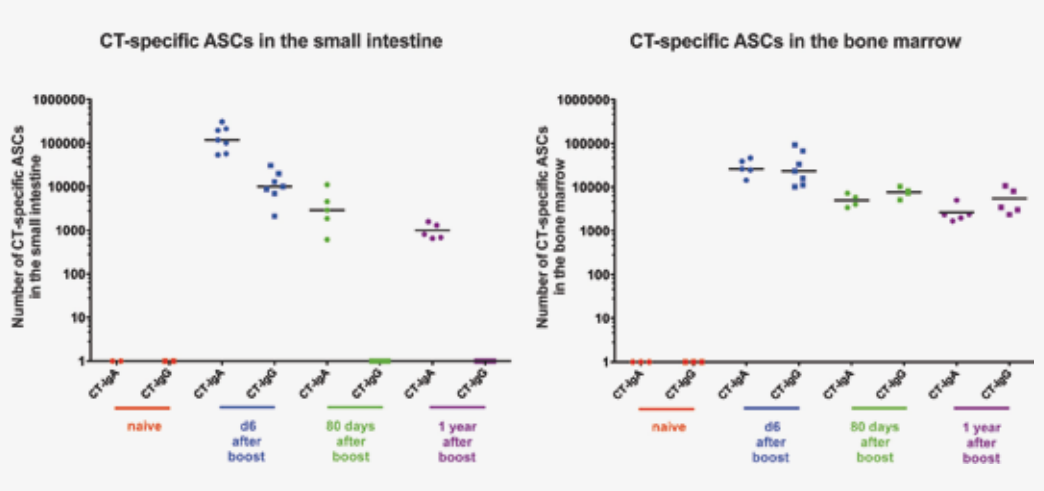

Figure 1: Detection of cholera toxin (CT)-specific plasma cells by ELISPOT. (A) IgA+ CT-specific plasma cells can be detected in the lamina propria up to at least on year after immunization. (B) IgA+ and IgG+ CT-specific plasma cells are present in the bone marrow one year after oral immunization.

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flow cytometry or immunofluorescence microscopy. We could detect EdU+ plasma cells in the lamina propria up to days after the end of the EdU pulse by flow cytometric analysis and immunofluorescence histology. This implies that long lived plasma cells are generated in mucosal immune responses. Interestingly, we could also find EdU+ plasma cells in the bone marrow, indicating that plasma cells generated in a mucosal response can contribute to the long lived plasma cell pool in that organ (Fig. 2).

Perspectives:

Understanding the dynamics of plasma cell migration and their turnover will help to understand their function in protective immune responses as well as in autoimmunity.

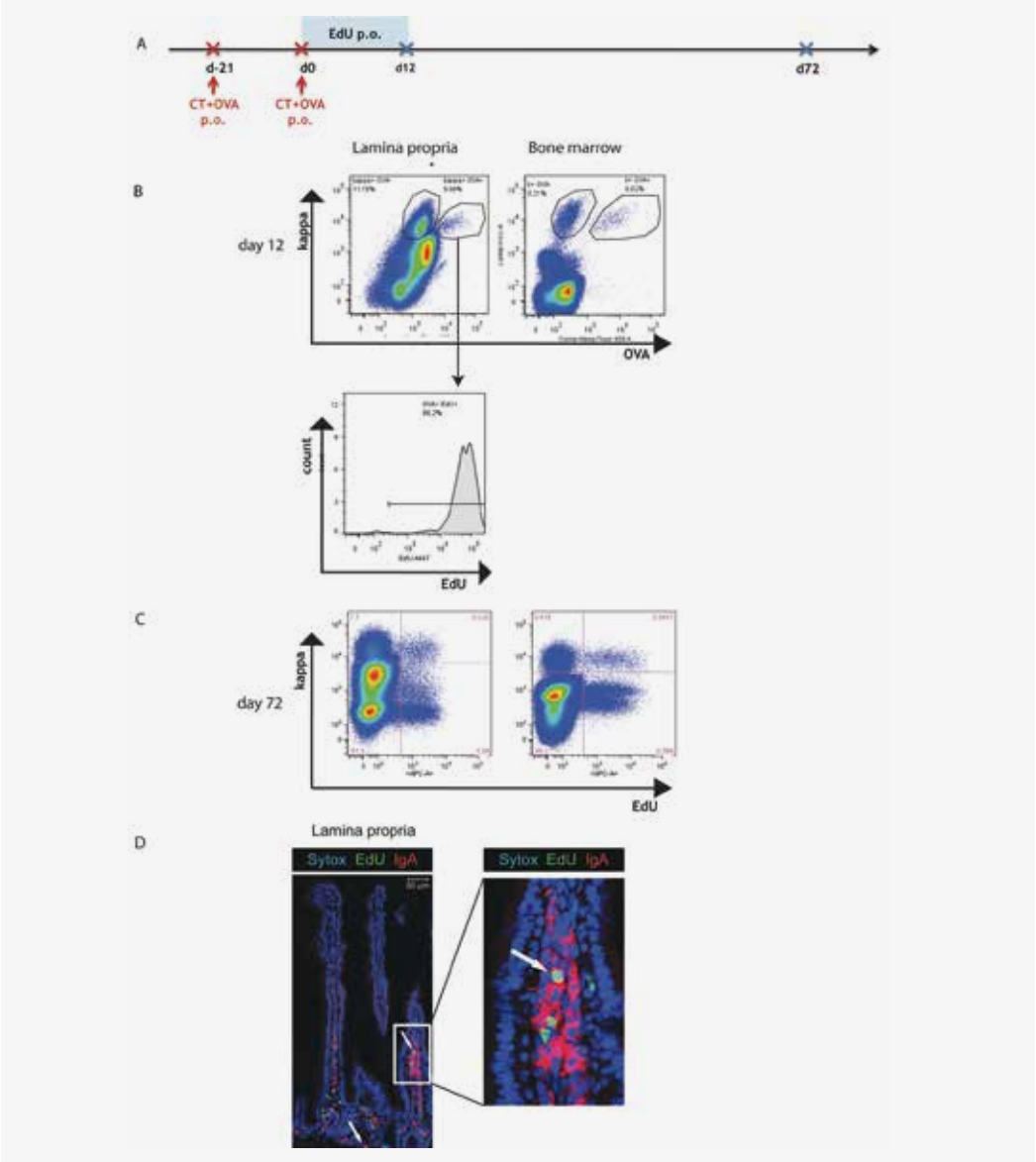

Figure 1: Long lived plasma cells are gnerated in mucosal immune responses.
(A) Experimental outline to assess the lifespan of ovalbumin-specific plasma cells using EdU-pulse-chase. (B) Ovalbumin-specific plasma cells are detected at day 12 after boost in lamina propria and bone marrow. They are almost all EdU+. (C)EdU+ plasma cells can be detected for at least 60 days after termination of the EdU-feeding in lamina propria as well as bone marrow by FACS. (D) EdU+ IgA+ plasma cells are present in the villous lamina propria as shown by immunofluorescence histology.

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*equally contributing senior authors

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Sergei Nedospasov

Inflammation Biology

Basic research that may result in innovative clinical applications

KEYWORDS

TNF
Lymphotoxin
Autoimmunity
Gene regulation
Antibody engineering

GROUP MEMBERS

Group Leader:
PhD, D.Sci. Sergei Nedospasow

Scientists:
Andrei Kruglov, PhD, Yuriy Shebzukhov, PhD.

PhD students:
Anna Kuchmiy (defended her Ph.D. in 2012);

Caroline Winsauser (since December 2011).

Bachelor/Diploma/Master students:
Taisiya Bezhaeva

Technical assistance:
Sandra Prepens, Tharsana Tharmilingam (till 12.2012),

Dirk Schlienz

Students:
Svetlana Bozrova (Summner 2012)

Others:
Visiting scientist, Marina Drutskaya, PhD (December 2012).

Our research group is interested in the physiological functions of the cytokines of the tumor necrosis factor (TNF) superfamily: TNF-α, lymphotoxin (LT) α and LTβ. TNF blockade in humans has emerged as an efficient treatment of autoimmune diseases, including rheumatoid arthritis (RA), and the therapeutic blockade of LT is under evaluation in cancer.

Using mouse models, we seek to better understand the immunological consequences of TNF ablation *in vivo*, as it occurs in patients on anti-TNF therapy. We also want to provide a rational for better and safer anti-TNF therapies. Previous mouse studies have suggested that beneficial effects of the therapy may be accompanied by partial immunodeficiency and by the loss of TNF-mediated host-defense functions.

We have generated panels of useful mouse models to investigate the pathogenic mechanism mediated by TNF. In the "knockin" mouse producing human TNF all clinically used TNF blockers can be studied and compared side-by-side in various disease models, such as septic shock, collagen-induced arthritis, experimental autoimmune encephalomyelitis and autoimmune colitis. Using mice with conditional ablation of either TNF or LT in specific cell types we are dissecting non-redundant functions of these cytokines in mucosal immunity. Finally, we are studying transcriptional regulation of mouse and human TNF/LT genes in various cell subsets, with specific focus on the epigenetic level of control. We are addressing the role of signalling pathways on the chromatin configuration of TNF and LTα promoters using cutting edge molecular biology methods. Altogether, our studies will help to better understand the role of TNF and LT produced by various cell types and may lead to the development of new therapeutic approaches.

COOPERATION PARTNERS

Prof. Dr. T. Blankenstein, MDC, Berlin, Germany

Dr. M. Drutskaya, Engelhardt Institute of Molecular Biology, Moscow, Russia

Dr. C. Gouttefangeas, Interfaculty Institute for Cell Biology, Tübingen, Germany

Dr. Sergei Grivennikov, Fox Chase Cancer Center, Philadelphia, USA

Prof. Dr. Mathias Heikenwalder, TU Munich, Munich, Germany

Prof. Dr. D. Kuprash, Engelhardt Institute of Molecular Biology, Moscow, Russia

Dr. Ronald Naumann, Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany

Prof. Dr. Steffan Rose-John, Christian-Albrechts-Universität zu Kiel, Kiel, Germany

Prof. Dr. J. Scheller, Institut für Molekularbiologie und Biochemie, Heinrich-Heine-Universität, Düsseldorf, Germany

Prof. Dr. Ari Waisman, Institute for Molecular Medicine, Mainz, Germany

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Role of TNF produced by distinct cellular sources during autoimmune diseases

SCIENTISTS

A. Kruglov C. Winsauer, S. Prepens, D. Schlienz, S. Nedospasov

COOPERATION PARTNERS

Dr. Sergei Grivennikov, Fox Chase Cancer Center, Philadelphia, USA

Prof. Dr. Mathias Heikenwalder, TU Munich, Munich, Germany

Prof. Dr. D. Kuprash, Engelhardt Institute of Molecular Biology, Moscow, Russia

Dr. Ronald Naumann, Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany

Prof. Dr. Steffan Rose-John, Christian-Albrechts-Universität zu Kiel, Kiel, Germany

Prof. Dr. J. Scheller, Institut für Molekularbiologie und Biochemie, Heinrich-Heine-Universität, Düsseldorf, Germany

TNF, Lymphotoxin α and β are cytokines expressed by mutiple cell types in the immune system. They have a broad range of physiologic functions during development and maintenance of immune system, as well as during inflammation (1). In the reporting period we studied the role of TNF/LT produced by various lymphocytes during autoimmune disease models, such as collagen-induced arthritis and autoimmune colitis. We uncovered the critical role of TNF produced by various cell subsets in the development of CIA and autoimmune colitis, suggesting different TNF-dependent mechanisms in these diseases.

Results and discussion

Elevated TNF levels are associated with pathology in many autoimmune disorders. TNF blockade is beneficial in rheumatoid arthritis, autoimmune psoriasis and Crohn's disease (1, 2).

We aimed to dissect the pathogenic and protective cellular sources of TNF during autoimmune arthritis and colitis. We found that TNF produced by macrophages and neutrophils played a pathogenic role in collagen-induced arthritis while TNF from T cells provided protection possibly via the control of Th1 and Th17 development in this disease (Fig. 1A, B, C). Mice with TNF deletion in B cells, characterized by reduced germinal centre formation and diminished anti-collagen antibody titres, developed arthritis with significantly reduced severity (Fig. 1A, B, D), suggesting a critical role for TNF in autoantibody production and arthritis development. Thus, our data uncover non-redundant effects of TNF in arthritis: direct pathogenic role of TNF derived from myeloid cells in arthritis induction, control of autoreactive T cell development by T-cell derived TNF, and regulation of autoantibody production via control of FDCs development and GC formation, presumably by soluble TNF produced by B cells.

To study the role of TNF in autoimmune colitis we employed T-cell transfer model into RAG1$^{-/-}$ recipients. Transfer of WT naïve T cells into RAG1$^{-/-}$ mice resulted into subsequent weight loss and development of strong inflammation in the colon (Fig. 1E). Surprisingly, naïve T lymphocytes from TNF deficient recipients were unable to induce disease (Fig. 1F). Altogether, these data indicate for the critical role of T-cell derived TNF in pathogenesis of autoimmune colitis and suggest that T-cell specific TNF ablation as a potential therapy for IBD.

Perspectives

Our data revealed pathogenic and protective functions of TNF expressed by various cell types during collagen-induced arthritis and autoimmune colitis. Thus, our results argue for the benefits of cell-specific blockade of TNF as a next generation of anti-TNF therapy in autoimmune diseases. We are currently developing reagents that may evaluate such a possibility in collagen-induced arthritis.

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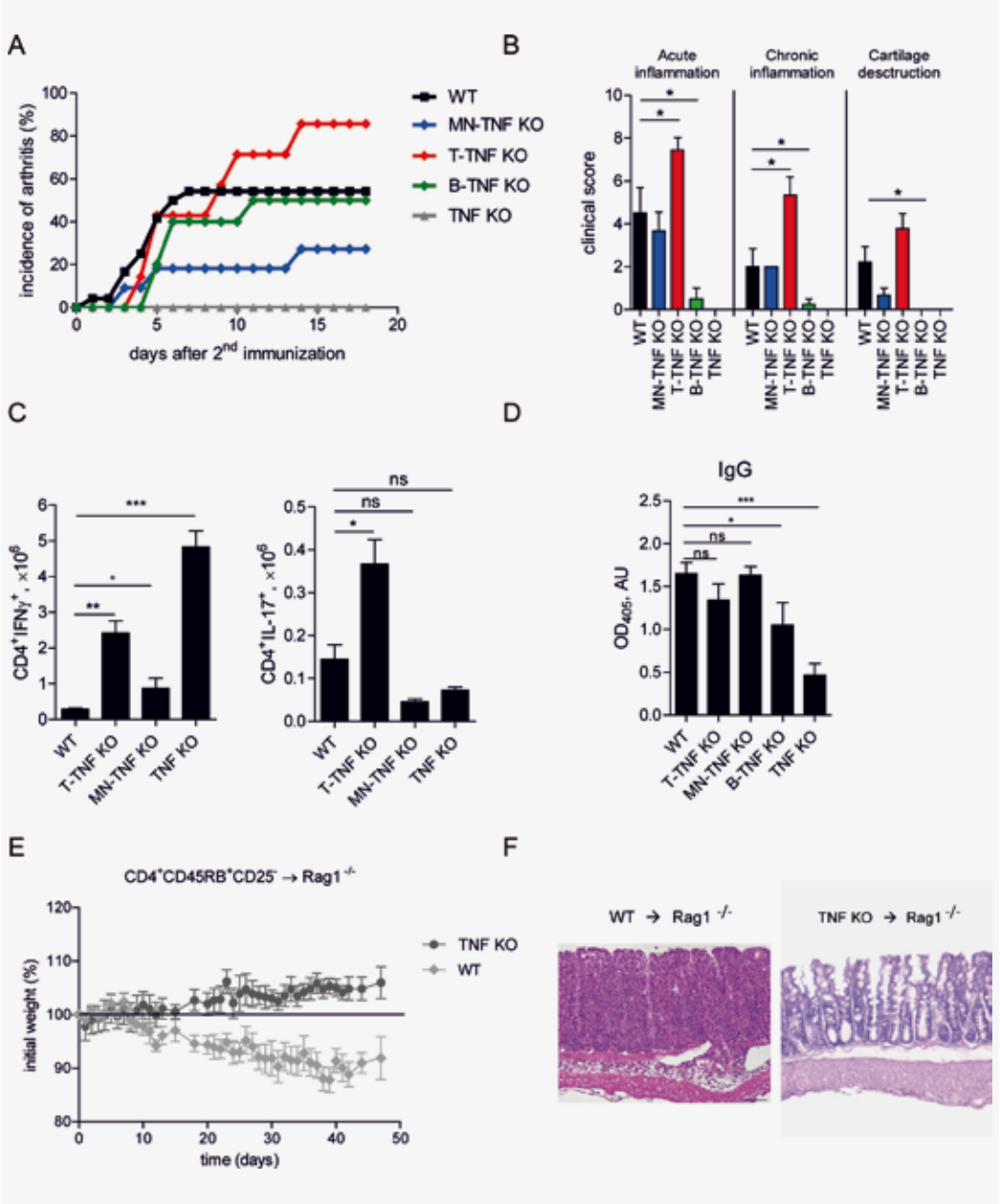

Figure 1. Pathogenic and protective functions of TNF during autoimmunity are defined by its cellular sources.
A. Collagen-induced arthritis incidence in WT, T-TNF KO, MN-TNF KO, B-TNF KO and TNF KO animals. Data are representative of three independent experiments. **B.** Clinical score of arthritic joints in WT, T-TNF KO, MN-TNF KO, B-TNF KO and TNF KO animals at day 14 after second immunization. Data are representative of two independent experiments. **C.** Antigen-specific Th1 and Th17 $CD4^+$ T cells in WT, T-TNF KO, MN-TNF KO and TNF KO mice at day 14 after second immunization during collagen-induced arthritis. Data are representative of three independent experiments. **D.** Anti collagen IgG titres in WT, T-TNF KO, MN-TNF KO, B-TNF KO and TNF KO mice at day 14 after second immunization during collagen-induced arthritis. Data are representative of three independent experiments. **E.** Weight loss upon transfer of naive ($CD4^+CD45Rb^{high}CD25^-$) T cells from WT and TNF KO animals into $Rag1^{-/-}$ recipients. Data are representative of three independent experiments. **F.** Hematoxylin and eosin staining of colonic sections of $Rag1^{-/-}$ mice transferred with WT or TNF KO naive T cells. Data are representative of three independent experiments.

PUBLICATIONS

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FUNDING

SFB633, TR52, DFG

Role of T cell lineage-specific transcription factors in regulation of genes at Tnf/Lymphotoxin (Lt) locus

SCIENTISTS

Y. Shebzukhov, T. Tharmalingam, T. Bezhaeva and S. Nedospasov

COOPERATION PARTNERS

Prof. Dr. D. Kuprash, Engelhardt Institute of Molecular Biology, Moscow, Russia

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Various subsets of T cells produce different amounts of Tnf and Ltα (1) and levels of expression of these cytokines correlate with activity of TCR-activated signaling cascades (2-3). Recent advances of genome-wide analysis of active chromatin and its interactions with regulatory proteins revealed binding of T cell lineage-specific trancription factors (TFs) to regulatory elements of Tnf/Lt locus, particularly to promoters of Tnf and Ltα genes (Fig.1). In this project we investigate the role of T cell lineage-specific TFs in regulation of transcriptional activity of genes at Tnf/Lt locus and TCR signal transduction.

Results and discussion

Bioinformatic analysis of ChIP-Seq data from ENCODE and GEO databases revealed that TCR-activated and lineage-specific TFs are binding to the same regulatory elements of Tnf/Lt locus (Fig.1). We are investigating the role of lineage-specific TFs in activity of regulatory elements of Tnf/Lt locus. Using a luciferase-based reporter assay we found that expression of the Th1-related transcription factor T-bet increases the activity of Tnf proximal promoter and enhancer in intron 3 of the Tnf gene.

Additional level of transcriptional regulation of Tnf/Lt locus may be provided by CCCTC-binding factor (CTCF)-dependent chromatin insulators, mediating interactions between regulatory elements and promoters. One of the CTCF-binding insulators is located in the last exon of the Ltβ gene (4) and coincided with a strong CpG island, already demethylated in embryonic stem cells (Fig. 2A). This insulator has an open chromatin structure regardless of the transcriptional activity of the Lt gene (for example, in macrophages) (Fig. 2A), contains a protein-protected DNA footprint and binds CTCF in different types of tissues and cells (Fig. 2B). We confirmed the open chromatin conformation of this CTCF-binding insulator by MNase assay (Fig. 2A) and will analyze its role in regulation of transcriptional activity of Tnf/Lt locus in primary T cells.

T cell lineage-specific TFs can potentially modify the TCR signaling cascade. We found that the expression of tyrosine kinase Lck - the major enzyme, providing the very first events of TCR signal transduction upon activation is decreased in Th2 cells. Analysis of ChIP-Seq data deposited to GEO database identified putative binding sites of T cell lineage specific factors of STAT family and T-bet in the promoters of Lck gene, and we expect to find the factor, responsible for the supression of Lck expression in Th2 cells.

Perspectives:

We will investigate the role of T cell lineage-specific TFs in the transcriptional regulation of TNF/LTα–genes and TCR signal transduction in T cell subsets, modulating expression of TFs by transfection of corresponding vectors and siRNA. This research will provide us with potential targets for regulation of Tnf and Lta expression in T cells.

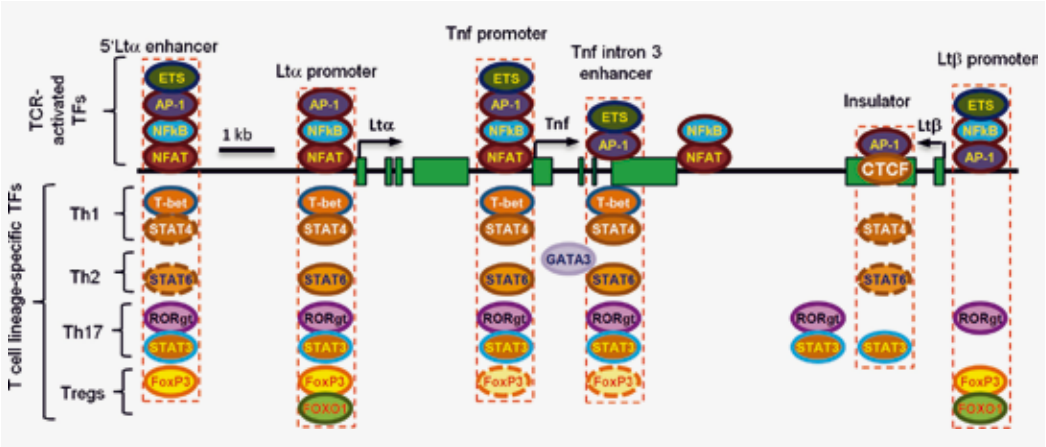

Figure 2. TCR-activated and lineage-specific TFs are binding to the same regulatory elements of the Tnf/Lt locus.

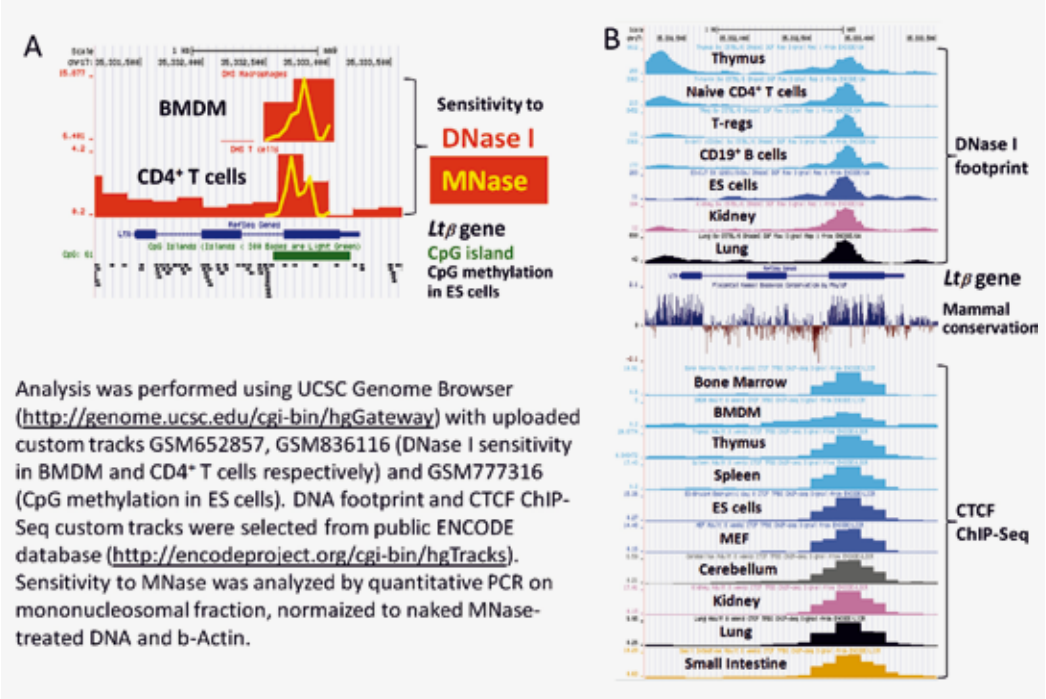

Figure 3. CTCF-binding insulator in the last exon of Ltb gene.

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FUNDING

DFG (SFB 633 and SFB/TR 52)

Raluca Niesner

Biophysical Analytics

Advancements in dynamic intravital multi-photon imaging

KEYWORDS

intravital two-photon laser scanning microscopy (TPLSM)

GRIN-based microendoscopy

fluorescence lifetime imaging (FLIM)

improvement of deep-tissue resolution

cellular interactions in the immune and central nervous system

GROUP MEMBERS

Group Leader:
Raluca Niesner

Postdoc:
Zoltan Cseresnyes

PhD Student:
Karolin Pollok

MD Student:
Agata Mossakowski

Master Student:
Hanna Zimmermann

MD Student:
Julian Pohlan

Technical Assistent:
Robert Günther

The development of new techniques which better meet the general requirement in biosciences and biomedicine to monitor vital processes at high spatial and temporal resolution, on a molecular basis, in their genuine environment is of central relevance for biophysics in general, and for microscopy in particular. In this frame, we focus on the improvement and extension of two-photon laser-scanning microscopy (TPLSM) with application to intravital microscopy (imaging in living anesthetized rodents) as far as the optical performance, e.g. spatial resolution, imaging depth, photobleaching or phototoxicity, and the molecular selectivity are concerned. The improved deep-tissue resolution and imaging depth due to IR-excitation by OPO (optical parametric oscillator) have already been demonstrated intravitally and will be extended to new areas including multi-photon microendoscopy and striped-illumination TPLSM. The linear "striped-illumination"-based TPLSM improves the axial resolution of both fluorescence signal and of second harmonic generation (SHG) by a factor of up to three and allow for the first time dynamic high-resolution microscopy in living tissue. The technique, which is particularly adequate to dynamically visualize cell-cell interaction within tissue, has been employed to image contacts between FDC and B-cells in the germinal center, which are presumably responsible for the clonal selection of high-affinity B cells. We currently extend the "striped-illumination" technique to the infrared excitation range to achieve larger penetration depth within organs and better deep-tissue resolution due to improved algorithms. Complimentary, we develop multi-photon microendoscopy based on GRIN optics technology (gradient refractive index = GRIN) to reach areas up to 1 cm deep within organs and intend to extend the technology to be suitable for longitudinal studies.

Furthermore, we demonstrate the molecular specificity of intravital Fluorescence Lifetime Imaging (FLIM) on the example of NADPH oxidase visualized via intracellular NADPH-FLIM in activated microglia, monocytes, neurons and astrocytes of healthy mice versus mice affected by experimental autoimmune encephalomyelitis (EAE). FLIM is further implemented to intravitally quantify the correlation between NADPH Oxidase activation and neuronal dysfunction in autoimmunity, i.e. the neuronal $Ca2^{+}$-level by FRET-FLIM in CerTN L15 mice.

COOPERATION PARTNERS

Jan-Leo Rinnenthal, Dr. rer. Nat., Institute for Neuropathology, Charité – University of Medicine, Berlin

Anja Erika Hauser, Prof. Dr., Immundynamics, DRFZ, Berlin and Charité - Berlin

Martin Behne, PD Dr. med., Eppendorf Universtity of Medcine, Hamburg

Helena Radbruch, Dr. med., Institute for Neuropathology, Charité – University of Medicine, Berlin

Carmen Infante-Duarte, Dr. rer. nat., Experimental Neuroimmunology, Charité – University of Medicine, Berlin

Friedemann Paul, Prof. Dr. med., NCRC, Charité – University of Medicine, Berlin

Frank Heppner, Prof. Dr. med., Institute for Neuropathology, Charité – University of Medicine, Berlin

Volker Andresen, LaVision Biotec GmbH, Bielefeld

Salim Seyfried, PD Dr. rer. Nat., Max-Delbrück Center for molecular medicine, Berlin

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Fluorescence lifetime imaging for *in vivo* MPLSM: quantifying cell function

SCIENTISTS

A. Mossakowski, J. Pohlan, R. Niesner

COOPERATION PARTNERS

H. Radbruch (Institute for Neuropathology, Charité – University of Medicine, Berlin); J.-L. Rinnenthal (Institute for Neuropathology, Charité – University of Medicine, Berlin); M. Behne, C. Börnchen (Eppendorf University of Medicine, Hamburg, Germany); A.E. Hauser (Immundynamics and Intravital Microscopy, Charité – University of Medicine, Berlin);

Due to the fact that the fluorescence lifetime is a molecule-specific parameter influenced only by the microenvironment, fluorescence lifetime imaging (FLIM) improves the capacity of fluorescence microscopy to monitor cellular and tissue function. To maintain the genuine environment of biological processes under study is of central relevance for biosciences and biomedicine. This results in two important requirements on microscopy, in general, and on FLIM, in particular: marker-free imaging, i.e. the use of endogenous chromophores such as the ubiquitous coenzymes NADH and NADPH (hereafter NAD(P)H) as probes of cellular function, and intravital imaging, i.e. microscopy in the genuine environment: the organism. We are concerned with these aspects starting from the development and validation of the method to its application to answer biologically relevant questions: in our case the role of NADPH oxidase activation and its correlation with neuronal dysfunction in chronic neuroinflammation.

Enzyme-selective marker-free FLIM

NAD(P)H fluorimetry belongs to the well established routines to monitor the cellular metabolism. However, this technique implies a loss of information since there is no direct possibility to distinguish between coenzyme molecules involved in cellular processes, i.e. bound to a specific enzyme, and the free (resting) coenzyme. Currently, it is widely accepted that for this purpose, i.e. to resolve between free and enzyme-bound NAD(P)H the method of choice is the fluorescence lifetime imaging (FLIM). Furthermore, it has been shown under cell free conditions that the fluorescence lifetime of the bound NAD(P)H strongly depends on the enzyme to which it is bound to.

We showed that NAD(P)H-FLIM can be used to selectively and intracellularly detect enzymes and apply this technique to study for instance the dynamic activation of NADPH oxidase in cell-mediated phagocytosis or the oxidative burst in plants of Nicotiana tabacum. Currently, we are interested to apply this technique to study the NADPH oxidase (NOX) function in activated microglia, macrophages, astrocytes and neurons of healthy mice versus mice affected by EAE in the central nervous system (Fig. 1). Further, we investigate whether the detection of NOX activation in blood-derived monocytes (CD11b) by FLIM can be used as a biomarker in MS diagnosis. Therefore, we measured and compared the NOX activation in spleen monocytes of healthy vs. EAE affected mice, which were either untreated, treated with glatiramere acetate or with EGCG (green tea extract). The same experiments were performed in blood-derived monocytes of healthy donors and of MS patients, either untreated or treated with glatiramere acetate or with glatiramere acetate and EGCG. These investigations were performed in the frame of a clinical trial led by Prof. Dr. F. Paul. Taken together, the results indicate that the activation of NOX in monocytes is significantly higher in untreated MS/EAE as compared to healthy controls. The treatment with glairamere acetate leads to a partial reduction of NOX activation. The treatment with EGCG leads to a reduction of NOX activation to the levels measured in healthy donors.

Intravital FRET-FLIM

FRET (Förster Resonant Energy Transfer) allows for investigating biochemical reactions, which imply changes in atomic distance in the range of 10-30 nm. The great power of this phenomenon lays in its compatibility with measurements in the living organism as currently possible due to the design of transgenic mice expressing FRET-based Ca-biosensors, membrane-potential-biosensors, apoptosis(caspase3/6/8)-biosensors, etc. Intravital FRET imaging has been already performed, however, only by means of two-photon-excitation fluorimetry. Though, it is known that repeated and reliable quantification of the FRET signal as necessary in dynamic intravital imaging is possible only by FLIM, a calibration-free FRET-quantification technique.

For the first time we were able to show that using parallelized TCSPC techniques dynamic, intravital FRET-FLIM is possible under the conditions of standard intravital two-photon laser-scanning microscopy, i.e. dynamic (approx. 1 minute / 100x100x50 μm^3 3D-stack), sub-cellularly resolved (down to 300 nm), deep-tissue imaging (down to 150 µm depth in brain tissue).

Furthermore, we used this method to quantify the correlation between NADPH oxidase activation in macrophages and neuronal dysfunction in the experimental autoimmune encephalomyelitis. Thereby we performed experiments in CerTN L15 x LysM tdRFP mice expressing in neurons (Thy1 expression cassette) a Ca-biosensor based on Troponin-C bound to derivatives of CFP and YFP as a FRET-pair. In tight cooperation with our collaborators from the Neuroimmunology, Charité, we also employ the intravital FRET-FLIM technique in CerTN L 15 mice to study the impact of other immune cells on the neuronal function in autoimmune processes in the brain.

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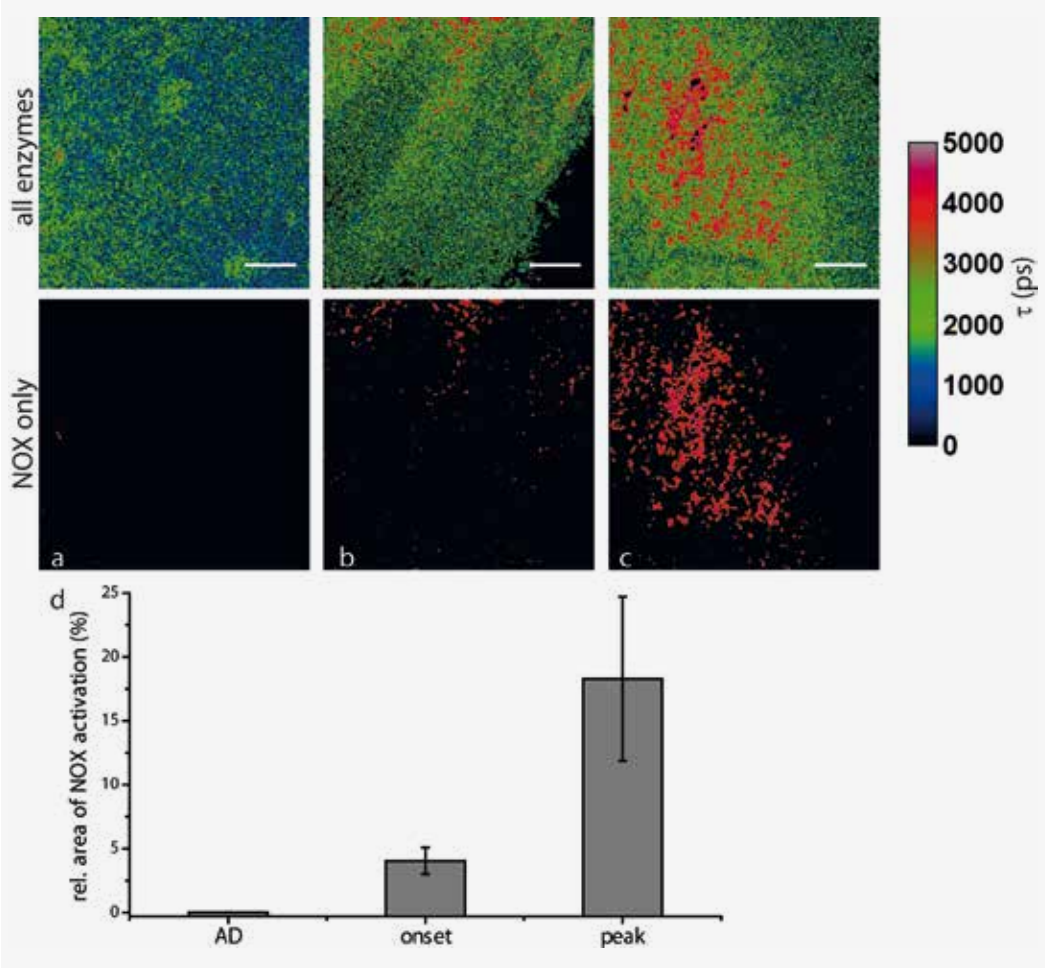

Figure 1: Fluorescence lifetime images of the enzyme-bound NAD(P)H in the brain stem of AD mice (a) and of mice affected by EAE, both at the onset of the disease (b) and at its peak (c). A fluorescence lifetime of approx. 3650 ps is typical for the activation of NADPH oxidase. (d) Quantification of the relative area of NOX activation in AD and at the onset and peak of EAE. Scale bar = 50 µm.

Improvement of optical performance in (intravital) microscopy: deeper, crisper, longer-lasting imaging

SCIENTISTS

Z. Cseresnyes, H. Zimmermann, K. Pollok, R. Niesner

COOPERATION PARTNERS

A.E. Hauser, Charité – University of Medicine, Berlin; H. Radbruch, Charité – University of Medicine, Berlin; H.-D. Chang, DRFZ, Berlin; V. Andresen, LaVision Biotec, Bielefeld; J.-L. Rinnenthal, Charité – University of Medicine, Berlin, S. Seyfried, MDC, Berlin;

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In the last 15 years the well established, dogmatic image of fluorescence microscopy in biosciences, i.e. molecularly highly flexible but of limited spatial resolution, was completely earth-quacked by novel nanoscopy techniques like STED, PALM, STORM or structured-illumination, which showed that the diffraction limit can be broken. However, these techniques cannot be applied or cannot be yet applied to deep-tissue intravital imaging. Our work is focusing on finding solutions for the improvement of resolution and of over-all optical performance for dynamic intravital microscopy.

Introduction

Since its development 1990, the two-photon laser scanning microscopy (TPLSM) has revolutionized our view on cellular and molecular dynamics especially of immune and neural processes in health and disease. Due to the advantages of near-infrared two-photon excitation, e.g. reduced scattering in tissue, optical sectioning and reduced photobleaching and phototoxicity at the out-of-focus regions, TPLSM first allowed cellular- and subcellular-resolved dynamic deep-tissue imaging in vital organ models and, even more important, in many organs of anesthetized small animals, i.e. intravital imaging.

Although the standard Ti:Sa-based TPLSM was able to answer many questions in biosciences and biomedicine by intravital imaging, there are still limitations referring to imaging depth, deep-tissue spatial resolution and photobleaching/phototoxicity. On one hand, the NIR excitation of the standard Ti:Sa lasers limits the penetration depth in hardly accessible organs and do not allow the use of red-fluorescent proteins transgenic-mouse technique, on the other hand, the depth-dependent deterioration of spatial resolution in tissue prevents us from unequivocally identifying dynamic cellular interactions in the living organisms, which build a central and general mechanism of tissue and organ function, e.g. as in the neuronal synapse, the immune synapse or newly in the neuro-immune synapse. Furthermore, the optics laws limit the imaging depth within organs, while current organ preparations for imaging limit the duration of an experiment to 10-12 hours.

Expanding intravital imaging capacities by means of GRIN-based multi-photon microendoscopy

In order to counteract the MPLSM limitation of imaging depth within organs (max. 1-1.6 mm), we use GRIN-based (gradient refractive index) microlenses, which allow focusing of high-power, ultra-short pulsed lasers necessary for multi-photon microendoscopy. Their dimensions vary between 350 µm diameter (4 mm length) and 1 mm diameter (1 cm length), while their NA (0.5 for 350 µm diameter and 0.8 for 1 mm diameter) allow multi-photon imaging similar to that achieved by MPLSM.

Currently, we are developing a system which allows the implantation of such lenses in the mouse to perform longitudinal studies in one and the same subject over few weeks. Concrete designs are concerned with the implant of a GRIN lens of 350 µm in diameter in the femoral bone marrow (Fig. 2) to characterize the survival niche of plasma cells and the implant of a 1 mm thick GRIN lens to image the cortex in mice affected by AD models or substantia nigra in mice affected by Parkinson disease models.

Resolution improvement in intravital microscopy: striped-illumination multi-beam TPLSM

The immune response implies the interaction between different subsets of immune cells as well as between immune cells and cells of the target organ. To quantitatively monitor this cellular communication and its implications for the cellular fate, truly high-resolved dynamic imaging in deep tissue is indispensable. Here we present the striped-illumination multi-beam two-photon laser scanning microscopy (SI-MB-TPLSM), which improves the depth-dependent axial resolution up to 3 fold and the lateral resolution up to 30% at similar acquisition speed, low photobleaching and photodamage and similar imaging depth as compared to standard TPLSM (Fig. 3). The technique is similar to structured-illumination imaging in wide-field microscopy but uses the scanned multi-beam line of a specialized two-photon microscope instead of a grid in the excitation pathway or the interference of two or three laser beams to create the known periodical illumination pattern. The algorithms used for evaluation are either a minimum-maximum algorithm or a standard Fourier-transform algorithm. Currently, we are extending the technique further to the infrared as far as the excitation wavelength range is concerned in order to take advantage to the increased imaging depth and to the reduced scattering effects as shown by us in Herz et al, Biophys. J., 2010.

In this way, we are for the first time able to quantify the dynamic interactions between the processes of antigen carrying follicular dendritic cells (FDC) and B cells within the germinal center involved in the clonal selec-

tion of high-affinity B cells. The B-cells are B 1-8 GFP$^+$ cells transferred in a non-fluorescing mouse, which is immunized in the foot pad with NP-CGG to observe the immune response in the popliteal lymph node. The staining of FDCs is performed 24 h before imaging by injection of CD21/CD35-Alexa586 or CD21/CD35-ATTO590 in the foot pad.

Expanding the simultaneous excitation range by asymmetric two-photon excitation

Currently, the number of chromophores, that can simultaneously be excited, detected and, finally, resolved by intravital MPLSM is still limited. By employing dual NIR/IR excitation at the same focus point, e.g. excitation at 850 nm (Ti:Sa) and at 1110 nm (OPO), we could simultaneously detect three fluorescent proteins, i.e. Cerulean and Citrine in neurons (FRET-pair of the calcium-biosensor in the CerTN L15 mouse) and tdRFP Th17 cells in the brain stem of mice affected by EAE (Herz et al, Biophys. J., 2010).

If in addition to the 850 nm excitation and to the 1110 nm excitation, the femtosecond laser pulses of the Ti:Sa and OPO are also temporally synchronized, a third excitation with one 850 nm and one 1110 nm photon can be achieved. This corresponds to a standard two-photon excitation at ≈963 nm. We demonstrated such an excitation for femtosecond pulsed lasers (Quentmeier et al, J. Phys. Chem. B, 2008), which has been later successfully employed to image the fluorescent proteins in the brain cortex of the Brainbow mouse by others (Mahou et al, Nat. Meth., 2012). The temporally and spatially matched dual NIR/IR excitation in intravital MPLSM (Fig. 4) will allow us an independent control of three different types of excitation, thus, extending the number of chromophores which can be simultaneously and optimally visualized from three to seven.

The adjustability of the spatial match of the NIR and IR foci will allow an improvement of the spatial resolution by changing the overlap of the illumination volumes. Only at the areas, where both NIR and IR photons are present, will the excitation of the chromophore molecules take place and will thus fluorescence appear. Furthermore, the effect of polarisation of the two different photons on the excitation efficiency as theoretically predicted in the 1970's (McClain, 1971) will be investigated. Hence, the possibility to distinguish between chromophores with similar or identical excitation and emission spectra via polarization-dependent asymmetric two-photon excitation will be explored.

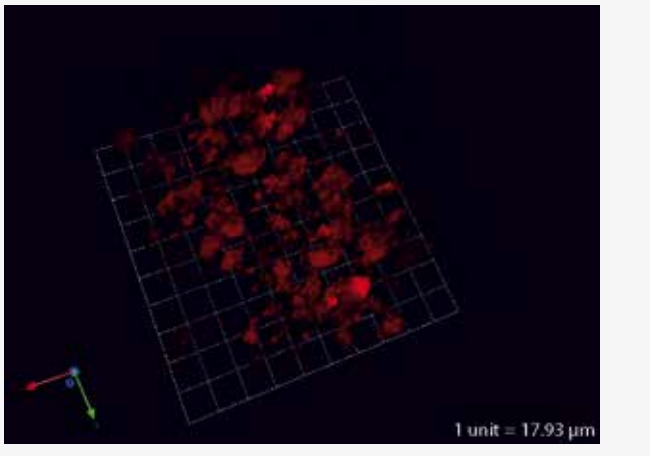

Figure 2: 3D view of fluorescence within the bone marrow of a LysM tdRFP mouse as recorded with a 350 µm thick GRIN lens (NA 0.3).

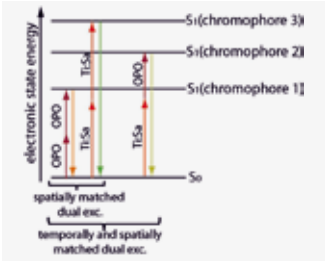

Figure 4: Principle of spatially and temporally synchronized NIR/IR dual two-photon excitation at 850 nm (Ti:Sa) and at 1110 nm (OPO). This will allow three parallel two-photon excitations and, thus, the simultaneous detection of an increased number of fluorophores.

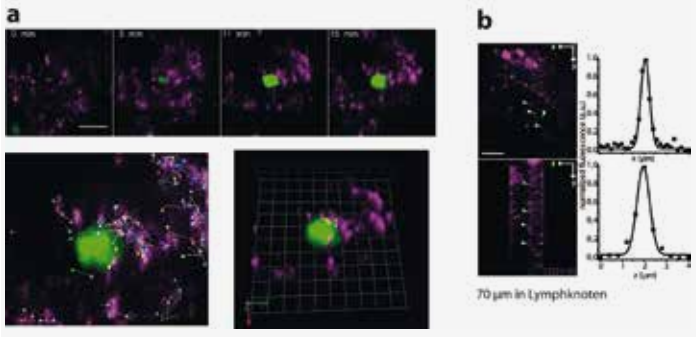

Figure 3: (a) Especially the axial resolution is dramatically improved in SI-MB-TPLSM as compared to standard TPLSM (PMT or CCD based). Due to this improved resolution, we could demonstrate how clusters of antigen carrying units are interacting with B cells within germinal centers of poplyteal lymph nodes. (b) Typical profiles of clusters of antigen carrying units in approx. 80 µm depth in the popliteal lymph node as recorded by SI-MB-TPLSM.

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Andreas Radbruch

Cell Biology

Immunological memory as driving force of rheumatic inflammation

KEYWORDS

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GROUP MEMBERS

Group leader:
Prof. Dr. rer. nat. Andreas Radbruch

Scientists:
Dr. rer. nat. Hyun-Dong Chang
Dr. rer. nat. Mir-Farzin Mashreghi
Alessandro Serra PhD
Evridiki Sgouroudis PhD
Dr. rer. nat. Özen Sercan
Adriano Taddeo PhD
Zhou Fang PhD
Dr. med. Jun Dong
Mairi McGrath PhD

PhD students:
Anna-Barbara Stittrich, Sandra Zehentmeier, René Riedel, Kerstin Westendorf, Claudia Haftmann, Melanie Weber, Kristyna Hradilkova, Silvia Kühnel, Jakob Zimmermann, Anna Okhrimenko, Haider Abid, Markus Bardua

Master students:
Daniel Schulz

Technical assistance:
Farah Hatam

Others:
Sandra Naundorf
Alessio Mazzoni
Konstantinos Papadakis

A key feature of inflammatory rheumatic diseases is an autoreactive, hyperactive immune system. State-of-the-art immunosuppressive therapies efficiently stop progression of disease but cannot cure the disease. If treatment is stopped, the disease usually relapses. We believe that one reason is that the immune system has acquired a memory for the rheumatic inflammation which is refractory to conventional immunosuppressive therapies. It is our goal to understand this "pathogenic" memory and its counterpart, the "protective" immunological memory.

In the past years, we could define cells of the immunological memory. We could demonstrate that plasma cells differentiate to memory plasma cells in the bone marrow, in survival niches organised by dedicated stromal cells. In these niches, the memory plasma cells survive longterm and continuously secrete antibodies.

Other stromal cells of the bone marrow organise the survival niches for memory T helper (Th) lymphocytes. Until recently, the predominant view has been, that memory Th cells are maintained by homeostatic proliferation and recirculate through the body in search for the cognate antigen. We could show, that after clearing the antigen, memory Th lymphocytes migrate to the bone marrow and settle in their niche and survive as resting cells for extended periods of time. On the other hand, memory Th cells driving chronic rheumatic inflammation are constantly confronted with autoantigens and reactivated. We are investigating how such proinflammatory Th cells adapt to the chronic inflammation enabling them to survive and perpetuate the inflammation. We could recently show, how coordinated signals lead to the differentiation and imprinting of proinflammatory Th cells, how proliferation is regulated by microRNA, and we have identified genes which are specifically expressed in such cells and are important for their survival and function. "Protective" memory Th cells apparently are not requiring these genes. Thus, our results have opened the way for new strategies to target selectively the "pathogenic" immunological memory in rheumatic diseases.

COOPERATION PARTNERS

PD Dr. Ria Baumgrass, Signaltransduktion, Deutsches Rheuma-Forschungszentrum Berlin

Dr. Andreas Bosio, Miltenyi Biotec GmbH, Bergisch Gladbach

Dr. Ute Bissels, Miltenyi Biotec GmbH, Bergisch Gladbach

Prof. Dr. Gerd-Rüdiger Burmester, Klinik für Rheumatologie und klinische Immunologie, Charité-Universitätsmedizin Berlin

Dr. Wei Chen, Max-Delbrück-Centrum für Molekulare Medizin und Berlin Institute for Medical Systems Biology, Berlin

Prof. Dr. Thomas Dörner, Klinik für Rheumatologie und klinische Immunologie, Charité-Universitätsmedizin Berlin

Dr. Zhou Fang, Max-Delbrück-Centrum für Molekulare Medizin und Berlin Institute for Medical Systems Biology, Berlin

Dr. Joachim Grün, Immunmonitoring, Deutsches Rheuma-Forschungszentrum Berlin

Dr. Andreas Grützkau, Immunmonitoring, Deutsches Rheuma-Forschungszentrum Berlin

Prof. Dr. Alf Hamann, Experimentelle Rheumatologie, Klinik für Rheumatologie und klinische Immunologie, Charité-Universitätsmedizin Berlin

Dr. Thomas Häupl, Klinik für Rheumatologie und klinische Immunologie, Charité Universitätsmedizin Berlin

Dr. Anja Hauser, Immunodynamik, Deutsches Rheuma-Forschungszentrum Berlin

Prof. Dr. Falk Hiepe, Klinik für Rheumatologie und klinische Immunologie, Charité-Universitätsmedizin Berlin

Prof. Dr. Thomas Höfer, Research Group Modeling of Biological Systems, Deutsches Krebsforschungszentrum, Heidelberg

Prof. Dr. Thomas Kamradt, Institut für Immunologie, Universitätsklinikum, Friedrich-Schiller-Universität, Jena

Prof. Dr. Christoph Loddenkemper, Institut für Pathologie, Charité-Universitätsmedizin Berlin

Prof. Dr. Max Löhning, Experimentelle Rheumatologie, Klinik für Rheumatologie und klinische Immunologie, Charité Universitätsmedizin Berlin und Deutsches Rheuma-Forschungszentrum Berlin

Prof. Dr. Fritz Melchers, Max Planck Institut für Infektionsbiologie, Berlin

Prof. Dr. Matthias Merkenschlager, Division of Clinical Sciences, Imperial College, London, U.K.

Prof. Dr. Toshinori Nakayama, Department of Immunology, Chiba University, Chiba, Japan

Silvia Pade, Klinik für Rheumatologie und klinische Immunologie, Charité-Universitätsmedizin Berlin

Prof. Dr. Nikolaus Rajewsky, Max-Delbrück-Centrum für Molekulare Medizin und Berlin Institute for Medical Systems Biology, Berlin

Dr. Anne Richter, Miltenyi Biotec GmbH, Bergisch Gladbach

PD Dr. Alexander Scheffold, Immunmodulation, Deutsches Rheuma-Forschungszentrum Berlin und Miltenyi Biotec GmbH, Bergisch Gladbach

Prof. Dr. Jochen Sieper, Medizinische Klinik I, Charité-Universitätsmedizin Berlin

Prof. Dr. Andreas Thiel, Berlin-Brandenburg Center for Regenerative Therapies BCRT, Berlin

Prof. Dr. Martin Zeitz, Medizinische Klinik I, Charité Universitätsmedizin Berlin

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Bone marrow mesenchymal stromal cells organize immunological memory

SCIENTISTS

S. Zehentmeier, Ö. Sercan, R. Riedel, D. Schulz, J.Dong, A.Okhrimenko, M. McGrath, S. Naundorf, A. Serra, A. Taddeo, E. Sgouroudis, H.D. Chang, A. Radbruch

COOPERATION PARTNERS

K.Tokoyoda[1], A. E. Hauser[2] M. Löhning[3], A.N. Hegazy[3] B. Hoyer[4], F. Hiepe[4] G.R. Burmester[4],C. Berek[5]

[1] Department of Immunology, Chiba University, Japan
[2] Immune Dynamics, DRFZ
[3] Experimental Immunology, DRFZ
[4] Charité Universitätsmedizin, Berlin
[5] B Cell Immunology, DRFZ

PUBLICATIONS

Shinoda K, Tokoyoda K, Hanazawa A, Hayashizaki K, Zehentmeier S, Hosokawa H, Iwamura C, Koseki H, Tumes DJ, Radbruch A, Nakayama T. 2012. Type II membrane protein CD69 regulates the formation of resting T-helper memory. Proc Natl Acad Sci U S A. 109:7409-14.

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FUNDING

Berlin-Brandenburg School of Regenerative Therapies (BSRT), ZIBI Graduate School, DFG Priority Programme 1486 „Immunobone", ERC Advanced Grant "IMMEMO"

A key feature of the adaptive immune system is immunological memory, which provides protection and enhanced reactivity against pathogens previously encountered, but can also contribute to rheumatic diseases when directed against self antigens. How immunological memory is maintained over a lifetime has been a matter of debate, with the most prevalent view being that specialized memory cells circulate in the body constantly scanning for recall antigens, while being maintained by homeostatic proliferation. Contrary to this view, we could show for memory plasma cells and memory T helper (Th) cells that, following an immune reaction, precursors of these memory cells migrate to the bone marrow, dock onto specialized stromal cells, differentiate to memory cells and persist as resting cells. Understanding the biology of bone marrow stromal cells, and in particular, how they organize the survival niche, will help us to elucidate the principles behind the long-term maintenance of protective, but also of pathogenic, immunological memory.

Establishment of immunity to pathogens is a key feature of the adaptive immune system. Immunity is maintained by specialized memory cells, such as memory plasma cells secreting protective antibodies or memory T cells, which play an essential role in the formation of memory B lymphocytes as well as long-lived memory plasma cells, and support the expansion and maintenance of memory cytotoxic T cells. However, the memory cells are not intrinsically longlived, and their longterm survival is conditional on the provision of specific survival signals.

For memory plasma cells we and others could demonstrate that specialized CXCL12-expressing stromal cells in the bone marrow provide survival signals to the memory plasma cells and attract other cell types, such as eosinophils, which contribute to the maintenance of plasma cell memory by provision of additional survival signals.

For memory T cells it was less clear how they are maintained over long time periods. It has been the predominant view that memory T cells circulate through the body and are maintained by persistent antigenic stimulation or by homeostatic proliferation regulated by IL-7 in the absence of antigen. Contrary to this view, we could show that also memory Th, similarly to memory plasma cells, are maintained in the bone marrow as resting cells in dedicated survival niches, defined by IL-7 expressing stromal cells. Thus, our results imply a novel, unrecognized role for bone marrow stromal cells in the organisation and maintenance of immunological memory.

Bone marrow stromal cells are stable organizers of a dynamic memory plasma cell niche

Memory plasma cells are found in direct contact to stromal cells expressing VCAM-1+ and the chemokine CXCL12. However, the stromal cells alone are not sufficient to support the survival of memory plasma cells. It was recently shown by the group of Claudia Berek that eosinophils contribute to the memory plasma cell niche by provision of APRIL and IL-6. Similarly, megakaryocytes have been shown to be important for memory plasma cell survival.

Together with the DRFZ group "Immune Dynamics" we have investigated how such "accessory" niche cells can contribute to a long-lived stable survival niche as the accessory cells are known to be relatively short-lived (Fig. 1). We have studied the proliferation dynamics of the memory plasma cell-associated stromal cells and eosinophils *in vivo* by pulse-chase labeling experiments. We found that the stromal cells of the niche are not proliferating and are a stable component of the memory plasma cell survival niche. In contrast, all eosinophils, whether associated with memory plasma cells or not displayed a constantly high turnover. Thus, the memory plasma cell survival niche in the bone marrow is a composite niche having a stable component, the stromal cells, and dynamic accessory cells. The question remains how the stromal cells organize the dynamic exchange of the accessory niche cells.

CD69 is required for Th memory establishment in the bone marrow

We have previously shown that following immunization Th cells migrate from the peripheral lymphoid organs (spleen and lymph nodes) to the bone marrow (BM) (Fig. 2) similarly to plasma cell precursors. In the bone marrow the memory Th cells dock onto IL-7-expressing stromal cells and are maintained there as resting cells in terms of proliferation and transcription. The resting bone marrow memory Th cells specifically express the surface markers Ly6c and CD69.

Although CD69 is generally regarded as marker of recent T cell activation, memory Th cells expressing CD69 in the bone marrow do not show any signs of activation and are resting. In collaboration with the group of Koji Tokoyoda we demonstrated that CD69 expression of Th cells is required for their establish-

ment as resting memory Th cells in the bone marrow. Mice lacking CD69 show normal primary immune responses but lack memory Th cells in the bone marrow. Apparently, CD69 is required for efficient homing of memory Th cells into the bone marrow. The molecular pathways which control memory T cell migration into the bone marrow, and then to their stromal niches, have not yet been deciphered and we are currently investigating the chemotactic signals needed for this migration. Furthermore, we know that in the bone marrow, Th cells which have docked onto stromal cells no longer proliferate, and are generally resting in terms of their transcriptional activity. Therefore, we are also currently analyzing whether such cells also become anergic to the multitude of chemotactic signals which are present in the bone marrow, which could potentially lure them away from their survival niches.

Heterogeneity of bone marrow stromal cells

The survival niches in the bone marrow for memory plasma cells and memory Th cells are defined by stromal cells expressing CXCL12 and IL-7, respectively (Fig. 3). Thus, there is a hitherto little appreciated heterogeneity among bone marrow stromal cells. The stromal cells were described as VCAM1+ fibroblast-like cells. The CXCL12+ stromal cells comprise about 17% of all VCAM1+ bone marrow cells. The IL-7+ stromal cells are a VCAM1+ population completely distinct from CXCL12+ stromal cells and comprise about 46% of all VCAM1+ bone marrow cells. IL-7+ stromal cells also contact B cell precursors and presumably also memory cytotoxic T cells, suggesting an even greater heterogeneity of bone marrow stromal cells. It will be important to understand the biology of the bone marrow stromal cells to understand how immunological memory is formed and maintained.

Perspectives

Contrary to common concepts, our data suggest that after a successful immune response, immunological memory is maintained by resting cells surviving in dedicated survival niches organized by specialized stromal cells. Interestingly, only one memory cell pairs with one stromal cell, suggesting that the stromal cells not only maintain immunological memory but also limit the number of available niches in which memory cells can survive in the absence of antigen. Furthermore we want to examine, whether the survival of memory cells in molecularly defined niches is a general principle in the organization of immunological memory and if this concept extends to the maintenance of memory B lymphocytes and cytotoxic T cells. While different accessory cell types have been described for the memory plasma cell niche, it is also not clear, whether the IL-7 expressing stromal cells require "accessory" niche cells to form the survival niche for memory T cells in the bone marrow. As we are now able to examine the resting protective immunological memory and compare its features to those of pathological memory in chronic inflammatory diseases, we will exploit these differences for the development of more selective therapeutic strategies targeting only pathogenic memory.

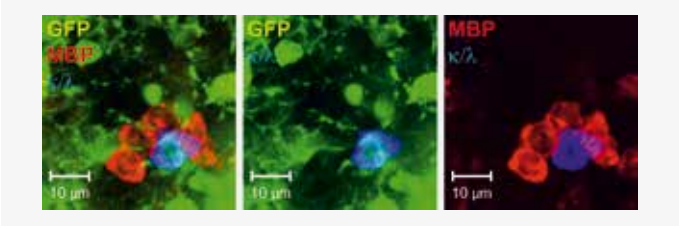

Figure 1: On day 30 after secondary immunization plasma cells (white) are mainly located in the bone marrow parenchyme, contacting the stromal reticular network (green). "Accessory" eosinophils (red) are surrounding the plasma cell. (Two-dimensional projection of a z-stack from a 30µm femoral bone marrow section; blue: nuclear staining).

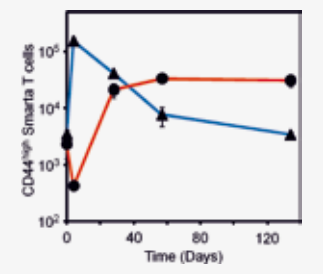

Figure 2: We have followed the number and localisation of antigen-specific T helper cells in a defined immune response. On day 60, more than 80% of antigen-specific T cells are found in bone marrow (red line) in comparison to the spleen (blue line). While the numbers of antigen-specific T cells in bone marrow remain constant over time, they continuously decrease in splenic tissue.

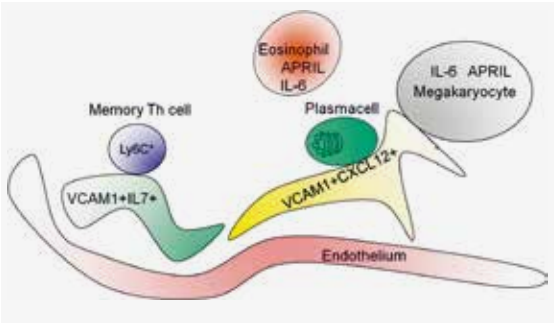

Figure 3: The survival niches for immunological memory cells are organized by dedicated stromal cells in the bone marrow. While IL-7+ stromal cells organize the survival niche for memory CD4+ T cells, CXCL12+ stromal cells organize the niche for memory plasma cells. Hematopoetic accessory cells like eosinophils and megakaryocytes contribute to the survival niches of memory plasma cells by provision of survival factors, such as IL-6 and APRIL.

Small ribonucleic acids control T lymphocyte survival and function in inflammation

SCIENTISTS

C. Haftmann, M. Bardua, A.-B. Stittrich, Z. Fang, K. Hradlikova, K. Westendorf, R. Riedel, J. Zimmermann, F. Hatam, H.-D. Chang, A. Radbruch and M.-F. Mashreghi

COOPERATION PARTNERS

Christian Neumann[1], Joachim Grün[2], Ria Baumgrass[3], Thomas Häupl[1], Helena Radbruch[1], Joachim Sieper[1], Marina Backhaus[1], Alexander Scheffold[1], Wei Chen[4], Nikolaus Rajewsky[4], Martina Porstner[5], Jürgen Wittmann[5], Hans-Martin Jäck[5], Andreas Bosio[6], Ute Bissels[6]

[1]Charité – Universitätsmedizin, Berlin

[2]Immune monitoring group, DRFZ Berlin

[3]Signal transduction group, DRFZ Berlin

[4]Laboratory of Systems Biology of Gene Regulatory Elements, Max-Delbrück-Center for Moleculare Medicine, Berlin

[5]Division of Molecular Immunology, Friedrich-Alexander University, Erlangen Nuernberg

[6]Miltenyi Biotec, Bergisch Gladbach

T helper (Th) lymphocytes are important components of the protective immunological memory but can also contribute to the initiation and perpetuation of rheumatic inflammatory diseases. Their function and survival depends on the expression of certain genes. The regulation of a cell's gene expression is strictly regulated on different layers. The first level is transcriptional, when the DNA of a gene is transcribed into messenger ribonucleic acid (mRNA). This form of gene regulation is followed by the post-transcriptional level of regulation in which the translation of the gene information into the protein is controlled. Only recently the importance of the post-transcriptional gene regulation mechanism has become more evident. Small regulatory ribonucleic acids called microRNAs (miRNAs) are key player in this process as they specifically find and degrade their target mRNA. We have assessed how many and which miRNAs are expressed in proinflammatory Th lymphocytes and which genes they could potentially target. Among those miRNAs we found some which can control the proliferation and survival of Th lymphocytes of a chronic inflammation and their location within the diseased tissue. Interestingly, miRNAs can be inhibited by so called antagomirs which are oligonucleotide with a complementary sequence. Furthermore, the regulatory function of miRNAs can be mimicked by synthetic siRNAs. Knowledge of the function and role of single miRNAs gives rise to completely new therapeutical strategies for the specific targeting of inflammation promoting Th lymphocytes.

What are microRNAs and how do they function?

MiRNAs are short, highly conserved, noncoding RNAs of ~22 nucleotide length that mediate post-transcriptional gene silencing by specifically binding to complementary mRNA sequences. They are usually encoded in introns of coding genes and in noncoding DNA regions. The generation of functional mature miRNAs from their primary transcript (primiRNAs) is a multi-step process requiring several cleavage steps and many different enzymes (Fig. 1). Finally the mature miRNA strand is incoorperated into the RNA-induced silencing complex (RISC), guides it to the target mRNA and mediates repression of protein translation and degradation of the mRNA. Until now, over 1000 miRNAs have been identified and are thought to control at least 30% of all genes.

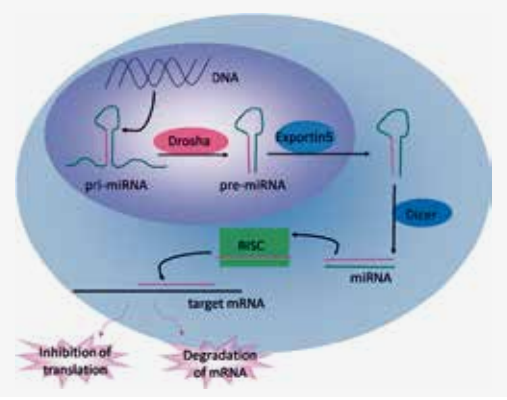

Figure 1: Generation and function of miRNAs.

Which miRNAs exist in T lymphocytes?

We have compared the global miRNA expression pattern of *in vitro* generated Th1, Th2 and Th17 cells. These cells have been treated with multiple restimulations in cell culture mimicking a constant restimulation occurring in inflamed tissues and were compared to only once stimulated Th lymphocytes representing a functional T cell memory. MiRNAs that are differentially expressed in the restimulated Th lymphocytes do most likely play a role for the pathology of the Th lymphocytes in a chronic inflammation. Deep sequencing and miRNA microarray hybridization analysis revealed over 300 Th lymphocyte specific miRNAs (Fig. 2). We have identified more than 100 miRNAs that were differentially expressed in the restimulated Th lymphocytes. Of particular interest were miRNAs expressed in restimulated Th1 lymphocytes, a Th subset associated with many chronic inflammatory diseases. Each of these about 30 miRNAs is now subject of detailed investigation to understand its function in Th1 lymphocytes.

How does miR-CH control the survival of Th1 lymphocytes in inflammation?

One miRNA that is specifically expressed in Th1 cells and is highly upregulated in repeatedly stimulated lymphocytes of this subset is miR-CH. Its expression depends on both, the Th1 specific transcription factor Tbet and a transcription factor that is a specific marker for repeatedly activated Th1 cells of the inflamed tissue, Twist1 (Niesner et al., 2008) (Fig. 3). We were able to show that the expression of miR-CH is inhibited in lymphocytes with a defect of expressing either of this transcription factors.

MiR-CH inhibits the expression of the pro-apoptotic gene Bim. Bim leads to cell death (apoptosis) and antagonizes the survival function of a gene called Bcl2. By decreasing Bim expression, miR-CH promotes survival of repeatedly stimulated Th1 cells. In repeatedly sti-

mulated Th1 lymphocytes inhibition of miR-CH by specific antagomirs, results in an increase of Bim over Bcl2 expression, which in turn leads to a reduced number of viable Th1 cells due to induced apoptosis. Most strikingly, an additional block of the increased levels of Bim after antagomir treatment using siRNAs is sufficient to increase the number of living cells in culture to comparable levels seen in the controls.

Perspectives

Based on the global miRNA screen, already the investigation of the first candidate miRNAs proved the important role of miRNAs for gene regulation in Th lymphocytes in the context of chronic inflammation. MiRNAs not only control survival: completed (Haftmann et al., 2012; Stittrich et al., 2010; Wittmann et al.) as well as preliminary investigations of other differentially regulated miRNAs of our screen showed that they also regulate persistence and motility of Th cells. Further studies will give greater insight into the biological relevance of miRNAs for the pathological capacitiy of repeatedly activated Th lymphocytes. MiRNA blocking antagomirs and siRNAs that mimic miRNA function are potent tools for new therapeutical approaches in treating chronic inflammation.

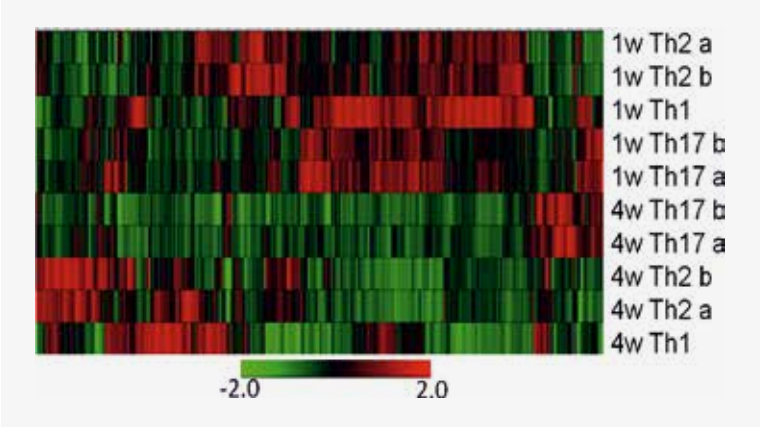

Figure 2: global miRNA expression analysis in once and repeatedly stimulated Th lymphocytes (green – induced, red – repressed).

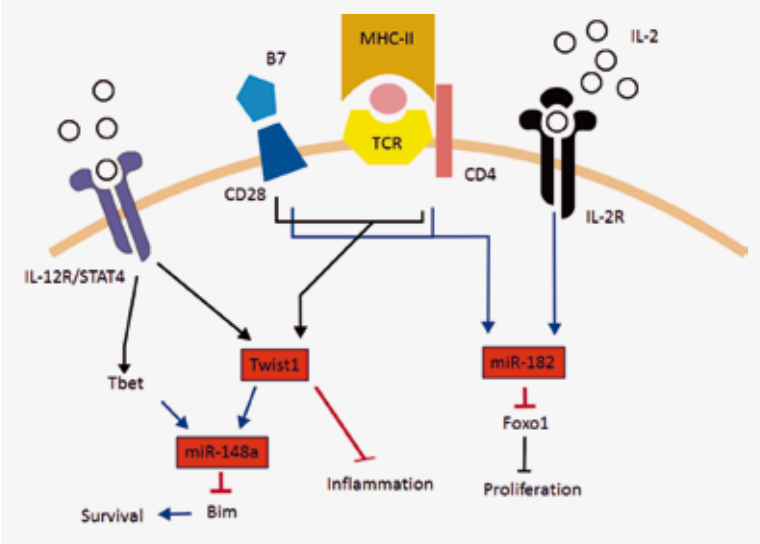

Figure 3: Induction of miR-CH promotes survival of repeatedly activated Th1 lymphocytes. Both, T cell receptor (TCR) and IL-12 receptor (IL-12R) signaling leads to expression of miR-CH via the transcription factors Tbet and Twist1. As a consequence, Bim translation is inhibited and the survival of the repeatedly activated Th1 cell is warrented.
The miR-182 is induced by IL-2/STAT5 signaling and targets the transcription factor Foxo1. Foxo1 plays an important role in regulating proliferation since it blocks cell cycle progression. Therefore, miR-182 is required for proper expansion of activated Th cells.

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Molecular adaptations of pathogenic Th cells to chronic inflammation

SCIENTISTS

K. Westendorf, K. Hradilkova, M. Weber, E. Sgouroudis, Sandra Naundorf, J. Zimmermann, A. Radbruch, H.D. Chang

COOPERATION PARTNERS

M. Löhning[1], J. Grün[2], A. Grützkau[2], A. Scheffold[3], T. Häupl[4], S. Pade[4], A. Kühl[4], F. Hiepe[4], T. Dörner[4], G.R. Burmester[4], J. Sieper[4], B. Siegmund[4], M. Zeitz[4], T. Höfer[5], A. Richter[6], Martin Böttcher[7], T. Kamradt[7]

[1]Experimentelle Immunologie, DRFZ

[2]Immunmonitoring, DRFZ

[3]Immunmodulation, DRFZ

[4]Charité Universitätsmedizin, Berlin

[5]Deutsches Krebsforschungszentrum, Heidelberg

[6]Miltenyi Biotec GmbH, Bergisch Gladbach

[7]Friedrich Schiller Universität, Jena

PUBLICATIONS

Dong J, Chang HD, Ivascu C, Qian Y, Rezai S, Okhrimenko A, Cosmi L, Maggi L, Eckhardt F, Wu P, Sieper J, Alexander T, Annunziato F, Gossen M, Li J, Radbruch A, Thiel A. Loss of methylation at the IFNG promoter and CNS-1 is associated with the development of functional IFN-γ memory in human CD4(+) T lymphocytes. 2013. Eur J Immunol. 43:793-804.

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Currently, rheumatic diseases are treated by immunosuppression, which in most cases effectively prevent progression of the disease. However, rarely therapy-free remission is achieved and cessation of therapy results in relapse. Apparently, the patients have acquired a memory for the rheumatic inflammation which is refractory to state-of-the-art immunosuppressive therapy. Especially pro-inflammatory T helper (Th) lymphocytes could play a key role in the initiation and maintenance of chronic inflammation. Such cells are functionally imprinted and have adapted to the chronic inflammatory environment making them refractory to physiological and therapeutic regulation. Thus, the proinflammatory Th cells and in particular their molecular adaptations to the chronic inflammation could represent a novel, selective therapeutic target for the treatment of rheumatic diseases.

The therapy of chronic inflammatory diseases represents a major challenge for scientists and physicians. While state-of-the-art immunosuppressive drugs can very efficiently suppress inflammation and ameliorate disease symptoms, they are not curative. We speculate that immunosuppressive therapies do not target the pathogenic immunological memory, which can rekindle the inflammation once therapy is interrupted. One molecular reason could be the immunological memory which is epigenetically and functionally modified and imprinted becoming insensitive to therapies effectively targeting activation of naïve cells. The proof-of-principle that cells of the immunological memory are involved in the pathogenesis and maintenance of rheumatic inflammation comes from the clinical observation following complete immune ablation. In patients with refractory rheumatic diseases immune ablation followed by reconstitution of the immune system from autologous hematopoietic stem cells resulted in long-lasting, therapy-free remission (Alexander et al. 2009). This therapeutic strategy, however, results in the complete loss of protective, acquired immunity, temporary immunodeficiency, and infection-related morbidity and mortality. Thus, we aim at the selective depletion of the pathogenic immunological memory selectively targeting molecular adaptations of such cells to chronic inflammation as possible curative treatment of rheumatic diseases.

Twist1 and Hopx are molecular adaptations of pathogenic Th memory cells to chronic inflammation

Memory Th lymphocytes play an important role in the initiation and maintenance of inflammation through the secretion of proinflammatory cytokines. Especially the pro-inflammatory Th type 1 (Th1) and type 17 (Th17) lymphocytes have been implicated in autoimmunity. With the rationale that pro-inflammatory memory Th lymphocytes have encountered their antigen repeatedly in the course of a chronic rheumatic inflammation, we have analyzed the transcriptome of repeatedly stimulated Th cells to identify genes which are regulated in response to chronic inflammation.

We could identify and have further analyzed two genes, Twist1 and Hopx, which were specifically and selectively upregulated in repeatedly stimulated, pro-inflammatory Th1 cells. Twist1 and Hopx were also highly expressed in CD4+ T cells isolated directly from the site of inflammation in patients with rheumatic diseases.

Artificial downregulation of Twist1 expression in Th1 cells by RNA interference (RNAi) resulted in the exacerbation of inflammation in a pre-clinical murine arthritis model, the ovalbumin-induced arthritis, indicating that Twist1 is an intrinsic regulator of inflammation (Fig. 1A). However, Twist1 also promotes the survival of pro-inflammatory Th1 cells by inducing a particular microRNA (see report Small ribonucleic acids control T lymphocyte survival and function in inflammation) and, thus, also contributes to the chronification of inflammation.

Inhibition of Hopx expression by RNAi in Th1 cells resulted in an impaired ability of the Th1 cells to survive *in vivo* (Fig. 1B). Consequently, Th1 cells, with a genetic deletion of the Hopx gene, were unable to support an inflammation in the ovalbumin-induced arthritis model (Fig. 1A). Thus, Twist1 and Hopx represent persistence genes of proinflammatory Th1 cells which are upregulated as adaptation to inflammation and represent interesting targets for novel therapeutic approaches aiming at the selective deletion of an immunological memory for rheumatic inflammation.

The relative role of Th1 and Th17 cells in chronic rheumatic inflammation

Both Th1 and Th17 cells have been implicated in the pathogenesis of chronic inflammation. Apparently, Th1 and Th17 cells share tasks in the control of inflammatory immune responses. Th1 and Th17 cells are considered distinct lineages of effector/memory cells, imprinted for reexpression of IFN-γ and IL-17, by up-

regulated expression of T-bet and RORγt, respectively. However, Th cells coexpressing IFN-γ and IL-17 have been observed *in vivo*, in particular in the context of chronic inflammation, but it remained elusive, how these cells had been generated, whether they represent a distinct lineage of Th differentiation, and how they contribute to inflammation. We could show that in Th17 cells IFN-γ expression can be induced very similar to naive Th cells by combined IFN-γ and IL-12 signalling (Fig. 3). IFN-γ is required to upregulate expression of the IL12Rβ2 chain, and IL-12 for Th1 polarization. These Th1/17 cells stably coexpress RORγt and T-bet on the single cell level, and are imprinted for reexpression of both IFN-γ and IL-17. Th17+1 cells could, thus, potentially combine the pro-inflammatory potential of Th1 and Th17 cells and represent an interesting therapeutic target. It has been shown that also Th2 cells can acquire the functional profile of Th1 cells by similar pathway (Fig. 3) (see Experimental Immunology group).

Perspectives:

We could identify genes, which are specifically expressed in chronically activated pro-inflammatory Th lymphocytes. Twist1 and Hopx represent molecular adaptations of pro-inflammatory Th1 cells to chronic inflammation, and allow us to identify Th cells of the pathogenic memory. Both candidates are now subjected to a detail molecular analysis to better understand their function in pro-inflammatory Th1 cells. In addition, we are developing strategies to selectively eliminate pathogenic memory Th cells via targeting Twist1 and Hopx. We are also analyzing the relative roles of Th1, Th17, and Th17+1 cells in their ability to induce and maintain chronic inflammation, how the cytokine memory of differentiated pro-inflammatory Th cells can be "erased", and how we can turn off the expression of inflammatory cytokines.

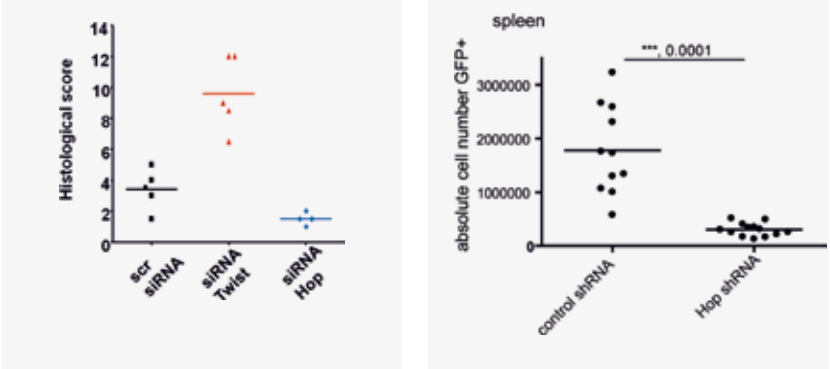

Figure 1: Twist1 and hopx regulate function of pro-inflammatory Th1 cells. (A) Inflammation in the murine ovalbumin-induced arthritis model is exacerbated when Th1 in which twist1 expression has been downregulated by RNA interference were adoptively transferred. Hopx-deficient Th1 cells are unable to support arthritic inflammation. (B) Knockdown of hopx expression in Th1 cells results in their inability to persist in vivo following adoptive transfer into wild type recipients.

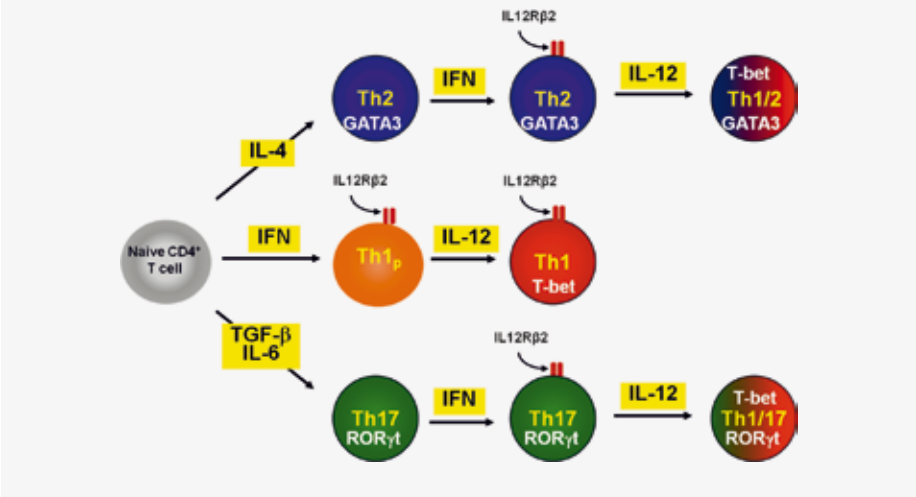

Figure 2: The IFN/IL-12 axis regulates the acquisition of pro-inflammatory Th1 function in Th lymphocytes. Similar to the two-step process required for Th1 differentiation in naïve Th cells, type 1 or 2 IFN lead to the upregulation of IL12Rβ2 in differentiated Th cells making Th2 or Th17 cells responsive to IL-12 signaling. IL-12 can then lead to the stable upregulation of T-bet and additional acquisition of Th1 functions generating qualitatively and functionally distinct pro-inflammatory Th1/2 or Th1/17 cells.

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Chiara Romagnani

Innate Immunity

Innate lymphocytes in protection and regulation

KEYWORDS

ILC,
NK cells,
cytokines,
inflammatory diseases

GROUP MEMBERS

Group leader:
Chiara Romagnani, PhD, MD

Scientists:
Daniela Paclik, Marina Babic Câc

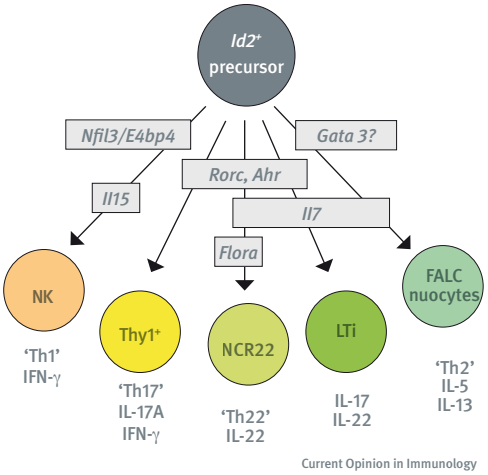
Ph.D. students:
Monica Killig, Merlin Lütke-Eversloh, Timor Glatzer

Diploma and MD students:
Isabelle Ommert

Students:
Luiz Gustavo Teixeira Alves

Our research interest mainly focuses on the family of innate lymphoid cells (ILC). ILC are heterogeneous concerning their cytokine profile and function in response to different pathogens, thus resembling the complexity of CD4+ helper T cell subsets. ILC family includes IFN-γ producing Natural Killer (NK) cells, IL-13/IL-5 producing natural helper (NH) cells, and a population of RORγt+ ILC mainly secreting IL-22 and IL-17. Although it is now clear that innate and adaptive cells share most of their effector molecules, activation requirements of ILC and T cells still largely differ and defining ILC´s exquisite role during infections and inflammatory diseases is of great importance. In our lab, we are currently trying to dissect ILC heterogeneity and to identify the signals and molecular switches responsible of ILC effector functions and "memory-like" properties. In our previous work, we have focused on NK cell differentiation and identified a new subset of polyfunctional NK cells. Currently, we are investigating NK cell IFN-γ regulation and in cooperation with our clinical partners, exploiting adoptive transfer of NK cells for the treatment of cancer and prevention of adverse autoimmune responses in patients undergoing hematopoietic stem cell transplantation. Moreover, we study ILC activation requirements and evaluate the role of these cells during inflammatory diseases.

In parallel to our extensive work on human ILC, we are currently generating conditional knock-out mice, in which different ILC populations are selectively depleted. Furthermore, their contribution in inflammatory disease models, such as colitis and arthritis, as well as their role during secondary immune responses is determined.



Current Opinion in Immunology

COOPERATION PARTNERS

Bluethgen N, Meisig J, Medical Systems Biology, Charité Universitätsmedizin, Berlin

Gröne J, Seifarth C, Chirurgische Klinik und Hochschulambulanz I, Charité Universitätsmedizin Berlin

Moretta A and L, Pende D, Pietra G, University of Genova, Italy

Richter A, Huppert V, Miltenyi Biotec GmbH, Bergisch-Gladbach

Schüler T, Institut für Immunologie - Charité Universitätsmedizin, Berlin

Seidl R, HNO-Klinik, Unfallkrankenhaus, Berlin

Stölzel K, Hals-Nasen-Ohrenklinik und Poliklinik, Charité Universitätsmedizin CBF, Berlin

Thiel A, Charité- BCRT, Berlin

Uharek L, B. Friederichs, Hämatologie, Charité Universitätsmedizin CBF, Berlin

Vivier E, Centre d'Immunologie de Marseille-Luminy, France

SELECTED PUBLICATIONS

Juelke, K., Killig, M., Luetke-Eversloh, M., Parente, E., Gruen, J., Morandi, B., Ferlazzo, G., Thiel, A., Schmitt-Knosalla, I., and Romagnani, C. (2010). CD62L expression identifies a unique subset of polyfunctional CD56dim NK cells. Blood 116, 1299-1307.

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NK cell differentiation and acquisition of effector functions

SCIENTISTS

M. Killig, M. Lütke-Eversloh, K. Juelke

COOPERATION PARTNERS

B. Friedrichs, L. Uharek, Hämatologie, Charité Universitätsmedizin, Berlin

J. Meisig J, Bluethgen N, Medical Systems Biology, Charité Universitätsmedizin, Berlin

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Juelke K, Killig M, Luetke-Eversloh M, Parente E, Gruen J, Morandi B, Ferlazzo G, Thiel A, Schmitt-Knosalla I, and Romagnani, C. CD62L expression identifies a unique subset of polyfunctional CD56dim NK cells. Blood 2010;116:1299-1307.

FUNDING

DFG-SFB 650
DFG-SFB TR 36

Human Natural Killer (NK) cells comprise two main subsets, CD56^{bright} and CD56^{dim} cells, that differ in function, phenotype and tissue localization. We could show that expression of CD56 and CD62L allows the identification of three NK cell subsets and that CD56^{dim} CD62L^{+} cells represent an intermediate stage of NK cell maturation between CD56^{bright} CD62L^{+} and CD56^{dim} CD62L^{-} cells. Importantly, we could demonstrate that during this differentiation process, NK cells display a progressively decreasing ability to respond to cytokines, while gradually acquiring the capacity to display effector functions in response to NK cell activating receptors (ActRec). NK cell differentiation can be defined even more precisely, when further markers such as CD16, CD57, KIR and NKG2A are included. By the use of principal component analysis (PCA), we could show that NK cell differentiation appears to be a continuum. According to this model, we have tracked NK cells in the course of a phase I/II clinical trial, where patients were transplanted with CD34^{+} cells followed by transfer of NK cells derived from the same donor. PCA analysis of donor NK cells allows tracking of adoptively transferred NK cells and reconstituting ones. Importantly, adoptively transferred NK cells proliferate *in vivo* and mostly belong to the CD56^{dim} CD62L^{+} polyfunctional NK cell subset. Altogether, our data show that NK cells undergo a differentiation process *in vivo* and that polyfunctional NK cells represent ideal candidates for adoptive NK cell transfer for the treatment of acute leukemias and modulation of graft versus host disease.

In the last years, it became clear that Natural Killer (NK) cells do not represent a homogenous population of cells "ready to kill", but also undergo a defined differentiation program, which includes education, priming and even generation of memory during recall responses. This complexity implies the existence of distinct stages of NK cell differentiation, which can guarantee an efficient division of labour, as it has been shown for T cells. However, understanding NK cell increasing complexity is hampered by the lack of adequate markers that would enable us to distinguish defined steps of NK cell differentiation history. In humans, two NK cell subsets have been characterized, namely CD56^{bright} and CD56^{dim} NK cells (Cooper et al, 2001). Recent reports provided evidence that CD56^{dim} NK cells may be derived directly from the CD56^{bright} NK subset (Romagnani et al, 2007; Chan et al, 2007). However, CD56^{dim} NK cells represent a heterogeneous population concerning the expression of several markers. The aim of this study was to dissect the heterogeneity of CD56^{dim} NK cells in order to identify intermediate stages of NK cell maturation and to better define the differentiation history of human NK cells.

Results and discussion:

In our study, we performed analysis of human NK cell subsets dissected according to KIR, NKG2A, CD27 and CD62L expression ex vivo or after stimulation with inflammatory cytokines or via ActRec. Our data show for the first time that CD56^{dim} CD62L^{+} cells represent a newly identified subset of human NK cells, displaying intermediate phenotype and functions between CD56^{bright} and CD56^{dim} CD62L- NK cell subsets and combines the ability of CD56^{bright} cells to proliferate and produce IFN-γ after cytokine stimulation, with the capacity to kill and produce cytokines via ActRec, a typical feature of CD56^{dim} cells. Moreover, CD56^{bright}, CD56^{dim} CD62L^{+} and CD56^{dim} CD62L- correspond to sequential steps of differentiation and maturation (Figure 1, left). In addition to CD56 and CD62L, combination of different markers such as

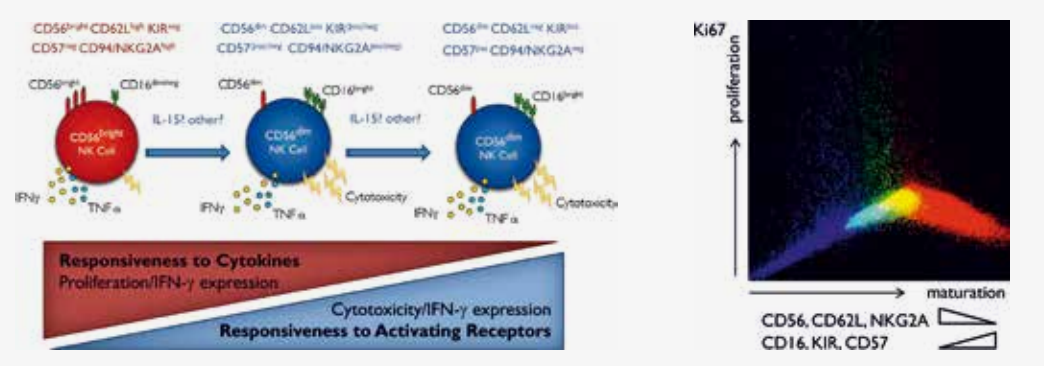

Figure 1: Continuum of NK cell differentiation. Model of NK cell differentiation (left) and principal component analysis of NK cells in healthy donors according to the indicated markers (right).

CD16, CD57, KIR and NKG2A allows a detailed dissection of NK cell maturation stages and, by the use of principal component analysis (PCA), we could show that distribution of these markers during NK cell differentiation appears to be a continuum (Figure 1, right).

According to this model, we have tracked NK cells in the course of a phase I/II clinical trial with patients transplanted with CD34⁺ cells, followed by transfer of NK cells derived from the same donor. PCA analysis of donor NK cells allows tracking of adoptively transferred NK cells and reconstituting ones (Figure 2, left). In the first week after transplantation, we were able to track adoptively transferred donor NK cells, which could be distinguished from recipient NK cells as well as from those reconstituting from the donor CD34⁺ graft. Transferred NK cells largely displayed a mature phenotype and their proliferative ability *in vivo* was confined to the CD62L⁺ subset (Figure 2, right). In the second week after transplantation, we detected a massive peak of highly proliferating immature NK cells, which progressively differentiated into mature NK cells. Clustering of NK cell populations recapitulates NK cell differentiation stages at steady state and led to a model in which 3 distinct phases can be distinguished during time (Figure 2, left). Our data show that NK cells undergo a differentiation process *in vivo* which can be reliably tracked by the combination of several markers. Due to their double responsiveness to cytokines and ActRec, polyfunctional CD62L⁺ NK cells combine the ability to proliferate and to display effector functions and therefore represent ideal candidates for adoptive NK cell transfer for the treatment of acute leukemias and modulation of graft versus host disease.

Perspectives:

In our future studies, we aim to investigate genes and mechanisms crucial for NK cell differentiation and acquisition of effector functions and manipulate NK cells in order to preserve their polyfunctionality.

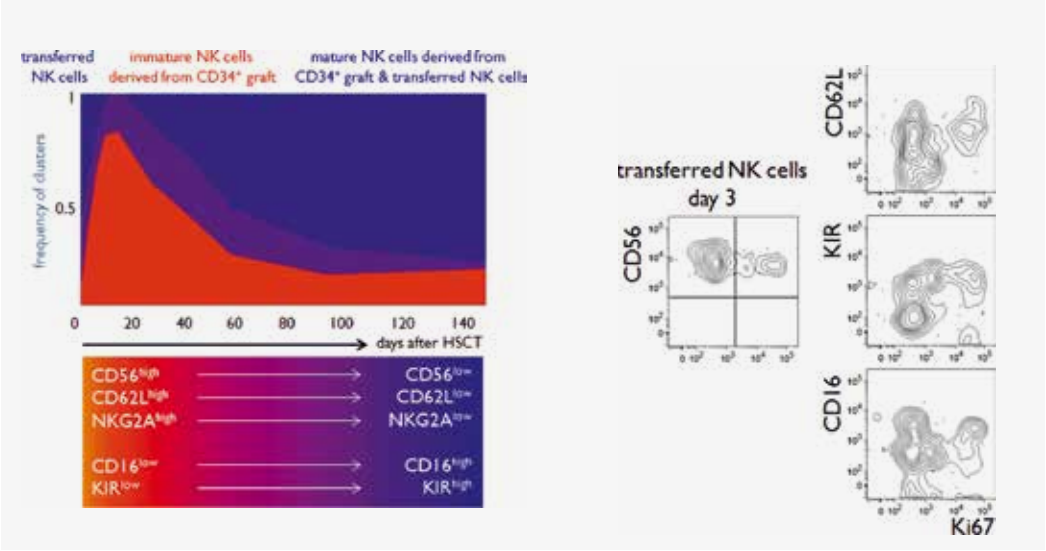

Figure 2: Tracking NK cells *in vivo*. PCA clustering of NK cells after HSCT (left) and Flow cytometric analysis of recipient PB 3 days after donor NK cell transfer (right).

Role of RORγt⁺ ILC in inflammation

SCIENTISTS

T. Glatzer, M. Killig, M. Lütke-Eversloh, D. Paclik, L.G. Teixeira Alves

COOPERATION PARTNERS

J. Meisig, N. Bluethgen - Medical Systems Biology, Charité Universitätsmedizin Berlin, Germany.

J. Gröne, C. Seifarth - Chirurgische Klinik und Hochschulambulanz I, Charité Universitätsmedizin Berlin

R. Seidl, HNO-Klinik, Unfallkrankenhaus, Berlin

K. Stölzel - HNO-Klinik, Charité Universitätsmedizin Berlin

T. Schüler, Institut für Immunologie - Charité Universitätsmedizin Berlin

E. Vivier, Centre d'Immunologie de Marseille-Luminy, France

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FUNDING

DFG-SFB 633

RORγt⁺ innate lymphoid cells (ILC) represent a major source of IL-22, which plays an important role in mucosal homeostasis. Human RORγt⁺ ILC have been mostly studied in tonsils where they represent a phenotypically and functionally heterogeneous population. Moreover, signal requirements for IL-22 and other cytokine expression in these cells have not been entirely elucidated. We could show for the first time that the activating receptor NKp44 is functional in human RORγt⁺ ILC derived from tonsils and gut lamina propria (LP). Functional as well as transcriptome analysis of RORγt⁺ ILC after NKp44 or cytokine stimulation showed that NKp44 engagement leads to only partially overlapping signatures compared to cytokine stimulation. While NKp44 triggering leads to selective expression of TNF and other pro-inflammatory genes, cytokine stimulation induces preferentially IL-22. These data support the concept that RORγt⁺ ILC exhibit different properties depending on the contextual stimulus, and that selective engagement of activating or cytokine receptors can be instrumental to dissect anti-inflammatory or pro-inflammatory functions displayed by these cells.

RORγt⁺ innate lymphoid cells (ILC) are a new population of lymphocytes mainly residing in mucosal associated tissues, such as gut LP and tonsils. These cells represent a major source of IL-22, an important player in mucosal immunity and tissue homeostasis, and were able to modulate experimental colitis in mice. IL-22 effects on epithelial cells (EC) appear to depend on the cytokine milieu of the inflamed tissue and can be either pro-inflammatory or protective, by promoting EC regeneration after damage. Identification of signals leading to selective IL-22 expression could promote epithelial wound healing, without inducing inflammation. In humans, it was previously shown that RORγt⁺ ILC produced IL-22 as well as other cytokines; however the physiological signals leading to cytokine production in these cells have been only partially elucidated.

Results and discussion:

We have dissected human RORγt⁺ ILC heterogeneity and characterized their phenotype, cytokine- and transcriptome profile *ex vivo* or after stimulation with various cytokines and/or via different activating receptors (ActRec). We have identified the markers exclusi-

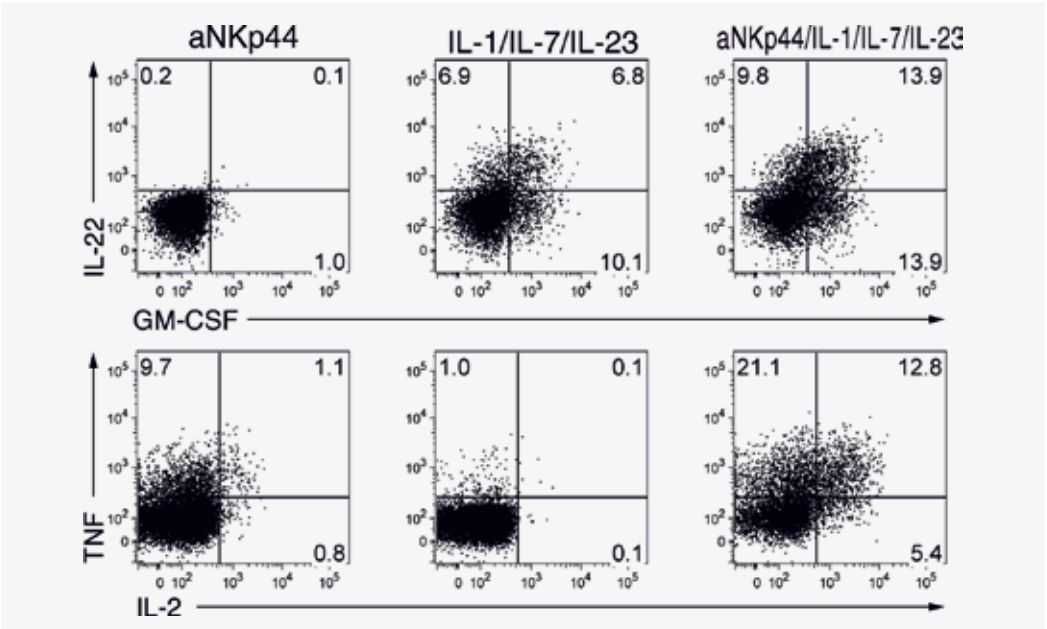


Figure 1: Stimulation of RORγt+ ILC via NKp44 or cytokines induces a divergent cytokine profile.

vely defining the IL-22 producing RORγt⁺ ILC. Furthermore we determined for the first time stimuli, which induce divergent cytokine profiles in RORγt⁺ ILC. We could show that NKp44 is the only functional ActRec in RORγt⁺ ILC and its engagement induces selective TNF, while cytokine stimulation induces preferential IL-22 and GM-CSF expression. NKp44 and cytokine stimulation worked in synergy to achieve optimal cytokine response (Figure 1).

Transcriptome analysis revealed that the two stimuli induce partially overlapping as well as specific signatures in RORγt⁺ ILC (Figure 2). Altogether, our data suggest that stimulation via NKp44 induces a pro-inflammatory response in RORγt⁺ ILC, while cytokine activation up-regulates several molecules involved in tissue protection.

Perspectives:

We aim to further dissect pro-inflammatory and anti-inflammatory properties of RORγt⁺ ILC *in vivo* by studying cells derived from non inflamed and inflamed LP of patients with Crohn's Disease. Moreover, we will study the role of different ILC subsets in the pathogenesis of experimental colitis and arthritis by selective depletion of different ILC populations.

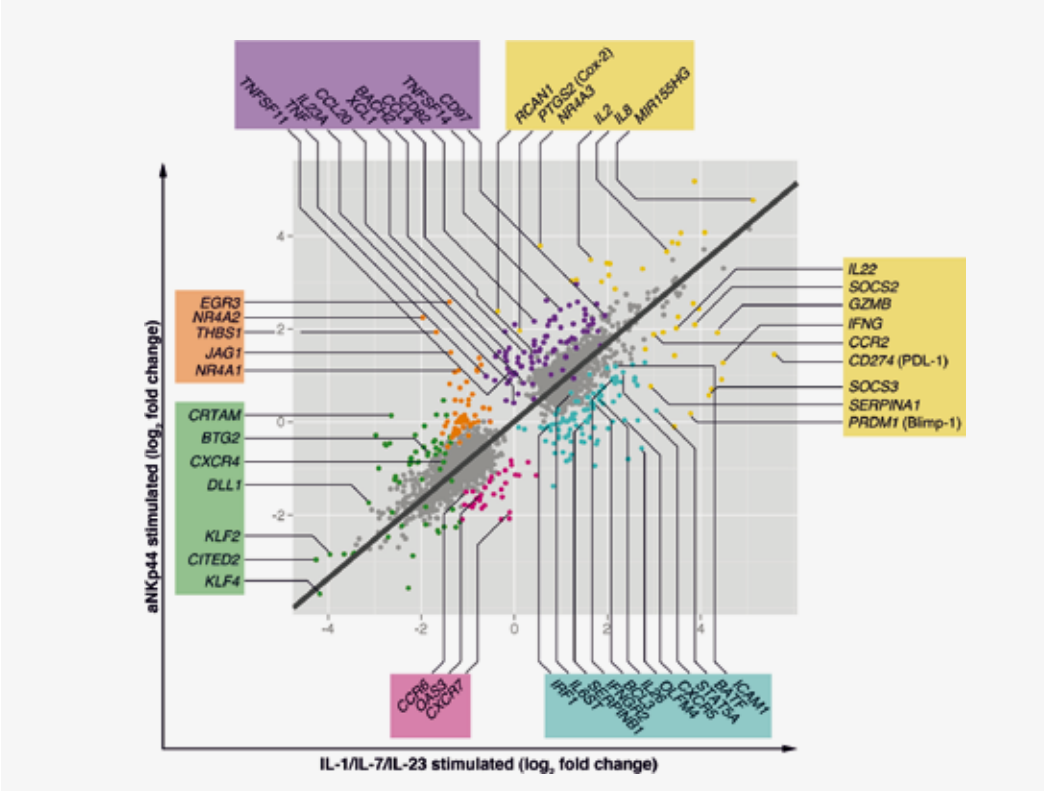


Figure 2: Analysis of RORγt+ ILC genes modulated after stimulation with cytokines or NKp44.

Koji Tokoyoda

Osteoimmunology

Roles of memory T helper lymphocytes in protective immunological memory ~ How do we memorize antigen information, keep it in mind and recall it? ~

KEYWORDS

Immunological memory,
T helper lymphocytes,
Bone marrow,
Secondary immune response,
Humoral immunity

GROUP MEMBERS

Group leader:
Koji Tokoyoda, PhD

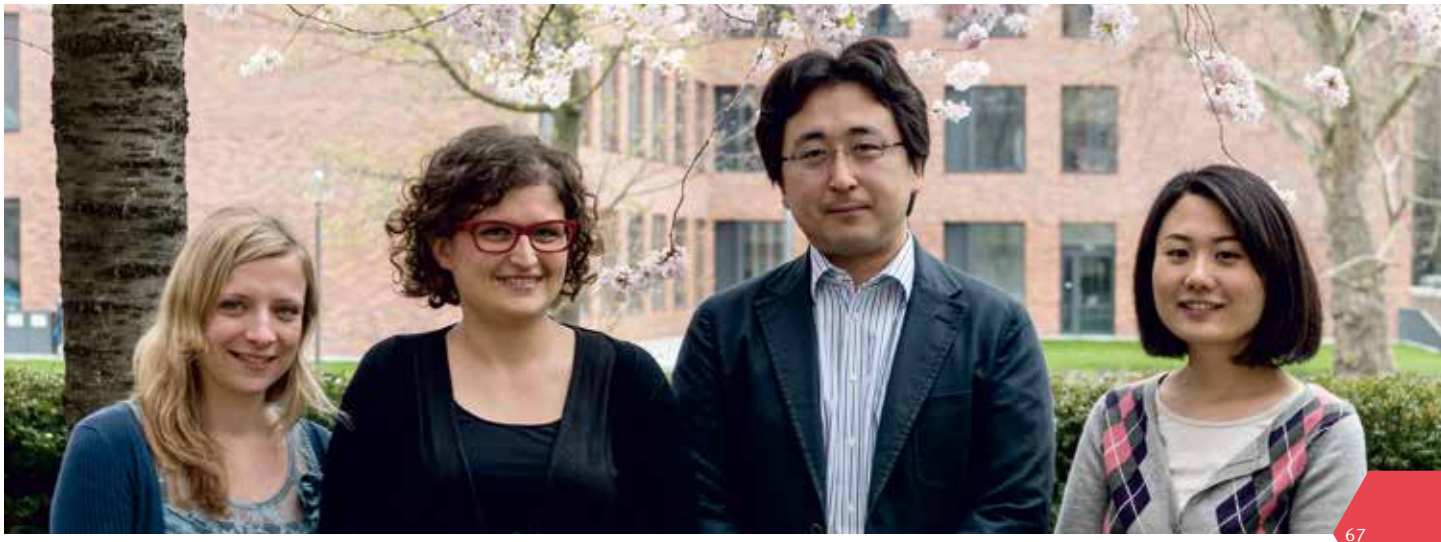
Scientists:
Asami Hanazawa, PhD
Caterina Curato, PhD

PhD Student:
Jana Sarkander

Memory T helper lymphocytes are critical for the generation, maintenance and reactivation of other memory cells that are essential for maintaining protective immunity to many kinds of infectious pathogens. Despite the important role, there is still much to discover about the diversity, generation, maintenance and reactivation of memory T helper lymphocytes in the body.

In 2004 and 2009, we have first found the survival niches for memory plasma cells and memory T helper lymphocytes in the bone marrow. Memory plasma cells adhere to CXCL12-expressing stromal cells and memory T helper lymphocytes are maintained on IL-7-expressing stromal cells. In 2012, we have reported that the absence of bone marrow-resident memory T helper lymphocytes induce the defective homing of memory plasma cell precursors into the bone marrow.

We try to understand our immune memory system, considering the localization and dynamics of memory lymphocytes in the body and also in the tissues or organs. Our goal is to clarify how our immune system removes the re-invaded pathogen efficiently. We have to understand the lifestyle and microenvironment of memory lymphocytes. Especially, we focus on the "where" and "when" of immunological memory.



COOPERATION PARTNERS

Prof. Dr. Max Löhning, DRFZ, Experimental Immunology, Berlin, Germany

Prof. Dr. Toshinori Nakayama, Chiba University, Immunology, Chiba, Japan

Prof. Dr. Andreas Radbruch, DRFZ, Cell Biology, Berlin, Germany

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Generation of memory T helper lymphocytes in the bone marrow via CD69

SCIENTISTS

K. Tokoyoda, A. Hanazawa

COOPERATION PARTNERS

M. Löhning, A. Radbruch, DRFZ
T. Nakayama, Chiba University

PUBLICATIONS

1. Tokoyoda K, Zehentmeier S, Hegazy AN, Albrecht I, Grün JR, Löhning M, Radbruch A. Professional memory CD4 T lymphocytes preferentially reside and rest in the bone marrow. Immunity. 2009. 30:721-730.

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FUNDING

SAW: Senatsausschuss Wettbewerb for funding of the organization of an international Leibniz Research Cluster (ILRC) "ImmunoMemory"

Memory T helper (Th) cells are crucial for the maintenance of acquired immunity to eliminate infectious pathogens. We have previously demonstrated that most memory Th cells reside and rest on stromal niches of the bone marrow (BM) (1,2). Little is known, however, regarding the molecular basis for the generation and maintenance of BM memory Th cells. Here we show that CD69-deficient effector CD4 T cells fail to relocate into and persist in the BM and therefore to differentiate into memory cells (3). Consequently, CD69-deficient CD4 T cells fail to facilitate the production of high-affinity antibodies and the generation of BM long-lived memory plasma cells in the late phase of immune responses. Thus, CD69 is critical for the generation and maintenance of professional memory Th cells which can efficiently help humoral immunity in the late phase. The deficit of immunological memory in CD69-deficient mice also highlights the essential role of BM for the establishment of Th cell memory.

Loss of BM memory Th cells in CD69-deficient mice

CD69, well-known as one of activation markers, is highly expressed on resting BM memory Th cells. To investigate the role of CD69 in Th cell memory, we monitored CD69-deficient or wild-type antigen-specific CD4 T cells after immunization in an adoptive transfer model. Four days after immunization, activated CD69-deficient or wild-type OVA-specific TCR transgenic (DO11.10) CD4 T cells were similarly detectable in the spleen, whereas none were detected in the BM of either group (Figure 1A). In the memory phase, CD69-deficient DO11.10 CD4 T cells did not accumulate in the BM as compared to wild-type cells as detected by flow cytometry, although most wild-type and CD69-deficient DO11.10 CD4 T cells disappeared from the spleen (Figure 1A). Thus, CD69 is required for the establishment of professional resting Th cell memory in the BM.

Defective help of CD69-deficient CD4 T cells in the generation of BM plasma cells

To investigate the function of CD69 in Th cells, we tested whether CD69-deficient CD4 T cells can help B cells for antibody production *in vivo*. CD4 T cells were sorted from spleens of CD69-deficient or wild-type DO11.10 mice and were transferred into normal mice, and then the transferred mice were immunized with NP-OVA and were analyzed for NP-specific antibody titer (Figure 1B). CD69-deficient CD4 T cells failed to induce the production of high-affinity antibodies, especially in the late phase. Affinity maturation of antibodies in B cells occurs on antigen-bearing follicular dendritic cells of germinal centers (GC) of the secondary lymphoid organs and is assisted by T follicular helper (T_{FH}) cells. However, GC B cells and T_{FH} cells of

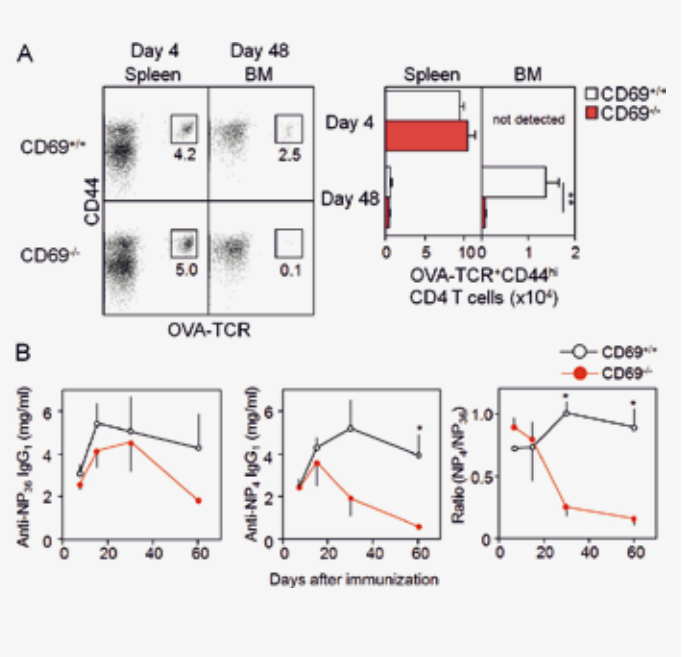


Figure 1: A role of BM memory Th cells in protective immunity.
(A) CD69 is required for the formation of memory Th cells. BALB/c mice transferred with CD4 T cells from CD69$^{+/+}$ and CD69$^{-/-}$ DO11.10 mice were immunized with OVA plus LPS. The number of OVA-TCR^{+} donor T cells was determined by flow cytometry. Representative OVA-TCR/CD44 staining profiles of B220- CD4 T cells in spleen of host mice on day 4 after immunization and in BM on day 48 are shown (left). The cell numbers (mean ± SD) of donor T cells was quantified as OVA-TCR+B220-CD44^{hi} CD4 T cells (right). P<0.01. (B) CD69-deficient CD4 T cells fail to induce the production of high-affinity antibodies and the generation of BM plasma cells in vivo. BALB/c mice transferred with CD4 T cells from CD69$^{+/+}$ and CD69$^{-/-}$ DO11.10 mice were immunized with NP-OVA plus LPS. Blood taken at each time point was analyzed for anti-NP_{36} IgG_1 and anti-NP_4 IgG_1 by ELISA. P<0.05.

the spleen were normally generated in CD69-deficient mice. To examine where and when CD69-deficient CD4 T cells exhibit the defects in the ability to help production of high-affinity antibodies, we enumerated antibody-secreting cells (ASCs) in spleen and BM of mice transferred with CD69-deficient or wild-type DO11.10 CD4 T cells. On days 14 and 28 after immunization, CD69-deficient CD4 T cells could generate splenic ASCs but not BM ASCs which include long-lived plasma cells. These results indicate that CD69-deficient CD4 T cells fail to induce the generation of long-lived plasma cells in the BM, although they can form GC normally.

Perspectives:

We have clarified the cellular and molecular mechanisms on the generation of memory Th cells (Figure 2; Question 1), i.e. CD69 actually functions as a homing receptor of memory Th cell precursors to the BM. Then, we are now focusing on maintenance and reactivation of memory Th cells in the BM (Figure 2; Questions 2 and 3). Understanding the lifestyle of memory Th cells leads to clarification of all immune memory system, as well as an improvement of vaccine and a therapy of allergy and autoimmune diseases. For instance, injection of anti-CD69 antibodies blocked the relocation of memory Th cell precursors to the BM. This may be used as a possible therapy for prevention of the persistence of harmful memory cells which are responsible for allergy and autoimmune diseases.

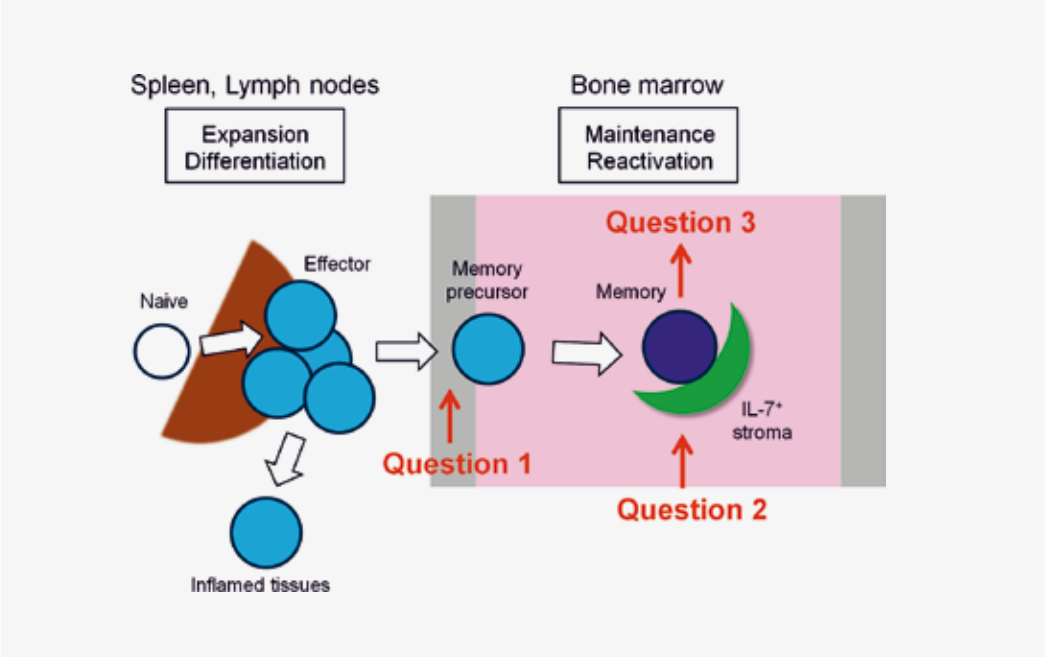


Figure 2: The lifestyle of memory Th lymphocytes.
(1) How are memory Th cells generated? (2) How are memory Th cells maintained? (3) How are memory Th cells reactivated in secondary immune response? These answers can lead to an understanding of the immune memory system in body.

Frank Buttgereit

Charité - University Medicine Berlin (CCM)
Department of Rheumatology and Clinical Immunology

Glucocorticoids & Bioenergetics

How do immune cells adopt to oxygen deficiency and a lack of nutrients in the inflamed tissue?

KEYWORDS

Bioenergetics of immune functions
Hypoxia and angiogenesis
Glucocorticoids
Autoimmune Diseases
Fracture healing

GROUP MEMBERS

Group Leader:
Prof. Dr. med. Frank Buttgereit

Scientists:
Dr. rer. nat. Timo Gaber
Dr. rer. nat. Cindy Strehl
Dr. med. Paula Hoff
Dr. med. Cornelia Spies

Ph.D. Students:
Dipl.-Ing. Martin Hahne
Dipl.-Ing. Saskia Schellmann
Annemarie Lang
Leonor Santos

MD students:
Claudia Hülso
Edgar Wiebe
Tazio Maleitzke
Susanne Schillo
Anne-Kathrin Kuhlmey

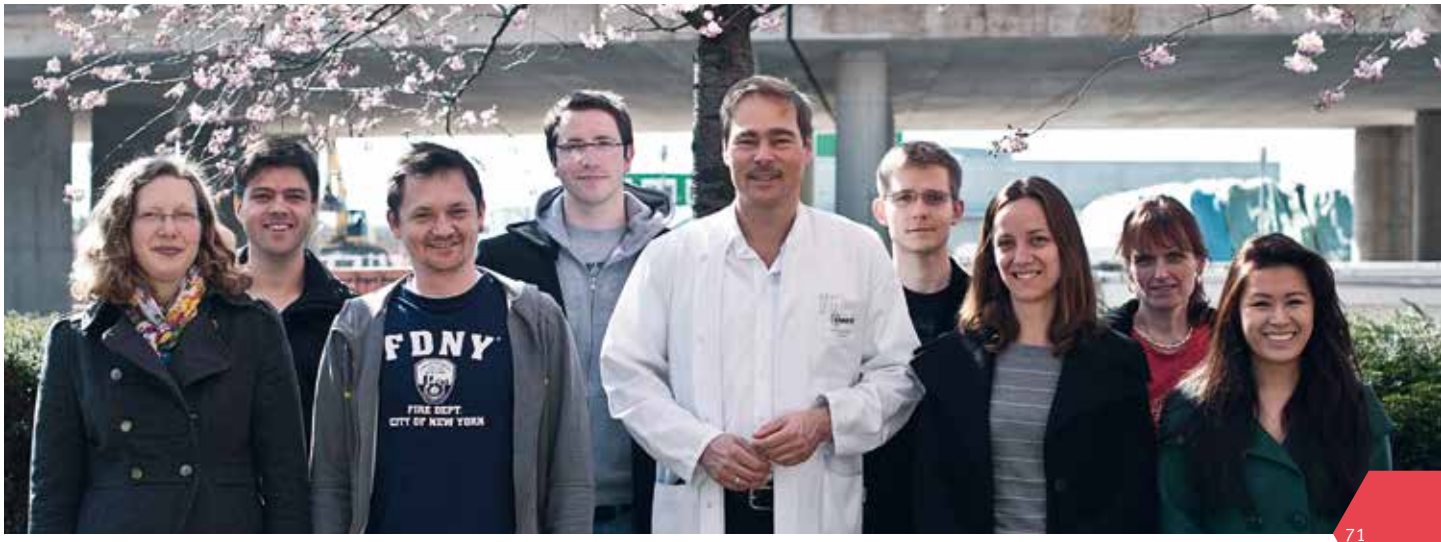
Bachelor/Diploma/Master students:
Katharina Blankenstein
Natalia Soboleva
Roman Rauch
Mathias Mursell
Cam Loan Tran
Kerstin Schönbeck

Technical Assistance:
Manuela Jakstadt

Microenvironmental conditions in infections and inflammatory diseases, e.g. rheumatoid arthritis, and in injured tissues such as in fractures are characterized by low oxygen tension (hypoxia), enrichment of oxidants, decreased pH-levels and low nutrient availability. Present immune cells, mesenchymal stem cells (MSCs) and endothelial cells (ECs) are enabled to adapt to these conditions by stabilization and activation of the transcription factors hypoxia inducible factor HIF-1 and/or HIF-2 to maintain cell viability and function. Therefore, one major focus of our work is to investigate the impact of HIFs on human $CD4^+$ T cells, monocytes, macrophages, mesenchymal stromal cells and ECs.

Glucocorticoids (GCs) represent the most potent and frequently-used class of anti-inflammatory drugs. The high clinical effectiveness of GCs is accompanied by a wide spectrum of adverse effects, which can not be completely avoided. GCs target immune cells in inflamed tissues rapidly and under bioenergetically restricted conditions. Their mode of action and their fast signaling events especially under these conditions are only barely understood and a second major focus of our research.

In the last year, we demonstrated the membrane-bound glucocorticoid receptor (GR) to be functionally active and to have the same origin as the cytosolic GR. Furthermore, we gave new insights into the mechanisms of adaptation to pathophysiological hypoxia and the different roles of HIF-1 and HIF-2 using human monocytes and macrophages, ECs, MSCs and T cells raising the knowledge about the pathogenesis of acute and chronic inflammation.



COOPERATION PARTNERS

Prof. Dr. Gerd-Rüdiger Burmester, Dr. Thomas Häupl, Dr. Bruno Stuhlmüller, Department of Rheumatology and Clinical Immunology, Charite – University Medicine Berlin, Germany

Prof. Dr. G.N. Duda, Dr. A.Ode, Berlin Brandenburg Center for Regenerative Therapies (BCRT), Charite – University Medicine Berlin, Germany

Dr. Stefan Kraus, Harvard University, Boston, USA

Prof. Dr. C. Perka, PD Dr. Schmidmaier, Dr. K. Schmidt-Bleek, Center for Musculoskeletal Surgery, Charité Berlin, Germany

Prof. Dr. Roland Lauster, Medical Biotechnology, TU-Berlin, Germany

Prof. Dr. Andreas Radbruch, Dr. Alexander Scheffold, Deutsches Rheumaforschungszentrum, Berlin, Germany

Prof. Dr. M. Seibel, Dr Hong Zhou, ANZAC Institute, University of Sydney, Australia

Dr. John Yewdell, NIH, Bethesda, USA

Magarethe und Heinrich Hoffmann, Ècole polytechnique fédérale de Lausanne (EPFL), Switzerland

Dick Heinegard, University of Lund, Sweden

SELECTED PUBLICATIONS

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Impact of hypoxia and the hypoxia-inducible factor-1 on osteogenesis and adipogenesis of human mesenchymal stromal cells

SCIENTISTS

M. Wagegg, T. Gaber, F. L. Lohanatha, M. Hahne, C. Strehl, M. Fangradt, C. L. Tran, K. Schönbeck, P. Hoff, M. Jakstadt, F. Buttgereit

COOPERATION PARTNERS

Ode - Berlin-Brandenburg Center of Regenerative Therapies and Julius Wolff Institute and Center for Musculoskeletal Surgery, Charité University Hospital, Berlin, Germany; C. Perka - Orthopaedic Departments, Charité University Hospital, Berlin, Germany; G. N. Duda - Berlin-Brandenburg Center of Regenerative Therapies and Julius Wolff Institute and Center for Musculoskeletal Surgery, Charité University Hospital, Berlin, Germany

PUBLICATIONS

Wagegg M, Gaber T, Lohanatha FL, Hahne M, Strehl C, Fangradt M, Tran CL, Schönbeck K, Hoff P, Ode A, Perka C, Duda GN, Buttgereit F. Hypoxia promotes osteogenesis but suppresses adipogenesis of human mesenchymal stromal cells in a hypoxia-inducible factor-1 dependent manner. PLoS One. 2012;7(9):e46483. doi: 10.1371/journal.pone.0046483. Epub 2012 Sep 27.

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Mesenchymal stromal cells (MSCs) are a pluripotent cell population capable of differentiating into a variety of cell types including osteoblasts, chondrocytes, adipocytes, and myoblasts. MSCs are essential for the repair and regeneration of damaged tissues, and can be easily isolated from numerous tissues. Therefore, cell therapy using MSCs represents a promising approach to promote wound healing and tissue regeneration, such as in repair of bone fractures. Bone healing is characterized by a series of cellular and molecular events that commence with hematoma formation and an inflammatory cascade, finally leading to MSC recruitment and terminal MSC differentiation. MSC recruitment is known to be essential for successful fracture repair, and recent studies have shown that migration of MSCs is strongly influenced by mechanical stimulation equivalent to conditions of the early bone-healing phase. This process takes place under low O2 tensions – so called hypoxia – which is mainly due to the disruption of supplying blood vessels. One key event in the cellular adaptation towards a hypoxic environment is the induction/stabilization of the transcription factor hypoxia-inducible factor (HIF)-1, which transactivates a number of genes whose products participate in a variety of cellular processes involved in adaptation to hypoxia, such as glycolysis, erythropoiesis, and angiogenesis. In this regard, only limited and inconsistent information is available on the impact of hypoxia and HIF-1a on the differentiation potential of primary human multipotent MSCs. Therefore, we analyzed the impact of hypoxia and HIF-1 on the competitive differentiation potential of hMSCs towards adipogenic and osteogenic lineages.

Hypoxia suppresses adipogenic and promotes osteogenic differentiation of human MSCs

To investigate the impact of HIF-1 on the differentiation of human MSCs, we induced the differentiation into adipocytes and osteoblasts under normoxic and hypoxic conditions, respectively. We found adipogenic differentiation to be suppressed under hypoxia, but osteogenic differentiation to be clearly promoted (fig. 1A). We also analyzed mRNA expression of HIF1A and VEGFA during adipogenesis and osteogenesis. In the case of adipogenesis and osteogenesis, HIF1A mRNA is differentially expressed in MSCs incubated under normoxic and hypoxic conditions. In adipogenesis, the expression significantly decreases after 2 weeks under hypoxia compared to normoxia ($p<0.05$). In contrast, during osteogenesis there is an increase of expressed HIF1A mRNA under hypoxic conditions compared to normoxia (fig. 1B, $p<0.05$). Surprisingly, expression of VEGFA transcription is up to 20 fold higher under hypoxic conditions during osteogenesis than during adipogenesis. It increases with time and is higher under hypoxia than under normoxia (fig. 1C, $p<0.05$). In contrast, VEGFA mRNA expression is decreased during adipogenesis. Furthermore, we analysed the expression of PPARG (a key marker for the adipogenic switch), and RUNX2 (a key marker for the osteogenic switch). In adipogenesis the expression of PPARG is significantly higher after 2 weeks under normoxia compared to hypoxia (fig. 1D, $p<0.001$). In the case of RUNX2, we observed a significant up-regulation of gene expression after 2 weeks under hypoxia compared to normoxia which is much more pronounced during osteogenic differentiation (fig. 1E, $p<0.01$) than during adipogenic differentiation ($p<0.05$). Furthermore, we found that osteogenesis of human MSC was facilitated by chemical inducers of HIF-1α, such as the iron chelating agent desferrioxamine mesilate (DFX) or the dimethyloxallyl glycine (DMOG), even under normoxic conditions, but to a lesser extent than under hypoxic conditions (fig. 1F). Taken together the obtained results indicate that hypoxia promotes osteogenesis but suppresses adipogenesis of human MSCs.

Reduction of HIF-1α expression in human MSCs (i) enhances adipogenesis under normoxic conditions, (ii) partially restores hypoxia-induced attenuation of adipogenesis and (iii) suppresses hypoxia-enhanced osteogenesis

In the next step, we considered HIF-1α to play a key role in regulating adipogenesis and osteogenesis. In order to investigate the function of HIF-1α in greater detail, a shRNA mediated knockdown of HIF-1α was performed using lentiviral transduction. To this end, we used two HIF-1α-silencing shRNAs (sh1 and sh2) and one non-silencing shRNA (scr) as control. Knockdown of HIF-1α was verified at the protein level (fig. 2A). In HIF-1α knockdown cells, adipogenesis and osteogenesis were induced for 4 weeks. In the case of adipogenesis, control cells (scr-treated cells) behaved as expected, i.e. adipogenesis was suppressed under hypoxia (fig. 2B). However, adipogenic differentiation of transduced MSCs was increased compared with controls, under both normoxic and hypoxic conditions (fig. 2B). Interestingly, osteogenic differentiation showed the converse, with promotion of osteogenesis under hypoxic conditions (fig. 2C). In the case of osteogenic differentiation of MSCs with HIF-1α knock-

down, we found suppression of hypoxia-enhanced osteogenesis (fig. 2C). From these results we conclude the ostoeogenic and adipogenic differentiation of human MSCs under hypoxia to be HIF-1 dependent.

Perspectives:

Hypoxia promotes osteogenesis but suppresses adipogenesis of human MSCs in a competitive and HIF-1-dependent manner. We therefore conclude that the effects of hypoxia are crucial for effective bone healing, which may potentially lead to the development of novel therapeutic approaches.

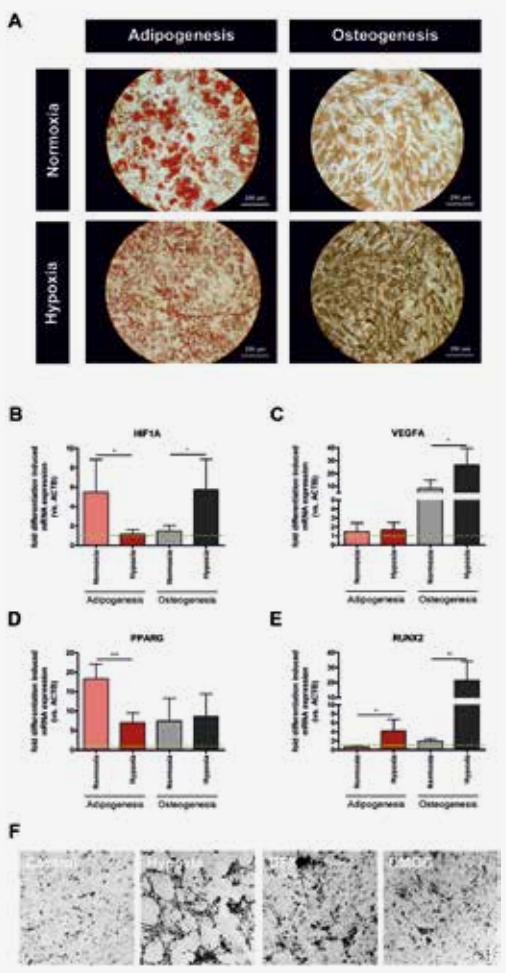

Figure 1: Hypoxia suppresses adipogenic and promotes osteogenic differentiation of human MSCs. (a) Oil-Red-O stain for the analysis of adipogenesis and von-Kossa stain for the analysis of osteogenesis of MSCs incubated under normoxia (<18% pO2) or hypoxia (1% pO2). (b) HIF1A, (c) VEGFA, (d) PPARG and (e) RUNX2 gene expression of MSCs incubated under normoxia (<18% pO2) or hypoxia (1% pO2). (f) Analysis of osteogenesis by von-Kossa stain of MSCs incubated under normoxia (<18% pO2) without treatment, or with either 250 mM DFX and 100 mM DMOG, respectively.

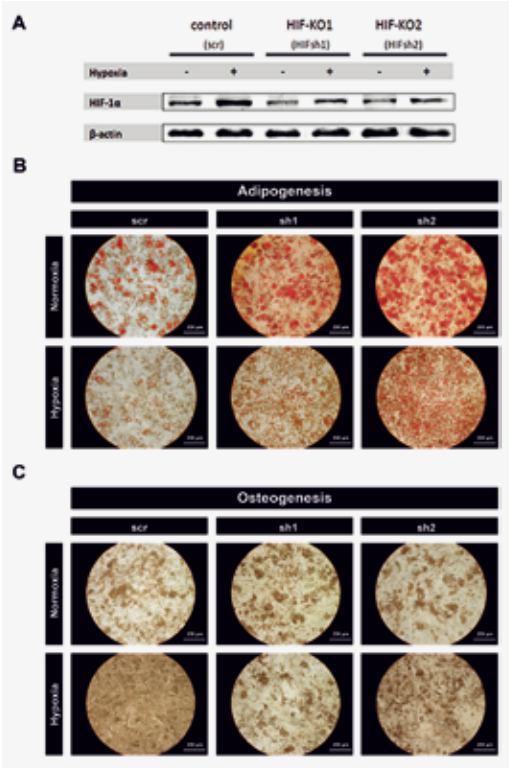

Figure 2: Reduction of HIF-1a of human MSCs (i) enhances adipogenesis under normoxic conditions, (ii) partially restores hypoxia-induced attenuation of adipogenesis and (iii) suppresses hypoxia-enhanced osteogenesis. (a) Transduction of anti HIF-1a-shRNAconstructs efficiently reduced HIF-1a protein expression as shown by immunoblot. (b) Oil-Red-O stain for the analysis of adipogenesis of shRNA-construct transduced MSCs and (c) von-Kossa stain for the analysis of osteogenesis of shRNA-construct transduced MSCs. Cells were maintained under normoxia (<18% pO2) or hypoxia (1% pO2).

Analysis of MIF function upon the angiogenic potential of HMECs

SCIENTISTS

M. Mursell, M. Hahne, C. Strehl, C. L. Tran, K. Schönbeck, M. Jakstadt, T. Gaber, F. Buttgereit

FUNDING

This work was supported by the Berlin-Brandenburg Center of Regenerative Therapies (BCRT) to TG and MJ and by the Berlin-Brandenburg School of Regenerative Therapies (BSRT) to MH

Angiogenesis is a hallmark of injured tissue, inflammation and progressing tumors. The multifunctional and proinflammatory protein macrophage migration inhibitory factor (MIF) participates in the regulation of angiogenesis. MIF was shown to be modulated via the hypoxia-inducable factor (HIF)-1, that controls cellular metabolic response to decreased oxygen tension thereby promoting angiogenesis.

Here, we focused on the effects of MIF in the process of angiogenesis and possible interactions with HIF-1. Therefore, we developed a human microvascular endothelial cells (HMEC) lentiviral based knockdown system for MIF and blocked MIF-signaling pathway via its receptor CD74, allowing us to analyse the interplay of MIF and HIF-1 during hypoxia-induced angiogenesis of HMECs.

Specific knockdown of MIF was achieved using lentiviral-based shRNA technology. The reduction of MIF was analysed on transcriptional and translational level by realtime RT-PCR and Western blot. Angiogenesis of transduced HMECs in comparison to scrambled and wildtype cells was studied by investigating both tubuli and node formation under hypoxia (<1% O2). Furthermore angiogenesis assay was also performed with cells treated with anti-CD74-IgG or rhMIF (0,1ng/ml).

Results and discussion:

It was previously shown that successful knockdown of MIF reduced HIF-1α protein expression. The MIF knockdown in HMECs led to significantly decreased tubuli formation (2-fold change under hypoxia, p=0.0019) compared to the scr control with similar effects by trend on node formation. Blocking of CD74 also led to a significantly decreased tubuli formation under hypoxia by a factor of 2.3 (control vs. wildtype + 4µg aCD74, p=<0.0001), whereas the addition of rhMIF did not influence tubuli formation.

Perspectives

Our findings reveal an essential regulatory function of MIF upon the angiogenic potential of HMECs. However, the underlying mechanisms and the influence on HIF-1 and other hypoxia driven factors have to be analysed. These provide new basic insights into angiogenesis in inflamed tissues *and moreover, targeting MIF locally might be useful to control inflammation. Therefore, we consider MIF to be of clinical importance in the control of inflammation.

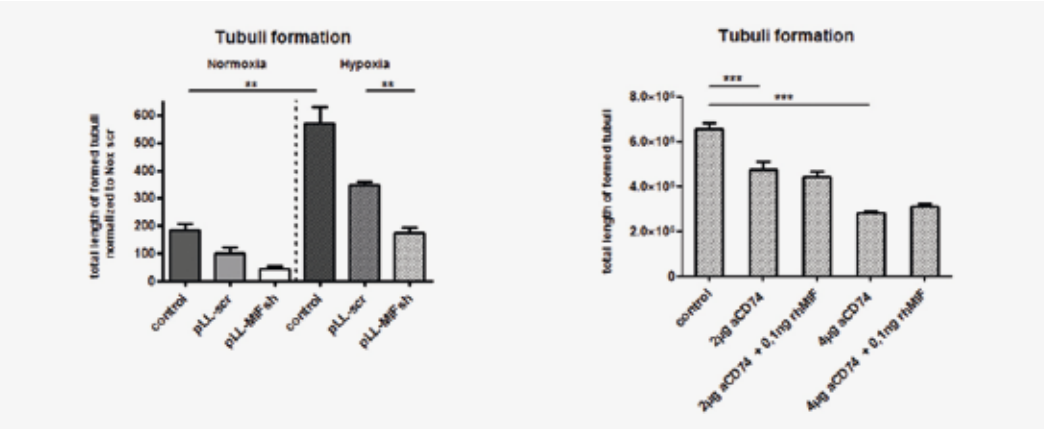

Figure 1: MIF knockdown and blocking of the MIF receptor (CD74) decreased tubuli formation

Pathophysiological hypoxia affects human CD4+ T cell function

Inflamed areas are characterized by infiltration of immune cells, local hypoxia and alterations of cellular redox states. We investigated the impact of hypoxia on survival, proliferation, cytokine secretion, intracellular energy and redox state of human CD4⁺ T cells.

We found that pathophysiological hypoxia (<2% O2) significantly decreased CD4⁺ T-cell survival after mitogenic stimulation. This effect was not due to an increased caspase-3/7-mediated apoptosis or ATP consumption/depletion. However, the ability of stimulated T cells to proliferate was reduced under hypoxic conditions, despite increased expression of CD25. Pathophysiological hypoxia was also found to modify intracellular ROS (iROS) levels in stimulated T cells over time as compared with levels found in normoxia. Physiological hypoxia (5% O2) did not decrease CD4⁺ T-cell survival and proliferation or modify iROS levels as compared with normoxia (Figure 1).

We conclude that pathophysiological hypoxia affects T-cell proliferation and viability via disturbed IL-2R signaling downstream of STAT5a phosphorylation, but not as a result of impaired cellular energy homeostasis. We suggest iROS links early events in T-cell stimulation to the inhibition of the lymphoproliferative response under pathophysiological hypoxic conditions. The level of iROS may therefore act as a mediator of immune functions leading to down-regulation of long-term T-cell activity in inflamed tissues.

Perspectives:
However, the role of Treg cells and HIF-1 during this cellular adaptive response to pathophysiological hypoxia will be investigeted more in detail.

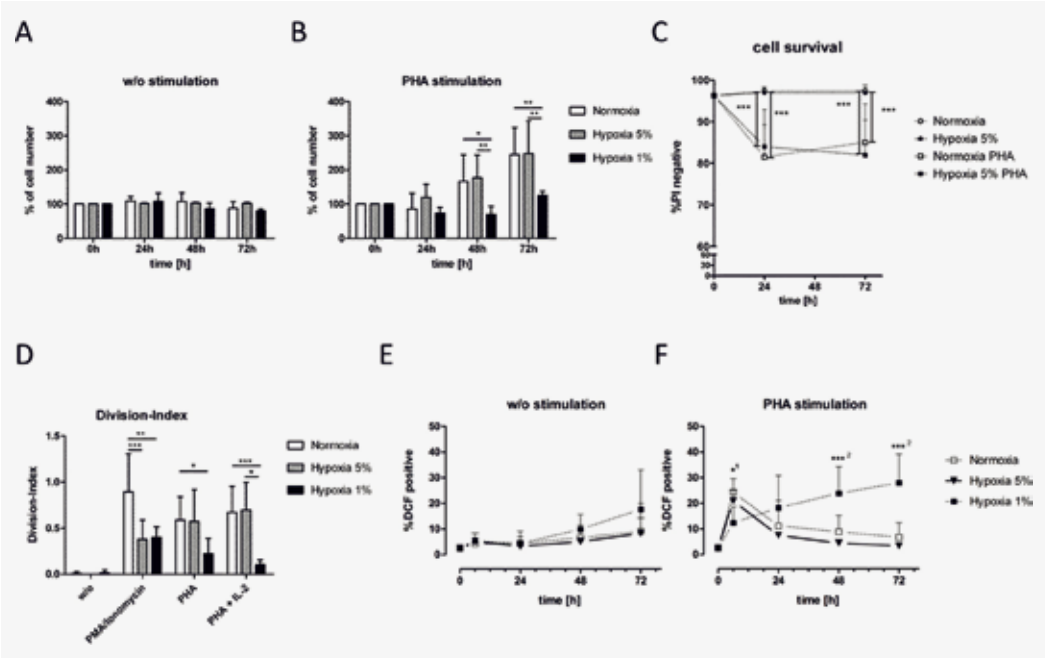

Figure 1: Physiological hypoxia does not decrease T-cell survival, proliferation and iROS response following PHA stimulation as compared with normoxia. (A, B) Comparative analysis of the development of T-cell count maintained for 72 h without (w/o) and with PHA stimulation is shown. (C) Cell survival is shown as PI-negative percentage of isolated CD3⁺/CD4⁺ T cells in relationship to normoxic or physiological hypoxic (5% O2) incubation conditions. Data are shown as mean + SD of n=4 independent experiments performed. ***p ‹ 0.001; two-way ANOVA with Bonferroni's multiple comparisonpost hoc comparison. (D) CFSE staining of CD4⁺ T cells after 96 h normoxic, physiological hypoxic (5% O2) or pathophysiological hypoxic (1% O2) incubation without stimulation, with PMA/ionomycine stimulation or with PHA stimulation and with or without initial treatment using recombinant human IL-2 as indicated, analyzed by flow cytometry. CD4⁺ T-cell proliferation is demonstrated by division-index and shown as mean + SD of n=6 independent experiments performed. *p‹0.05; **p‹0.01; ***p‹0.001, two-way ANOVA with Bonferroni's multiple comparison post hoc test. (E, F) Overview of DCF positive cells after normoxic, physiological hypoxic (5% O2) or pathophysiological hypoxic (1% O2) incubation with and without PHA stimulation as indicated. Data are shown as mean + SD of n=4-9 independent experiments performed. *p‹0.05; ***p‹0.001, two-way ANOVA with Bonferroni's multiple comparison post hoc test, 1hypoxia 1% O2 as compared with normoxia, 2hypoxia 1% O2 as compared with both normoxia and hypoxia 5% O2 respectively.

SCIENTISTS

T. Gaber, C.L. Tran, S. Schellmann, M. Hahne, C. Strehl, P. Hoff, M. Jakstadt, F. Buttgereit

COOPERATION PARTNERS

A. Radbruch: German Rheumatism Research Center (DRFZ), Charitéplatz 1, 10117 Berlin, Germany, Charitéplatz 1, 10117 Berlin

G.-R. Burmester: Department of Rheumatology and Clinical Immunology, Charité University Hospital, Charitéplatz 1, 10117 Berlin

PUBLICATIONS

Gaber T, Tran CL, Schellmann S, Hahne M, Strehl C, Hoff P, Radbruch A, Burmester GR, Buttgereit F. Pathophysiological hypoxia affects the redox state and IL-2 signaling of human CD4+ T cells and concomitantly impairs survival and proliferation. Eur J Immunol. 2013 doi: 10.1002/eji.201242754.

FUNDING

This work was supported by the Berlin-Brandenburg Center of Regenerative Therapies (BCRT) to TG and MJ and by the Berlin-Brandenburg School of Regenerative Therapies (BSRT) to MH

Thomas Dörner

Charité - University Medicine Berlin (CCM)
Department of Rheumatology and Clinical Immunology

B Cell Memory

Principles of induction and maintenance of memory B cells in protective and autoreactive immunity and therapeutic targeting

KEYWORDS
Autoimmunity,
Innovative B-cells Therapy,
Antigen-specific B-cell,
Immune-homeostasis,
Immunological memory

GROUP MEMBERS

Group leader:
Prof. Dr. med. Thomas Dörner

Scientists:
Henrik E. Mei, PhD
Capucine Daridon, PhD

PhD students:
Claudia Giesecke, MA
Ina Wirries, MA
Sarah J. Fleischer, Dipl-Ing

Diploma student:
Stefanie Schmidt, Dipl-Ing
(until 2011)

MD students
Julia Sieber
Daniela Blaßfeld (until 2012)
Nadine Sieger (until 2012)
Maximilian Koenig

Students:
Vanessa Fleischer (2012)

Master student:
Katarzyna Luda (until 2012)

Technical assistance:
Karin Reiter

Our team is focusing on immunological memory; in particular on memory B cells and plasma cells. Memory B cells play an important role in long-term protection of the body against pathogens as well as under autoimmune conditions. Our three different axes of investigation are: 1) basic mechanisms in the induction and maintenance of protective, antigen-specific memory B cells after vaccination, 2) maintenance of memory plasma cells and their interaction with human bone marrow and 3) targeting pathogenic memory B cells in autoimmunity, such as rheumatoid arthritis (RA), immune thrombocytopenia (ITP), primary Sjögren´s syndrome (pSS) and systemic lupus erythematosus (SLE).

First, kinetics and characteristics of memory B cell differentiation into plasma cells in human are studied following a primary (KLH) and secondary (TT) immunizations. Using in-house developed detection systems, antigen-specific cells generated in response to the vaccination can be tracked and analyzed by flow cytometry, molecular and functional assays. Second axis is the study of the plasma cells homeostasis in the bone marrow; and in particular, the analysis of the microenvironment of distinct plasma cell subsets by flow cytometry, RNA profiling and immune histology. Applying these technologies, investigations on autoimmune diseases, such as SLE, pSS, RA or ITP are in place searching for autoreactive cells.

Perspectives: New approaches for improved diagnostics for rheumatic diseases, fine-tuned evaluation of disease activity, up-to-date approaches for therapy monitoring as well as the identification and validation of new therapeutic targets and biomarkers will emerge.

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Immunopathogenic mechanisms of systemic autoimmune diseases. Wahren-Herlenius M. and Dörner T. Lancet 2013 in press.

COOPERATION PARTNERS

M. Brisslert, PhD, Sahlgrenska Academy at Gothenburg university Hospital, Gothenburg, sweden

Prof. G.R. Burmester, Charité – Universitätsmedizin, Department. of Medicine, Rheumatology and Clinical Immunology, Berlin, Germany

Dr. M. Dürr, Charité Universitätsmedizin, Department of Nephrology, Berlin, Germany

R Engelmann, PhD, University of Rostock, Institute of Immunology, Rostock, Germany

S. Fillatreau, PhD, DRFZ,immune regulation, Berlin, German

J. Grün, PhD, DRFZ, regine von ramin lab, Berlin, Germany

A. Grützkau, PhD, DRFZ, regine von ramin lab, Berlin, Germany

Prof. F. Hiepe, Charité – Universitätsmedizin, Department. of Medicine, Rheumatology and Clinical Immunology, Berlin, Germany

Dr. T. Häupl, Charité – Universitätsmedizin, Department. of Medicine, Rheumatology and Clinical Immunology, Berlin, Germany

Dr. A.A Kühl and Spieckermann S, Charité - Universitätsmedizin, Research Center ImmunoScience (RCIS) and Department. of Gastroenterology, Infectiology and Rheumatology, Berlin, Germany

Dr. R. Kuhly, Charité Universitätsmedizin,Department of Anesthesiology and Operative Intensive Care Medicine, Berlin, Germany

Prof. C. Loddenkemper, Dept. Pathology, Technical University Munich

Prof. P. Neuhaus, Charité Universitätsmedizin, Department of General, Visceral, Vascular and Thoracic Surgery, Berlin, Germany

Prof. C. Perka, Department of Orthopedics, Charité Universitätsmedizin, Berlin, Germany

Prof. A. Radbruch, DRFZ, cell biology, Berlin, Germany

Dr. C. Romagnani, DRFZ, innate immunity, Berlin, Germany

Dr. B. Rudolph, Charité Universitätsmedizin, Department of nephropathology, Berlin, Germany

Prof. A. Salama and A. Pruss, Charité Universitätsmedizin, Institute of Transfusion Medicine, Berlin, Germany

Dr. R. Ullrich, Charité Universitätsmedizin, Department of Gastroenterology, Infectiology and Rheumatology, Berlin, Germany

Prof. Dr. M. Wahren-Herlenius, Karolinska Hospital and Institute, Stockholm, Sweden

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Splenic proliferative lymphoid nodules distinct from germinal centers are sites of autoantigen stimulation in immune thrombocytopenia.Daridon C, Loddenkemper C, Spieckermann S, Kühl AA, Salama A, Burmester GR, Lipsky PE, Dörner T. Blood. 2012 Dec 13;120(25):5021-31. doi: 10.1182/blood-2012-04-424648. Epub 2012 Sep 6.

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CD22 ligation inhibits downstream B-cell receptor signaling and Ca(2+) flux upon activation. Sieger N., Fleischer S.J., Mei H.E., Reiter K., Shock A., Burmester G.R., Daridon C., Dörner T. Arthritis Rheum. 2012 Dec 11. doi: 10.1002/art.37818.

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Safety and clinical outcomes of rituximab therapy in patients with different autoimmune diseases: experience from a national registry (GRAID).Tony H.P., Burmester G., Schulze-Koops H., Grunke M., Henes J., Kötter I., Haas J., Unger L., Lovric S., Haubitz M., Fischer-Betz R., Chehab G., Rubbert-Roth A., Specker C., Weinerth J., Holle J., Müller-Ladner U., König R., Fiehn C., Burgwinkel P., Budde K., Sörensen H., Meurer M., Aringer M., Kieseier B., Erfurt-Berge C., Sticherling M., Veelken R., Ziemann U., Strutz F., von Wussow P., Meier F.M., Hunzelmann N., Schmidt E., Bergner R., Schwarting A., Eming R., Hertl M., Stadler R., Schwarz-Eywill M., Wassenberg S., Fleck M., Metzler C., Zettl U., Westphal J., Heitmann S., Herzog A.L., Wiendl H., Jakob W., Schmidt E., Freivogel K., Dörner T.; GRAID investigators. Arthritis Res Ther. 2011 May 13;13(3):R75. doi: 10.1186/ar3337.

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Hiepe F., Dörner T., Hauser A.E., Hoyer B.F., Mei H., Radbruch A.. Long-lived autoreactive plasma cells drive persistent autoimmune inflammation. Nat Rev Rheumatol. 2011 Mar;7(3):170-8. doi: 10.1038/nrrheum.2011.

Diminished B cell response and peripheral B cell abnormalities in patients with SLE are linked to disturbances of the spleen tyrosine kinase (Syk)

The B cell receptor (BCR) response as well as the BCR affinity plays a crucial role in the development and maintenance of autoimmunity. Therefore our analysis of the BCR dependent signaling pathway in patients with autoimmune diseases such as systemic lupus erythematosus (SLE) is of high interest. Our study reports beside a diminished but long lasting Syk phosphorylation after BCR activation and a reduced level of basal Syk and phosphorylated Syk in patients with SLE, a SLE associated CD27(-)Syk-bright population showing activated phenotype with a stronger Syk phosphorylation after BCR activation. Thus, SLE patients exhibit a CD27(-)Syk-bright B cell subset could be responsible for the hyper-reactive phenotype of SLE B cells and may play an important role in the maintenance of autoimmunity.

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by a breakdown of self-tolerance inducing production of autoantibodies (Ab), B cell hyper-reactivity as well as an impaired peripheral B cell homeostasis. Recent data suggest that also a disturbed B cell response could lead to the development of autoimmunity.

In this study, the BCR downstream signaling kinase Syk was analyzed from 26 healthy controls (HD), 38 SLE patients, 12 rheumatoid arthritis (RA) and 17 primary Sjögren's syndrome (pSS) patients in detail and revealed new insights into peripheral B cell abnormalities based on intracellular characteristics. In addition, the phosphorylation kinetic of Syk after BCR engagement was studied on peripheral blood B cells of SLE patients and healthy controls using phosphoflow analysis.

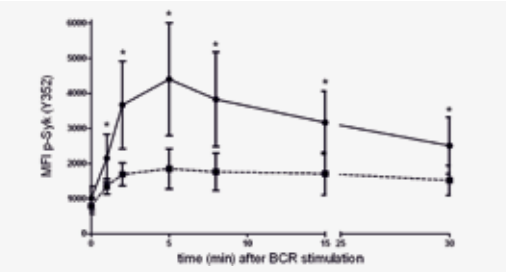


Fig.1: Diminished Syk phosphorylation after BCR activation in patients with SLE versus HD. The curves show the phosphorylation kinetics of Syk(Y352) in HD (•) compared to SLE patients (■) represented by the mean fluorescence intensity (MFI) (* p>0.5; Mann-Whitney-Test).

Fig.2: Enlarged $CD27^{(-)}Syk^{bright}$ subset in the peripheral blood of SLE. (A) Whole blood of healthy controls and SLE patients were. Representative dot plots show the Syk concentration within $CD27^{+}$ versus $CD27^{(-)}$ B cells with a distinct $CD27^{(-)}Syk^{bright}$ B cell subset in SLE patients. The histogram compares the Syk concentration of $CD27^{(-)}$ B-cells (grey) with those within T-cells as negative control (black). (B) Scatter plot shows the frequency of Syk^{bright} within $CD20^{+}CD27^{(-)}$ B cells of HD(•), SLE (■), RA(▲) and pSS patients (◆) (* p>0.5; Mann-Whitney-Test).

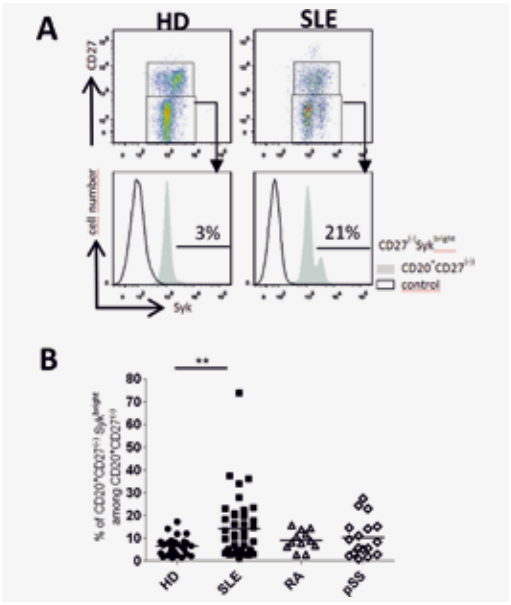
Reduced Syk phosphorylation after BCR activation in SLE patients

Interestingly, SLE B cells compared to HD revealed a significantly lower Syk (Y352) phosphorylation after BCR engagement at all time points analyzed, demonstrating a diminished B cell response in patients with SLE. However, a stable and long-lasting Syk phosphorylation after BCR engagement was observed in B cells from SLE patients (fig.1).

Increased frequency of a unique CD27(-)Sykbright subset in patients with SLE

A significant enlarged Sykbright B cell population has been identified within CD27(-) B cell subset in the blood of SLE patients (fig.2A). The frequency of CD27(-)Sykbright population was 6.4±4% in the blood of HD and 14.4±13.7% in SLE ($p<0.001$). No correlation between the frequency of CD27(-)Sykbright B cells and SLE disease activity could be observed. Of note, the frequency of CD27(-)Sykbright B cell subset was not significantly increased in HD, RA or pSS patients (fig. 2B).

Thus, SLE patients showed a diminished but long-lasting Syk phosphorylation and exhibit an enlarged CD27(-)Sykbright B cell subset with an activated phenotype that could be responsible for the hyper-reactivity of SLE B cells and may play an important role in the maintenance of autoimmunity. A better characterization of CD27(-)Sykbright B cells might be relevant for new target for SLE therapy.



■ SCIENTISTS

S.J. Fleischer, C. Daridon, T. Dörner

■ COOPERATION PARTNERS

G.R. Burmester, T. Alexander, R. Biesen, F. Hiepe, department of Rheumatology, Charité Universitätsmedizin Berlin

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■ FUNDING

SFB650/TP16, DFG 4D1/7-2

Induction, maintenance and re-activation of human memory tB cells

In this project we seek to understand induction, maintenance and re-activation mechanisms of human memory B cells (mBC). This line of research has not only implications for our knowledge of protective immunity but clearly may impact on our understanding of the maintenance of autoimmunity and potential therapeutic approaches. We have refined a method to directly detect antigen-specific B cells in peripheral blood and tissues. Based on this, we investigate primary and secondary vaccinations in healthy donors and autoimmune patients to delineate similarities and differences in their B cell responses. The spleen has been implicated not only in mBC differentiation, but also in their maintenance and storage. To investigate this further, we studied splenectomized patients after vaccination in order to delineate the distribution, frequencies and characteristics of antigen-specific mBC as well as mBC subpopulations within lymphoid tissues, e.g. spleen and tonsil compared to peripheral blood.

Memory B cells (mBC) constitute the reactive arm of immunological memory provided by antigen-experienced B lymphocytes. Upon secondary encounter with their cognate antigen mBC provide a faster, quality and quantity improved response compared to a primary one. The mechanisms and conditions how the survival of mBC over years is achieved under healthy as well as autoimmune conditions remain elusive. Recently, the spleen has been implicated in mBC differentiation and maintenance. However, human studies are incomplete addressing antigen-specific mBC and a potential role of the spleen for their maintenance.

To address this, we firstly identified the distribution and frequency of mBC and tetanus-specific (TT^+) mBC in the spleen in contrast to tonsil, bone marrow and peripheral blood (PB).

Using flow cytometry, human PB, bone marrow, spleen and tonsils were analyzed for their relative content of $CD19^+$ B cells, $CD19^+CD20^+CD27^+$ mBC and the respective frequency of TT^+ mBC (Fig.1). This analysis revealed that bone marrow, spleen and tonsils contain more $CD19^+$ B cells than are circulating in PB. However, mBC were only enriched in tonsils and spleen but not in bone marrow. Interestingly, TT^+ mBC were commonly found in spleen, tonsils and PB.

These results indicate that (TT^+) mBC are not restricted to the human spleen but appear to patrol and reside within different tissues as evidenced by their presence outside of antigenic challenge in PB and the tonsil.

We will further investigate the phenotypic, functional and molecular characteristics of antigen-specific mBC in PB and different lymphoid tissues including their tissue microenvironments. A current application seeks funding by the transregio "B cell immunology" and will further delineate conditions of mBC maintenance.

This research does not only extend our basic understanding of B cell memory generation and maintenance, it may also guide us to improve current therapies of autoimmune disease

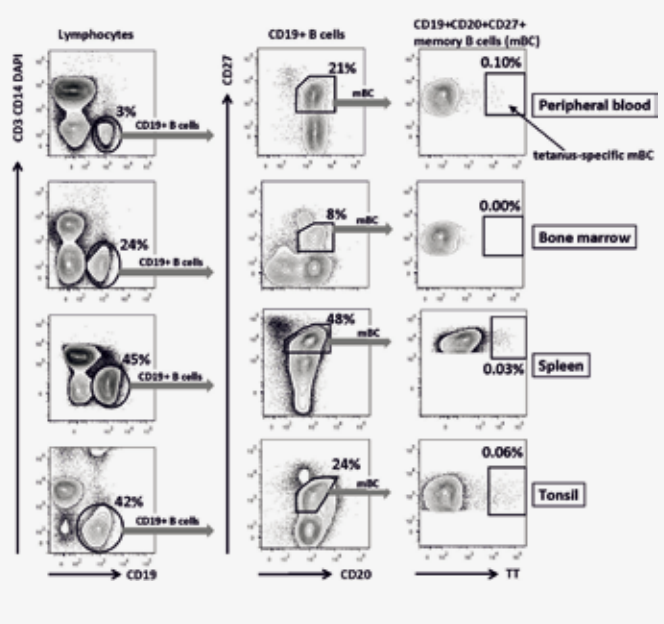

Fig.1: Identification of tetanus (TT)-specific B cells within memory B cells (mBC) in blood and lymphoid tissues outside of vaccination challenges suggest their survival in these organs and continuous recirculation. Numbers indicate median frequencies from all samples analyzed.

FUNDING

DFG Do 491/7-1, SFB633/A14

SCIENTISTS

C. Giesecke, T. Dörner

COOPERATION PARTNERS

R. Ullrich, Department of Gastroenterology, Infectiology and Rheumatology; A. Salama, Institute of Transfusion Medicine; P. Neuhaus, Department of General, Visceral, Vascular and Thoracic Surgery: R. Kuhly, Department of Anesthesiology and Operative Intensive Care Medicine; C. Perka, Orthopedics Department, Charité; C. Romagnani, DRFZ

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The composition of antibody-secreting plasmablasts in SLE

SCIENTISTS

H.E. Mei, S. Schmidt, A. Redlin, T. Dörner

COOPERATION PARTNERS

T. Alexander, B.H. Hoyer, F. Hiepe, Dept of Rheumatology, Charité – Universitätsmedizin Berlin

PUBLICATIONS

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FUNDING

DGRh start-up 2009, DFG Do491/5-3

Systemic Lupus erythematosus (SLE) is characterized by hypergammaglobulinemia, autoantibody production and increased presence of antibody-secreting cells (so-called plasmablasts) in the blood. In this project we confirmed the increase of plasmablasts in SLE patient´s blood. However, most plasmablasts in SLE patients and controls showed mucosal characteristics, and showed little or no autoreactivity. Vice versa, IgG plasmablasts in SLE patients constituted a minor cell fraction but secreted autoantibodies. Moreover, the expression of receptors expressed by plasmablasts indicative of mucosal immune responses but not those of systemic immune responses correlated with an inflammatory cytokine signature in the blood. These results suggest that cytokine-dependent as well as autoantigen-driven plasmablast differentiation contribute to overproduction of plasmablasts in SLE.

The majority of plasmablasts in SLE express a mucosal phenotype

Initally, B lymphopenia (p=0.0013) and increased plasmablasts levels in the blood (p=0.0002) were confirmed in SLE using flow cytometry. In SLE patients and controls, 57% and 55% of plasmablasts expressed the mucosal immunoglobulin IgA (Fig.1), the mucosal chemokine receptor CCR10 (39% and 44%) or the mucosal adhesion receptor β7-integrin (38% and 51%). Taken together, 73% and 80% of plasmablasts expressed at least one of these receptors, demonstrating that the majority of plasmablasts appears to be derived from mucosal immune responses. In support of the mucosal provenance of most plasmablasts in SLE, the phenotype of plasmablasts induced by an oral immunization with cholera vaccine (IgA+/-CCR10+/-β7 integrin+) but not by systemic immunization with tetanus vaccine (IgA-CCR10-β7-integrin-) was similar to SLE plasmablasts.

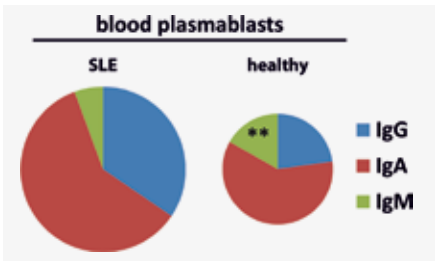

Fig.1: Isotype composition of blood plasmablasts in SLE patients and controls. Pie chart areas reflect the numbers of plasmablast/ml blood.

Mucosal plasmablasts in SLE show few autoreactivity and correlate with inflammatory serum cytokines

To analyze autoreactivity of plasmablasts, blood mononuclear cells were cultured and supernatants were analyzed for IgA-ANA (antinuclear antibody) and IgG-ANA by ELISA. While healthy controls did not secrete ANA, IgG-ANA was detectable in 75% of the SLE patients, and 32% of the same patients showed IgA-ANA production at lower magnitude compared to IgG-ANA. By contrast, expression of receptors induced in mucosal but not systemic immune responses such as IgA and CCR10 was positively associated with an inflammatory cytokine signature in the patients´ blood sera, e.g. IL-2, IL-6 and IL-17 (Fig.2).

These results suggest that distinct B cell differentiation pathways contribute to the expansion of plasmablasts in SLE; i.e. differentiation of autoreactive IgG plasmablasts and presumably cytokine-driven, unspecific differentiation of poorly autoreactive IgA plasmablasts. This provides insight into the dysregulation of B cells in SLE and may allow for development of treatments to specifically target autoreactive antibody-secreting cells.

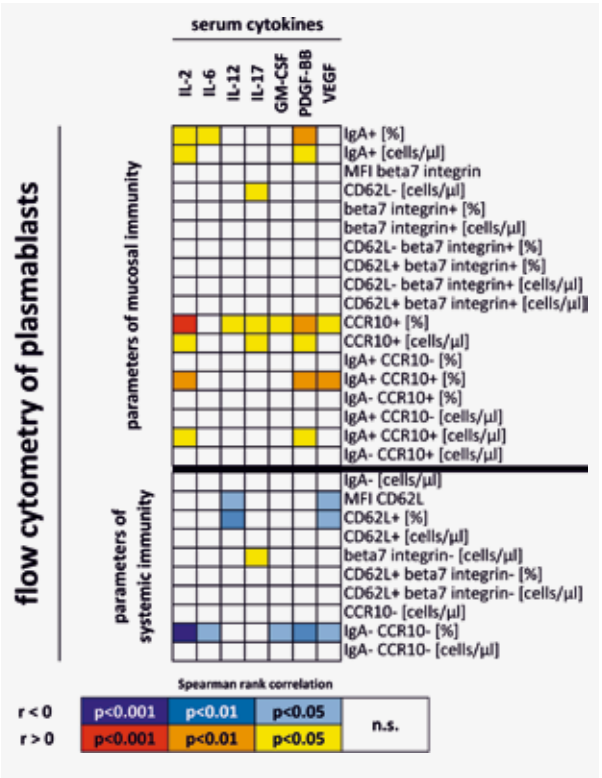

Fig.2: Correlation of inflammatory cytokine levels (columns) in blood serum and expression of mucosal receptors (top lines) but not ´systemic´ receptors (bottom lines) by plasmablasts in SLE. Yellow, orange and red colours indicate positive correlations, gradient of blue colours indicate inverse correlations.

Splenic proliferative lymphoid nodules distinct from germinal centers are sites of autoantigen stimulation in immune thrombocytopenia.

This study focused on understanding the cellular mechanisms involved in the breakdown of tolerance and resulting in the production of autoantibodies in human spleen. Sixty seven human spleens from immune thrombocytopenia (ITP) and controls, in particular their B-cell organization were investigated quantitatively and qualitatively. As a result, non-polarized aggregates of proliferating B cells distinct from classic germinal center (GCs) were identified in both ITP and controls spleens, but in ITP spleens platelet auto-antigen immune complex (IC) bound to follicular dendritic cells (FDCs) were frequently observed in these structures suggesting that they are sites of autoantigenic stimulation.

The aim of this work is to understand the *in situ* breakdown of tolerance in autoimmune disease. Spleens from ITP, a human autoimmune disease characterized by the production of autoantibodies against platelets has been chosen as a readily available model to investigate the breakdown of immune tolerance *in situ*. Therefore, 31 spleens from ITP patients and 36 control spleens were analyzed and compared by immunohistochemistry and immunofluorescence.

Two different splenic structures accommodating proliferating B cells within the white pulp, classical germinal centers (GCs) and proliferative lymphoid nodules (PLNs) were clearly identified (Fig.1). The number of typical GCs was decreased in ITP spleens whereas PLNs were prominent and comparable to the number found in normal spleens. Notably, PLNs were observed in control and ITP spleens. PLN were characterized by proliferating Ki67+ B cells in close contact with follicular dendritic cells lacking polarization into dark and light zones (Fig.1). As opposed to cells in GCs, proliferating B cells in PLNs lacked expression of Bcl6. Of note, the density of T cells was reduced in both GC and PLN in ITP in comparison to control spleens. T follicular helper cells were found within PLNs as well as in GCs, but the density of Foxp3+ regulatory T cells (Treg) was reduced in both structures in ITP. Strikingly, FDC networks of PLNs but not GCs from ITP spleens contained abundant platelet glycoprotein IIb/IIIa autoantigens in IgM containing immune complexes in close proximity to proliferating B cell (Fig.2).

These observations are consistent with the conclusion that PLN and not GC are the site of the development and maintenance of autoimmunity in ITP, which could be accentuated by the reduced density of Treg in these structures. PLN may also play a critical role in the breakdown of tolerance characterized by other autoimmune/rheumatic diseases. A good understanding of this breakdown would allow us to restore the tolerance in autoimmune diseases.

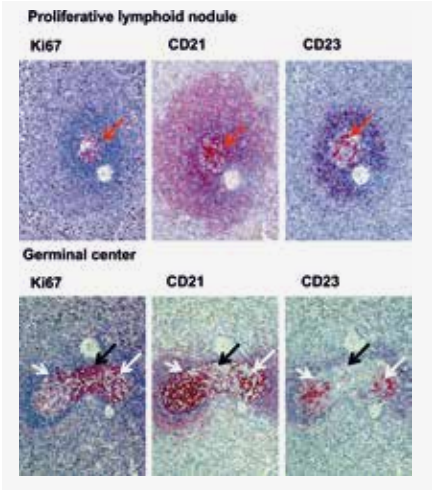

Fig.1: Serial sections of 10 AITP and 10 controls spleens were stained to evaluate the expression of Ki67, CD21 and CD23 in both PLN (upper panels) and GC (lower panels). The black arrows mark the dark zone (Ki67^{+}, CD21$^{+/-}$ and CD23^{-}) and the white arrows indicate the light zone (Ki67$^{+/-}$, CD21^{+}, CD23^{+}) of a GC. Visualization with Zeiss Axio Imager Z1 fluorescence microscope, magnification: 200x. The photomicrographs shown here are from AITP spleens.

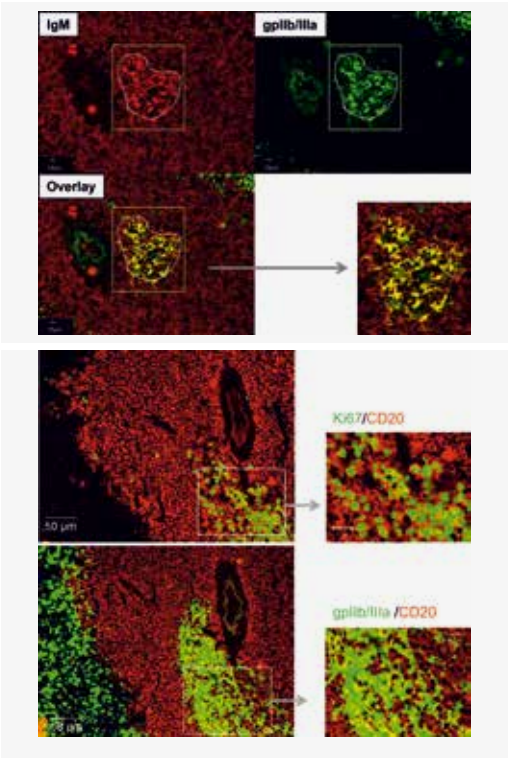

Fig.2: A- Sections from 2 spleens were examined by double immunofluorescence staining: IgM (red) with gpIIb/IIIa (green). The colocalization of IgM and gpIIb/IIIa represents gp IIb/IIIa autoantigens in IgM containing immune complexes. B-Sections from 2 AITP spleens were stained for CD20 (red), Ki67 (green) or gpIIb/IIIa (green) and assessed for the presence of proliferating B-cells and B-cells in proximity to gpIIb/IIIa. Visualization was carried out with a Zeiss Axio Imager Z1 fluorescence microscope, magnification: 200x.

SCIENTISTS

C.Daridon, T. Dörner

COOPERATION PARTNERS

Lipsky P.E. Formerly NIAMS/NIH, Autoimmunity branch, Bethesda, USA

Loddenkemper C., Spieckermann S, Kühl A.A., Dept. of Gastroenterology, Infectiology and Rheumatology, Charité - University Medicine Berlin

Kühl A.A., Research Center ImmunoScience (RCIS)

Salama A. Institute of Transfusion Medicine, Charité - University Medicine Berlin

Burmester G.R. Dept. of Medicine, Rheumatology and Clinical Immunology, Charité - University Medicine Berlin

PUBLICATIONS

Daridon C., Loddenkemper C., Spieckermann S., Kühl A.A., Salama A., Burmester G.R., Lipsky P.E., Dörner T. Splenic proliferative lymphoid nodules distinct from germinal centers are sites of autoantigen stimulation in immune thrombocytopenia. Blood 2012

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FUNDING

SFB650/TP16

DFG Do 491/7-1

Enric Esplugues

Neuroimmunology

Understanding Neuroimmunological disorders: the crosstalk between the Immune and the Central Nervous System

KEYWORDS

TH17 cells, autoimmunity, tolerance, citokines, chemokines.

GROUP MEMBERS

Group leader:
Enric Esplugues, PhD

Scientist:
Caterina Curato, PhD

Ph.D. students:
Daniel Cirera and Anna Pascual

Inflammation is a very important component of the host response to infections and tumors. However, excessive inflammation may lead to a variety of pathological states, including different autoimmune disorders.

The main interest of our lab is focused on understanding the cellular and molecular basis that lead to the induction, development and resolution of inflammatory disorders that are caused by the dysfunctions of the innate and/or the adaptive immune system.

In particular, we are interested in the study of the TH17 cells, a recently identified CD4+ T cell subset distinct from T helper type 1 (TH1) and T helper type 2 (TH2) cells. TH17 cells can drive antigen specific autoimmune diseases and are considered the main population of pathogenic T cells driving experimental autoimmune encephalomyelitis (EAE), the mouse model for multiple sclerosis. The factors that are needed for the generation of TH17 cells have been well characterized. However, where and how the immune system controls TH17 cells *in vivo* is one of our major interests.

To address these questions, our laboratory employs a wide range of experimental techniques to get insight from epigenetics (FISH, 3C, ChIP, EMSA, DHA, genome wide gene expression analyses…) to the whole animal level (genetic approaches including conditional targeting and generation of different reporter mice).

COOPERATION PARTNERS

Prof. Carlos Fernandez-Hernando, Division of Cardiology, (NYU), USA

Prof. Richard Flavell, Immunobiology, Yale University, New Haven, USA

Prof. Anja Hauser, Immunodynamics, DRFZ, Berlin

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Control of TH17 cells occurs in the Small Intestine. Esplugues E*#, Huber S*, Gagliani N, Hauser AE, Town T, Wan YY, O'Connor Jr. W, Rongvaux A, Van Rooijen N, Haberman AM, Iwakura Y, Kuchroo VK, Kolls JK, Bluestone JA, Herold KC, Flavell RA#. Nature. 2011 Jul 17; 475(7357):514-8. (*co-first authors, #co-directed the project)
F1000 Article Factor: 18

miR-33a/b contribute to the regulation of fatty acid metabolism and insulin signaling. Dávalos A, Goedeke L, Smibert P, Ramírez CM, Warrier NP, Andreo U, Cirera-Salinas D, Rayner K, Suresh U, Pastor-Pareja JC, Esplugues E, Fisher EA, Penalva LO, Moore KJ, Suárez Y, Lai EC, Fernández-Hernando C. Proc Natl Acad Sci U S A. 2011 May 31;108(22):9232-7

TH17 Cells Express Interleukin-10 Receptor and Are Controlled by Foxp3(-) and Foxp3(+) Regulatory CD4(+) T Cells in an Interleukin-10-Dependent Manner. Huber S*, Gagliani N*, Esplugues E*,O'Connor W Jr, Huber FJ, Chaudhry A, Kamanaka M, Kobayashi Y, Booth CJ, Rudensky AY, Roncarolo MG, Battaglia M, Flavell RA. Immunity. 2011 Apr 22;34(4):554-65. (*co-first authors)
F1000 Article Factor: 8

Control of TH17 cells *in vivo*

SCIENTISTS

A. Pascual, D. Cirera and C. Curato

COOPERATION PARTNERS

Prof. Richard Flavell, Immunobiology, Yale University, New Haven, USA

Prof. Anja Hauser, Immunodynamics, DRFZ, Berlin

REFERENCES

Esplugues E*#, Huber S*, Gagliani N, Hauser AE, Town T, Wan YY, O'Connor Jr. W, Rongvaux A, Van Rooijen N, Haberman AM, Iwakura Y, Kuchroo VK, Kolls JK, Bluestone JA, Herold KC, Flavell RA#. Nature. 2011 Jul 17; 475(7357):514-8. (*co-first authors, #co-directed the project)

TH17 Cells Express Interleukin-10 Receptor and Are Controlled by Foxp3(-) and Foxp3(+) Regulatory CD4(+) T Cells in an Interleukin-10-Dependent Manner. Huber S*, Gagliani N*, Esplugues E*,O'Connor W Jr, Huber FJ, Chaudhry A, Kamanaka M, Kobayashi Y, Booth CJ, Rudensky AY, Roncarolo MG, Battaglia M, Flavell RA. Immunity. 2011 Apr 22;34(4):554-65. (*co-first authors)

Interleukin (IL)-17-producing T helper cells (TH17) are a recently identified CD4+ T cell subset distinct from T helper type 1 (TH1) and T helper type 2 (TH2) cells. TH17 cells can drive antigen specific autoimmune diseases and are considered the main population of pathogenic T cells driving experimental autoimmune encephalomyelitis (EAE), the mouse model for multiple sclerosis. The factors that are needed for the generation of TH17 cells have been well characterized. However, where and how the immune system controls TH17 cells *in vivo* remains unclear.

Here, by using a model of tolerance induced by CD3-specific antibody, a model of sepsis and influenza A viral infection (H1N1), we show that pro-inflammatory TH17 cells can be redirected to and controlled in the small intestine. TH17-specific IL-17A secretion induced expression of the chemokine CCL20 in the small intestine, facilitating the migration of these cells specifically to the small intestine via the CCR6/CCL20 axis. Moreover, we found that TH17 cells are controlled by two different mechanisms in the small intestine: first, they are eliminated via the intestinal lumen and simultaneously pro-inflammatory TH17 cells acquire a regulatory phenotype with *in vitro* and *in vivo* immune-suppressive properties (rTH17). These results identify mechanisms limiting TH17 cell pathogenicity and implicate the gastrointestinal tract as a site for control of TH17 cells.

After polyclonal T cell activation via TCR, apoptotic T cells will be engulfed by phagocytes. IL-6 and TGF-b are going to be produced by APCs and consequently IL-17A+CCR6+CD4+ T cells (TH17) are generated in the periphery. TH17-mediated production of IL17 will induce upregulation of CCL20 in the small intestine attracting and trapping the pro-inflammatory TH17 cells. In the Small Intestine, part of the TH17 cells are going to be eliminated in the lumen and others will be reprogrammed in rTH17 cells with immuno-suppressor functions.

Perspectives:

A critical goal of current research is to understand the mechanisms that lead to immune-tolerance and hence alleviate symptoms on a long term basis in patients with autoimmune disorders like Rheumatoid Arthritis. TH17 cells are strongly implicated in the pathogenesis of numerous autoimmune diseases. The factors that are needed for the generation and expansion of TH17 cells have been well characterized. However, mechanisms controlling TH17 cells *in vivo* remain unclear.

Recently we have reported different cellular mechanisms controlling the fate of the TH17 cells *in vivo* (Esplugues et al. Nature. 2011) and our work will have a great impact in the design of new therapeutical approaches in many different immune related pathologies like Multiple Sclerosis or Rheumatoid Arthritis.

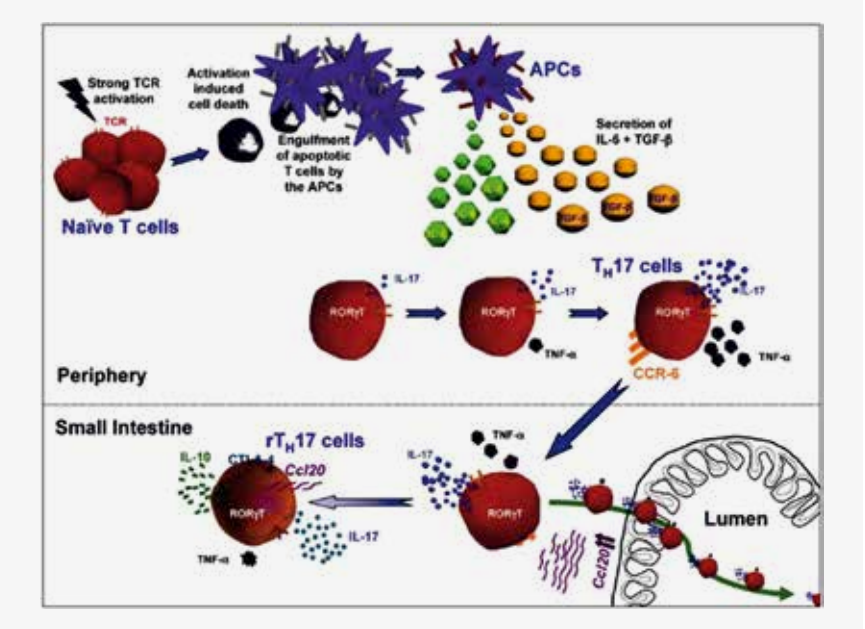

Figure 1: Fate of TH17 cells during tolerance induction after a polyclonal T cell activation *in vivo*.

PUBLICATIONS

Esplugues E*#, Huber S*, Gagliani N, Hauser AE, Town T, Wan YY, O'Connor Jr. W, Rongvaux A, Van Rooijen N, Haberman AM, Iwakura Y, Kuchroo VK, Kolls JK, Bluestone JA, Herold KC, Flavell RA#. Nature. 2011 Jul 17; 475(7357):514-8. (*co-first authors, #co-directed the project)

TH17 Cells Express Interleukin-10 Receptor and Are Controlled by Foxp3(-) and Foxp3(+) Regulatory CD4(+) T Cells in an Interleukin-10-Dependent Manner. Huber S*, Gagliani N*, Esplugues E*,O'Connor W Jr, Huber FJ, Chaudhry A, Kamanaka M, Kobayashi Y, Booth CJ, Rudensky AY, Roncarolo MG, Battaglia M, Flavell RA. Immunity. 2011 Apr 22;34(4):554-65. (*co-first authors)

FUNDING

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Photo: K.Zimmermann

Falk Hiepe

Charité - University Medicine Berlin (CCM)
Department of Rheumatology and Clinical Immunology

Autoimmunology

Development of new strategies for better treatments of autoimmune diseases

KEYWORDS

Memory plasma cells, Autoantibodies, Systemic autoimmune diseases, Cellular therapies, Autologous hematopoietic stem cell transplantation

GROUP MEMBERS

Group leader:
Univ.-Prof. Dr. med. Falk Hiepe

Scientists:
Dr. med. Tobias Alexander, Dr. med. Robert Biesen, Dr. med. Anne Bruns, Dr. rer. nat. Velia Gerl, Dr. med. Bimba F. Hoyer, Dr. med. Sandra Schneider, Adriano Taddeo

Ph.D. students:
Qingyu Cheng, Theresa Fischer, Julia-Carmen von Glischinski, Nadja Haasler, Laleh Khodadadi, Katrin Kozeck, Franziska Rauhut, Thomas Rose, Veronika Scholz, Lars Templin, Aderajew Waka,

Secretary:
Sabine Weber

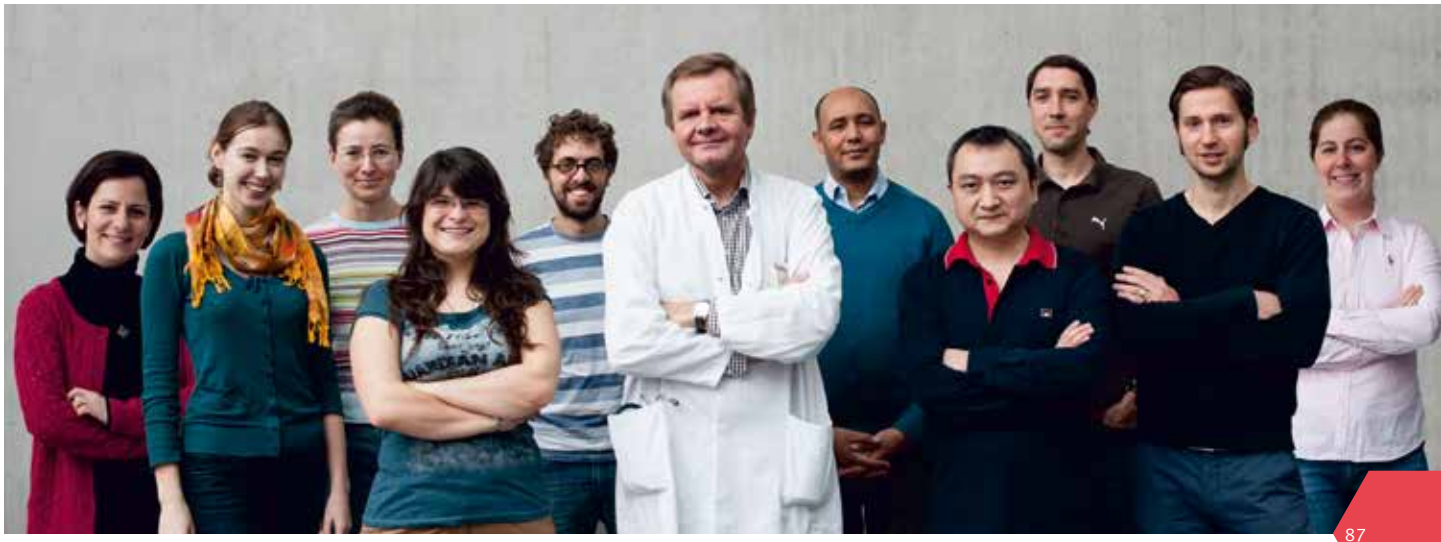
Our research group is fundamentally involved in analyzing the mechanisms, which contribute to and help maintain severe autoimmune diseases in order to develop new therapeutic concepts in the long term.

The main focus is on the role of long-lived autoreactive plasma cells in autoimmune diseases. Long-lived plasma cells reside in niches in the bone marrow and inflamed tissues where they are resistant to immunosuppressive/cytotoxic drugs or therapies targeting of B cells. In collaboration with the research group from Andreas Radbruch, we look for new therapeutic strategies targeting the autoreactive memory.

We demonstrated together with the Unit for Bone Marrow Transplantation (Renate Arnold) at the Charité – Universitätsmedizin Berlin and the research group of Andreas Thiel (BCRT) that the autoreactive memory could be eliminated by immunoablation followed by autologous hematopoietic stem cell transplantation done in patients with severe autoimmune diseases refractory to conventional immunosuppression. This may provide the fundament for the subsequent regeneration of a normal immune system. In some patients, however, the disease relapsed or secondary autoimmune disorders occurred. We investigate the reasons for this in a controlled clinical trial in SLE. In preclinical models we want to optimize the immunoablative protocol with regard to stability of tolerance and safety.

In another project we study the role of dendritic cells in SLE. These cells, in their function as antigen-presenting cells and producers of cytokines, play a significant role in the pathogenesis of SLE. They represent a possible target in the development of new therapies, which is why a precise characterization of these cells is so important.

Several biologics targeting different cytokines or cells (e.g. BAFF/BLys, APRIL, type I interferon, IL-10, B cells, PDC, co-stimulatory molecules), which are involved in the pathogenesis of SLE and other systemic autoimmune diseases have studied or will be tested in clinical trials. These different therapeutic approaches will bring us a big step forward to achieve the ambitious aim of a personalized therapy. For this, adequate biomarkers that identify the optimal therapeutic target and/or characterize disease activity are needed. We have identified several serologic and cellular biomarkers (autoantibodies, Siglec1 expression on monocytes, B and T cell subpopulations). Their relevance for the aforementioned aims is studied in the clinic.



COOPERATION PARTNERS

Univ.-Prof. Dr. med. Renate Arnold, Charité - Universitätsmedizin Berlin, CVK, Med. Klinik m. S. Hämatologie, Onkologie und Tumorimmunologie, Berlin, Germany

Prof. Dr. Klemens Budde und Dr. Michael Dürr, Charité - Universitätsmedizin Berlin, CCM, Medizinische Klinik m.S. Nephrologie, Berlin

Prof. Dr. Richard Burt, Northwestern University, Department of Medicine, Division of Immunotherapy, Chicago, USA

Univ.-Prof. Dr. med. Thomas Dörner, Charité - Universitätsmedizin Berlin, CCM, Medizinische Klinik m.S. Rheumatologie und Klin. Immunologie, Berlin

Dr. rer. nat. Henry Hess, Immunology & Pharmacology Research, Merck KGaA, Darmstadt, Germany

Univ.-Prof. Dr. Peter M. Kloetzel, Charité - Universitätsmedizin Berlin, Institut für Biochemie, Berlin

Prof. Dr. Igor Lisukov, ISt. Petersburg State Medical Pavlov University, Ratsa Gorbacheva Memorial Children`s Institute, Department of Hematology and Transplantology, St. Petersburg, Russia

Univ.-Prof. Dr. Rudolf Manz, Universität zu Lübeck, Institut für Systemische Entzündungsforschung ISEF, Lübeck, Germany

Prof. Dr. med. Thomas Porstmann, Seramun Diagnostica GmbH, Heidesee, Germany

Prof. Dr. Michael Reth, Max-Planck-Institut für Immunbiologie, Freiburg. Germany

Dr. Jürgen Schmitz, Dr. Andrzej Dzionek, Dr. Barbara Behle, Miltenyi Biotech, Bergisch Gladbach, Germany

Dr. Wolfgang Schlumberger, EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany

David Tarlinton, Walter und Eliza-Hall-Institut für Medizinische Forschung, Melbourne, Australia

Univ.-Prof. Dr. med. Reinhard E. Voll, Universitätsklinikum Freiburg, Med. Klinik, Abteilung Rheumatologie und Klinische Immunologie, Freiburg, Germany

Dr. rer. nat. Hedda Wardemann, Max-Planck-Institut für Infektionsbiologie, Berlin, Germany

Univ.-Prof. Dr. med. Margitta Worm, Charité - Universitätsmedizin Berlin, CCM, Klinik für Dermatologie, Venerologie und Allergologie, Berlin

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Helios-expressing Foxp3+ Regulatory T Cells are expanded in active systemic Lupus erythematosus

SCIENTISTS

L. Templin, G. Burmester, A. Radbruch, F. Hiepe

COOPERATION PARTNERS

A. Sattler, C. Groß, B. Sawitzki, S. Kohler, A. Meisel, R. Arnold, A. Thiel, Charité – Universitätsmedizin Berlin

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PUBLICATIONS

1. Alexander T, Sattler A, Templin L et al. Foxp3+ Helios+ regulatory T cells are expanded in active systemic lupus erythematosus. Ann Rheum Dis 2012; Dec 21.

FUNDING

This project was in part funded by SFB650 TP12.

Previous data demonstrated increased frequencies of peripheral blood Foxp3+ T cells in patients with systemic lupus erythematosus (SLE). However, it was unclear whether such cells resembled true regulatory T (Tregs) cells or activated, conventional T (Tconv) cells, as Foxp3 can be expressed by both cell types. By using expression analysis of Helios, a transcription factor that was recently identified as a marker for natural Tregs, we were able to demonstrate that the vast majority of circulating Foxp3+ T cells in SLE was indeed Helios+. Although Helios-expressing Tregs were highly proliferative in active SLE, as indicated by Ki-67 expression, and predominantly confined to a CD45RA- CD31- Foxp3low memory phenotype, such cells were highly demethylated at the Foxp3 locus (TSDR) and essentially produced no effector cytokines. These data indicate a proliferation of Tregs with functional capacity in active SLE, presumably to compensate for autoreactive effector responses.

Helios-expressing Foxp3+ Tregs are expanded in active SLE.

Frequencies of Foxp3+ Helios+ Tregs, unlike Foxp3+ Helios- T cells, were significantly increased in SLE patients compared to HD ($p<0.001$) and positively correlated with disease activity ($R2=0.75$) (Fig. 1A). Phenotypically, Helios+ Tregs in SLE showed decreased levels for CD45RA and CD31 ($p<0.001$) and increased levels for Ki-67 ($p=0.017$) and basal phosphorylated STAT5 ($p=0.007$) suggesting a peripheral Treg expansion in response to γ-chain signaling cytokines. In addition, Helios+ Tregs in SLE displayed similar chemokine receptor expression for CXCR3 and CCR4 compared to HD, indicating that such cells possess migratory potential to inflamed tissue and skin, respectively.

Helios-expressing Tregs in SLE lack effector cytokine expression and possess a highly demethylated TSDR.

When enriched CD4+ T cells were stimulated with PMA/Iono for 5 h and cytokine production by Foxp3+ T cells was analysed by intracellular staining, considerable numbers of IFN-γ and IL-2 secreting cells were detected; however, these cells were strictly confined to the Helios- T cell population in both HD and SLE patients while Helios+ Tregs essentially secreted no cytokines (Fig 1B). Next, the methylation status of the Treg specific demethylation region (TSDR) of the Foxp3 gene was investigated. Here, Helios+ Tregs in SLE showed a highly demethylated TSDR similar to that found in HD (Fig 1C), suggesting that such Tregs possess functional suppressive properties.

PERSPECTIVES:

Helios-expressing Foxp3+ Tregs are peripherally expanded in active SLE seem to possess functional suppressive capacity and migratory potential into inflamed tissue. Our data indicate that Helios+ Tregs in SLE are not enriched for effector T cells but rather actively involved in controlling chronic autoimmune responses. Nevertheless, although expanded *in vivo*, lupus Tregs may not fully compensate for the ensuing autoreactive effector responses. Based on these findings, Foxp3+ Helios+ Tregs may serve as a source for Treg-based interventions in future therapeutic approaches and may be utilizable as a biomarker for disease activity in SLE.

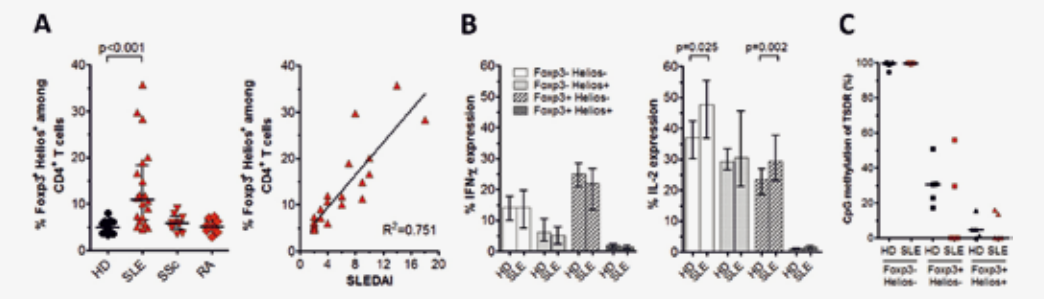

Figure 1: A) Expression analysis of Foxp3 and Helios in CD4+ T cells in healthy donors (HD, n=20), patients with SLE (n=20), systemic sclerosis (SSc, n=10) and rheumatoid arthritis (RA, n=10) (median/interquartil range values), and correlation between Helios+ Treg levels in SLE with their disease activity based on systemic lupus erythematosus disease activity index (SLEDAI). B) Purified CD4+ T cells from healthy donors and SLE patients were intracellularly analysed for production of IFN-γ and IL-2 after stimulation with PMA/ionomycin for 5h in the presence of Brefeldin A for 4h. C) TSDR methylation was performed in FACS-sorted Helios/Foxp3 T cell subsets from 5 healthy donors (HD) and 5 active SLE patients with. Median values are shown.

Blockade of CXCR4-CXCL12 interaction reduces homing and survival of plasma cells in NZB/W mice

SCIENTISTS

Qingyu Cheng, Laleh Khodadadi, Adriano Taddeo, Oliver Winter, Bimba Hoyer, Andreas Radbruch and Falk Hiepe

Previously, we demonstrated that long-lived plasma cells resistant to immunosuppressive/cytotoxic drugs and therapies targeting B cells contribute to the pathogenesis of antibody-mediated diseases and should therefore be considered as a promising therapeutic target. In bone marrow (BM), the plasma cells reside in survival niches consisting of several cells, cytokines and adhesion molecules. The niches are organized by stromal cells expressing the chemokine CXCL12, which is the ligand of CXCR4 expressed on plasma cells. In this study we investigated the contribution of CXCL12-CXCR4 interaction to the longevity of plasma cells in the murine model of lupus.

Material and methods

Plasmablasts and plasma cells purified from spleens of NZB/W mice were incubated with the CXCR4 blocker AMD3100 (500µg/ml) for 30 minutes and then adoptively transferred to immunodeficient Rag1-/- mice. After 14 days we analyzed the number of plasma cells in BM. Furthermore, OVA immunized NZB/W mice were treated with AMD3100 (20mg/kg, i.p.) 7 times every other day after boost; anti-OVA secreting plasma cells in BM were checked on day 3 and 15 after boost. The effect of plasma cell depletion was investigated in 3-4 months old NZB/W mice using AMD3100 (5mg/kg s.c. three times a week) alone or combined with bortebomib (0.5mg/kg i.p. twice a week) for two weeks.

Results

Two weeks after adoptive transfer the number of plasma cells in Rag1$^{-/-}$ mice treated with AMD3100 was lowered by 60% in BM compared to a control group. After secondary immunization with OVA the AMD3100 treatment resulted in a significant reduction of anti-OVA secreting plasma cells in BM by 33% on day 3 and by 23% on day 15. After 15 days the number of MHC class II negative anti-OVA secreting plasma cells significantly decreased by 42% in BM of treated mice comparing with control mice. In comparison with untreated NZB/W mice, AMD3100 efficiently depleted plasma cells including long-lived plasma cells. After two weeks of treatment, total plasma cell number was decreased by 69% in spleen and by 61% in BM; long-lived plasma cells were reduced by 67% in spleen and by 64% in BM. The combination of bortezomib with AMD3100 in NZB/W significantly enhanced the depletion of long-lived plasma cells compared to monotherapy with bortezomib or AMD3100 (Fig. 1).

Conclusions

CXCR4 blockade with AMD3100 can reduce the homing of plasma cells to the BM and the survival of long-lived plasma cells. The combination of bortezomib with AMD3100 shows synergistic effects on plasma cell depletion. The findings highlight the importance of the CXCR4-CXCL12 axis for the plasma cell niche.

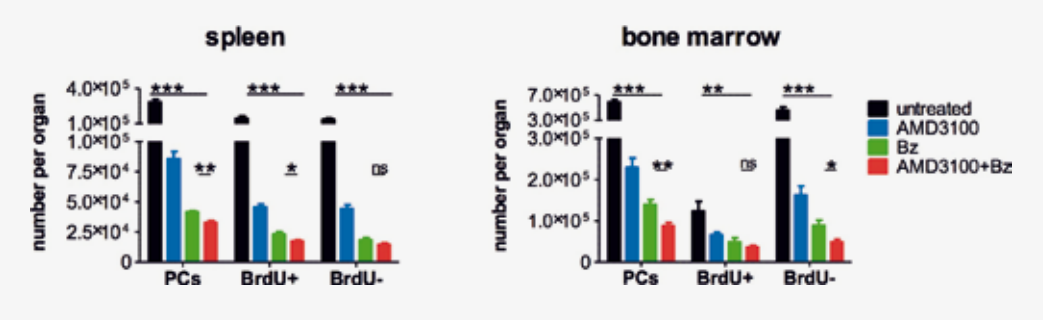

Figure 1: AMD3100 efficiently depleted plasma cells including long-lived plasma cells (BrdU-) in NZB/W mice, the combination of bortezomib with AMD3100 significantly enhanced the depletion.

SCIENTISTS

Gerl V., Fischer T., Winter O., Taddeo A., Alexander T., Biesen R., Hiepe F.

Department of Rheumatology and Clinical Immunology, Charité University Hospital, Berlin, Germany

and

German Rheumatism Research Centre, Berlin, Germany

Siglec-1$^+$/CD11c$^+$ plasmacytoid dendritic cells are promoted in peripheral blood in SLE.

Plasmacytoid dendritic cells (pDC) are potent producers of IFN-alpha. IFN-alpha serum levels correlate with disease activity in SLE and IFN-alpha is suggested to promote autoimmune responses. Thus, pDCs are considered a crucial element in SLE pathogenesis. However, only few studies focus on the development of distinct DC subsets and their lineage-relationship in the complex microenvironment of autoimmune-inflammation. It is believed that pDCs could differentiate into myeloid DCs in an IFN-alpha dependent manner. In SLE, where low expression of BDCA-2 is commonly seen, this differentiation could be relevant and point to such a lineage switch as well as to an activated state of pDCs.

Aim

To study the impact of autoimmune inflammation on pDC differentiation/distribution in SLE sub-characterizing blood pDCs with specific pDC and myeloid markers with particular attention to pDC differentiation in the context of SLE.

Methods

Multi-color-flowcytometric analyses were performed on whole blood of healthy donors and SLE patients. pDCs were identified by CD3$^-$/CD19$^-$/CD14$^-$/CD-123^{high}/HLA-DR$^+$/BDCA-2$^+$ expression and characterized for CD11c and Siglec-1. In parallel, pDCs were analyzed by confocal microscopy.

Results

We identified a small subpopulation of pDCs, which reveal a "myeloid-like" phenotype co-expressing Siglec-1 and CD11c (fig. 1, 2) in healthy donors and SLE. The percentage of Siglec-1$^+$ pDCs correlates with disease activity in SLE (fig. 3).

Conclusion

The SLE specific environment may favor the appearance of Siglec-1$^+$ pDCs. Their functional role in SLE pathogenesis is currently under investigation.

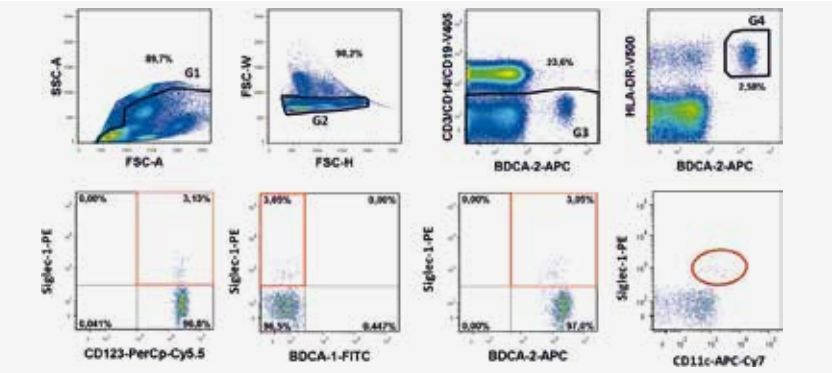

Figure 1: Identification of Siglec-1 expressing pDCs (red gates) by multicolour flow cytometry. Cell were first gated for PBMCs (G1) and doublet discrimination (G2). Siglec-1$^+$ PDCs were found inbetween classically characterized pDCs with lineage- (G3), HLA-DR$^+$/BDCA-2+ (G4) with CD123^{++} phenotype. They are negative for mDC marker BDCA-1 but positive for the classical myeloid DC marker CD11c.

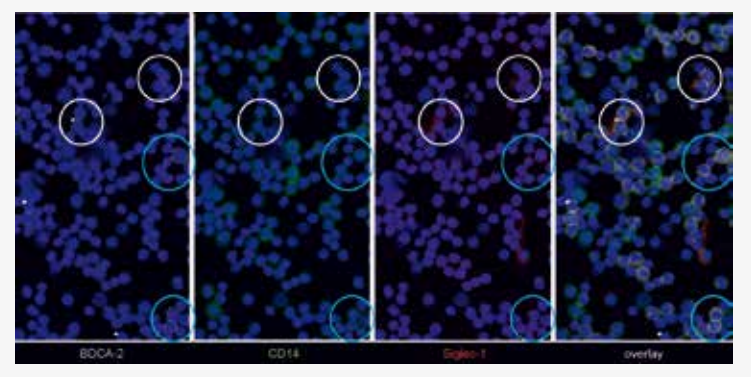

Figure 2: Siglec-1$^+$/BDCA-2$^+$ (white circles) and Siglec-1$^-$/BDCA-2$^+$ (blue circles) pDCs can be found in the peripheral blood of healthy donors.

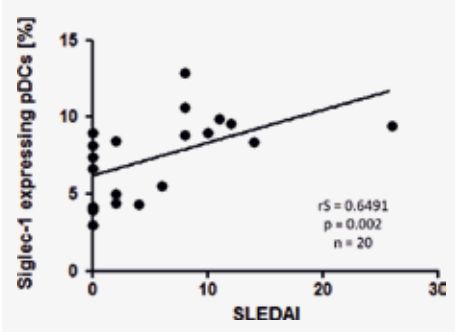

Figure 3: The percentage of Siglec-1 expressing pDCs correlates with disease activity in SLE. Spearman regression analysis.

Plasma cells as a biomarker for disease activity in patients with granulomatosis with polyangiitis (GPA, Wegener Granulomatosis)

B cells are thought to play an important role in GPA due to the presence of the autoantibodies (ANCA) [1] as well as the success of B cell depletion therapy [2]. Therefore, we analyzed the B cell subsets in the peripheral blood of patients with GPA who were characterized regarding disease activity and ANCA level.

Material and methods

17 patients with GPA (10 with active disease, 7 with inactive disease) were analyzed by flow cytometry as compared to 17 healthy donors. Disease activity measured by the BVAS (Birmingham vasculitis activity score). Staining for CD27, CD20, CD19, MHC class II, CD3, 4 and 8 was analyzed using flowjo software. Statistical analysis was performed using GraphPad Prism; p-values of <0,05 were considered as significant. The ethics committee of the Charité approved the study; all patients had signed informed consent.

Results

Significant differences could be observed in plasma cell counts as well as frequencies in GPA patients (6.4 ±5.0/µl) with a BVAS score >0 as compared to those with a BVAS score =0 (2.5± 1.6/µl) or healthy persons (2.3±1.15/µl). Plasma cell numbers as well as the frequency of plasma cells within all B cells correlated with the BVAS (r= 0.91, p<0.0001) as well as the ANCA level in the serum (r= 0,83, p=0,0013). No significant differences could be observed for naive and memory B cells or the overall B cell numbers as compared to healthy donors. Regarding T cells, there was a significant reduction of CD3 (p=0,01) and CD4 T (p=0,012) cells in patients with active GPA as compared to patients in remission, whereas CD8 T cells did not show any significant changes.

Conclusion

The increased number of circulating plasmablasts in active GPA suggests a disturbance of B cell homeostasis in pathogenesis. They may serve as a biomarker of disease activity and support therapeutic strategies targeting B cells and plasma cells.

SCIENTISTS

Bimba F. Hoyer[1,2]
Adriano Taddeo[2]
Laleh Khodadadi[2]
Qingyu Cheng[2]
Maika Rothkegel[2]
Gerd-Rüdiger Bumester[1]
Andreas Radbruch[2]
Falk Hiepe[1,2]

[1] Med. Klinik m.S. Rheumatologie und Klinische Immunologie, Charité - Universitätsmedizin Berlin

[2] Deutsches Rheumaforschungszentrum Berlin

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Targeting of long-lived plasma cells and their precursors is required to achieve persistent depletion of the autoreactive plasma cell memory.

Long-lived plasma cells (LLPC) have been identified as an important therapeutic target in systemic lupus erythematosus (SLE) which is refractory to conventional therapies. Indeed, the therapeutic depletion of LLPC using the proteasome inhibitor bortezomib leads to significant benefits in NZB/W lupus prone mice. However, it is assumed that after discontinuation of bortezomib-administration pathogenic PC can rapidly regenerate due to constant B cell hyperreactivity contributing to the maintenance of pathogenic memory. Therefore in this study we tested a new treatment approach aimed at depleting LLPC and, at the same time, preventing the generation of new autoreactive LLPC that results from B cell-hyperreactivity. We combined bortezomib with an anti-CD20 antibody or with the immunosuppressive drug cyclophosphamide (CY) and analysed the LLPC-B cells dynamics in bone marrow (BM) and spleen of NZB/W mice. Our data show that the bortezomib-monotherapy leads only to transient depletion of plasma cells that reappear after discontinuation of bortezomib due to persisting B cell hyperactivity (Fig. 1, black line). Bortezomib combined with anti-CD20 therapy was not efficient in suppressing the continuous supply of newly generated autoreactive PC although it could partially target the short-lived PC compartment (red line). Conversely, the continuous application of the anti-proliferative drug cyclophosphamide after PC depletion with bortezomib was able to keep the number of PC including autoreactive PC low (blue line).

Our data shed new light on the dynamics between B cells and PC in autoimmunity and it could show, for the first time, that strategies for depleting LLPC have to have two components a) initial depletion of LLPC and b) continuous prevention of regeneration of autoreactive PC trough the targeting of B cell differentiation.

SCIENTISTS

Taddeo Adriano, Hoyer F. Bimba, Alexander Tobias, Khodadadi Laleh, Cheng Qingyu, Winter Oliver,Chang Hyun-Dong, Radbruch Andreas, Hiepe Falk.

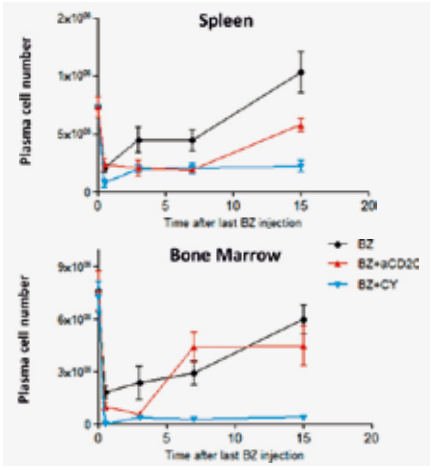

Figure 1: 12-16 weeks old NZB/W mice were treated with two bortezomib injections within 36h, alone (black line) or in combination with anti-CD20 once per week (red line) or cyclophosphamide every fourth day (blue line). The spleen (left) and the BM (right) of the mice were analyzed 12h, 3, 7 and 15 days after the last bortezomib injection by FACS to calculate the absolute numbers of plasma (mean and SE).

Andreas Hutloff

Robert Koch Institute, Berlin

Chronic Immune Reactions

How T and B cells talk to each other

KEYWORDS

T cell costimulation
ICOS
T follicular helper cells
T/B cooperation
Chronic inflammation

GROUP MEMBERS

Group leader:
Dr. rer. nat. Andreas Hutloff

Scientists:
Dr. rer. nat. Dana Vu Van

Ph.D. students:
Maysun S. Al Baz,
Franziska Fuhrmann,
Randi K. Franke,
Jan P. Weber

Our group is generally interested in the activation of T lymphocytes and their interaction with B cells. The main focus concentrates on the role of costimulatory receptors. These cell interaction molecules modulate T cell activation and play a central role for the regulation of the immune response. Absence of costimulation can result in severe immunodeficiency. On the other hand, overexpression of costimulatory receptors is often associated with exaggerated immune responses, resulting in autoimmunity or allergy. Therefore, costimulatory receptors are an attractive target for therapeutic intervention.

Using an *in vivo* T cell/B cell interaction model, which we have recently developed in our laboratory, we are testing how different costimulatory receptors influence distinct phases of the immune response. We are analyzing early events of T/B cooperation in the lymph node as well as T cell effector functions in inflamed tissues. We are especially interested in the subpopulation of T follicular helper cells (TFH), which have a crucial role for germinal center (GC) reactions. Without TFH cells B cells can not differentiate into long-lived plasma cells or memory B cells. This gatekeeper function for B cell differentiation is of central interest in the context of antibody-mediated autoimmune diseases. We are interested in factors determining early TFH differentiation, maintenance of their phenotype during the GC reaction, and their fate after the GC reaction has come to an end. We recently showed that TFH cells can indeed survive as long-term memory cells.

A major focus of our work is the inducible costimulator ICOS which was cloned and initially characterized in our group. Loss of ICOS results in a strongly diminished humoral immune response. This can be traced back to an almost complete absence of TFH cells in ICOS knock-out mice. We want to understand, how ICOS regulates the maintenance of the TFH phenotype on a molecular level.

COOPERATION PARTNERS

Dr. Katja C. Beier, Dr. Kerstin Gerhold, Department of Paediatric Pneumology and Immunology, Charité University Medicine Berlin, Germany

Prof. Dr. Joanna Cichy, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland

Prof. Dr. Marc Ehlers, Institute for Systemic Inflammation Research, University of Lübeck, Germany

Prof. Dr. Eckard Hamelmann, University Children´s Hospital, Ruhr University Bochum, Germany

Prof. Dr. Anja Hauser, Intravital Imaging and Immune Dynamics, Charité University Medicine Berlin, Germany

Dr. Mir-Farzin Mashreghi, Dr. Ria Baumgrass, Dr. Raluca Niesner, German Rheumatism Research Centre Berlin (DRFZ), Germany

Prof. Dr. Richard Kroczek, Dr. Hans-Werner Mages, Robert Koch Institute, Immunologic Defense Mechanisms, Berlin, Germany

Prof. Dr. Max Löhning, Department of Rheumatology and Clinical Immunology, Charité, Berlin, Germany

Dr. Geraldine Nouailles, Max Planck Institute for Infection Biology, Berlin, Germany

Dr. Anke Osterloh, Birte Kretschmar, Prof. Dr. Bernhard Fleischer, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany

SELECTED PUBLICATIONS

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T cell costimulators are critical regulators of the immune response

SCIENTISTS

M.S. Al Baz, F. Fuhrmann, R.K. Franke, A. Hutloff, D. Vu Van, J.P. Weber

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FUNDING

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Costimulatory receptors are important regulators of T cell activation and differentiation. Lack of costimulation can result in severe immunodeficiency whereas increased costimulation is often associated with autoimmune disease. Costimulatory pathways are therefore attractive targets for therapeutic intervention.

Results and discussion

Antigen-recognition via the T cell receptor alone is not sufficient for full T cell activation. Instead, additional costimulatory signals are required. They regulate the T cell response on different levels (Fig. 1) (1): 1) Initial activation. Already on first antigen contact costimulatory receptors on dendritic cells determine whether a T cell gets fully activated. This "2-signal" system of T cell activation helps to prevent unwanted immune responses against harmless environmental allergens or autoantigens. 2) T cell differentiation. In later phases of the immune response costimulatory signals are involved in the development of certain T cell subsets like T follicular helper cells (TFH) or tissue infiltrating effector cells. 3) T cell effector function. Costimulatory receptors regulate a wide range of effector functions like B cell help or cytokine secretion. 4) T cell memory. Costimulators can enhance long-term survival of memory T cells.

Beside CD28, one of the best characterized costimulators, several other molecules which are involved in the interaction of T cells with antigen-presenting cells have been identified (1). Structurally they all belong to the immunoglobulin superfamily (like CD28) or the TNF superfamily. They can transmit either (positive) costimulatory or coinhibitory signals into the T cell. The inducible costimulator ICOS was identified in 1999 as a molecule structurally and functionally related to CD28 (3, 4). In contrast to CD28, ICOS is not expressed constitutively but only on activated T cells. However, the ligand for ICOS (ICOS-L) has a broad expression range on almost all cells of the immune system with highest expression on B cells and dendritic cells. Under inflammatory conditions, even non-hematopoietic cells can upregulate ICOS-L, which might be important for immune reactions in inflamed tissues. The strong expression of ICOS on TFH cells early indicated an important role of ICOS for T cell-dependent B cell responses (4).

ICOS is a nice example to illustrate the importance of costimulators for the balance of the immune response. Loss of ICOS results in severe immunodeficiency. Human ICOS-deficiency patients present with the clinical picture of Common Variable Immunodeficiency (CVID). They have strongly reduced total B cell numbers and almost no memory B cells. Therefore, they are highly susceptible to infectious diseases (5). The phenotype of ICOS knock-out mice is quite similar. Their germinal center reaction against a specific antigen is strongly impaired resulting in a reduced production of antigen-specific immunoglobulins. On the other hand, overexpression of ICOS is associated with autoimmunity. Systemic lupus erythematosus patients have an increased frequency of ICOS-expressing T cells in the peripheral blood, which additionally infiltrate diseased organs (6). In a mouse model we recently showed that ICOS is a critical switch for the decision between immunity and tolerance (7).

Due to their important role for the fine regulation of the immune response, costimulatory molecules are an attractive target for therapeutic intervention (2). A soluble CTLA-4 chimera (Abatacept, Belatacept) which effectively blocks the CD28 pathway is already in clinical practice for the treatment of rheumatoid arthritis. However, due to the important role of CD28 for the initial activation of naive T cells, blockade of this pathway results in a general immunosuppression. Therefore, blockade of the ICOS pathway is an attractive option since ICOS is expressed on effector cells, only (3). A blocking monoclonal antibody against ICOS-L (AMG-557) is currently tested for the treatment of systemic lupus erythematosus (SLE). Another strategy is based on the high ICOS expression on chronically activated effector cells. Therefore, a depleting anti-ICOS antibody (MEDI-570) is also tested for the elimination of autoreactive T cells in SLE patients.

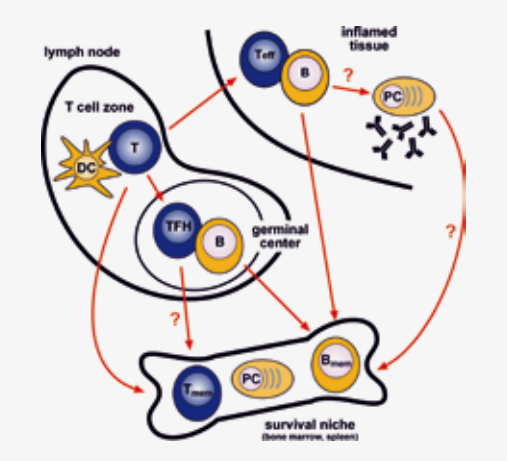

Figure 1: Costimulation in different sites and phases of the immune response.

Analysis of antigen-specific T and B cells *in vivo*

To analyze the interaction of T and B cells in vivo, we recently developed a mouse model, which allows tracking of antigen-specific T and B cells in all phases of the immune response. The system is based on the adoptive transfer of transgenic T and B cells into syngenic, immunocompetent recipients. Cell surface markers allow not only for detection of transferred cells in flow cytometry and histology, but also for re-isolation ex vivo without crosslinking of the antigen receptor. Using knock-out mice we are analyzing the role of ICOS for T follicular helper cells.

Results and discussion

The development of TFH cells can be divided in different phases (Fig. 1). Already very early the master transcription factor Bcl-6 is upregulated in some T cells which are in contact with dendritic cells in the T cell zone (day 2). Antigen-specific T cells then migrate to the T/B border and interfollicular regions where they get further signals for TFH differentiation in contact with antigen-specific B cells (day 3). A small fraction of T cells migrates deep into the B cell areas where they promote the development of GC B cells (day 8).

The analysis of immune reactions in vivo is hampered by the very low frequency of antigen-specific cells. This is most critical for early phases of the immune response. Adoptive transfer of antigen-specific T and B cells helps to elevate their frequency above the threshold for unambiguous detection. Our system is based on the cotransfer of ovalbumin- (OVA-) specific T cells from OT-2 mice and B cells from B1-8i mice which have a B cell receptor knock-in specific for the hapten nitrophenol (NP). After transfer, recipient mice are immunized with an NP-OVA conjugate. To analyze the role of specific molecules in T/B cooperation we have several possibilities to block their function: i) Knock-out mice crossed to the TCR or BCR transgenic mice ii) Blocking antibodies iii) shRNA-mediated knock-down or overexpression using a retroviral system.

As an example, Fig. 2 shows an experiment where wild-type T cells and ICOS knock-out T cells were co-transferred with B1-8i B cells into recipient mice. Due to the presence of wild-type T cells, B cells can normally differentiate into GC B cells (not shown). Therefore, this experiment shows that the defect of ICOS knock-out T cells to differentiate into TFH cells (characterized by co-expression of CXCR5 and PD-1) is T cell intrinsic.

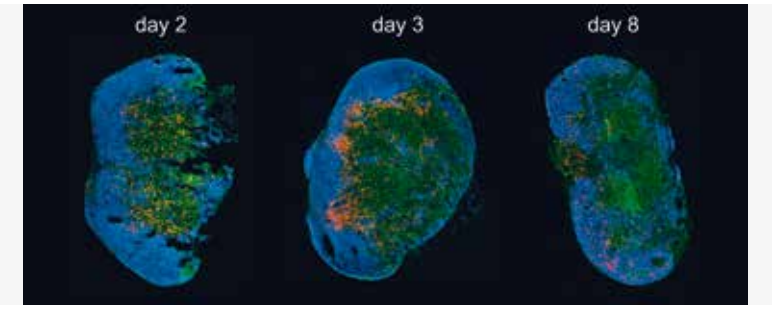

Figure 1: Histological analysis of antigen-specific T cells (red) in a draining lymph node. The B cell area is stained in blue, the T cell area in green.

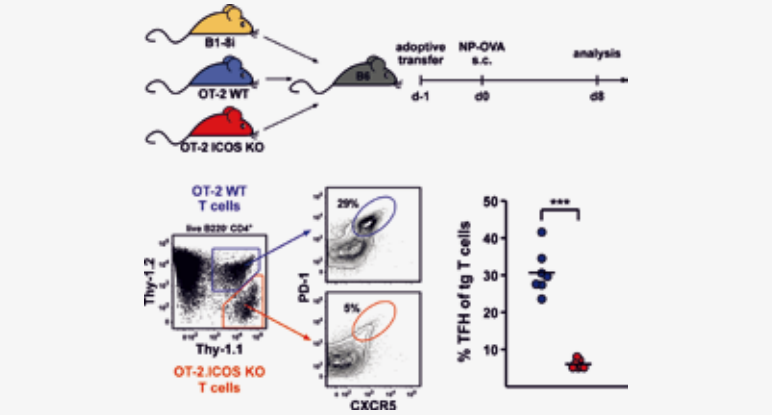

Figure 2: Role of ICOS for TFH differentiation.

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M.S. Al Baz, F. Fuhrmann, R.K. Franke, A. Hutloff, D. Vu Van, J.P. Weber

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FUNDING

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T follicular helper cells survive as long-term memory cells

SCIENTISTS

F. Fuhrmann, A. Hutloff, J.P. Weber

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TFH cells are now recognized as a separate T cell lineage. However, in contrast to other T cell subsets it has been controversial whether they represent short-lived effector cells or can also survive as long-term memory cells. To answer this question, we sorted antigen-specific TFH and non-TFH cells and transferred them into secondary hosts. With this approach we could show that TFH cells indeed can survive as memory cells. Upon re-encounter with the antigen they rapidly regain their effector functions although there seems to be some flexibility in the TFH program.

Results and discussion

The generation of memory T cells is an important function of the immune system and allows a very fast and effective response upon re-encounter with the same antigen. At the same time, auto-reactive memory cells are a severe problem in autoimmune patients. TFH cells represent the subpopulation of $CD4^+$ T cells which provides help for antigen-specific B cells in the germinal center response (1). Without TFH cells, neither memory B cells nor long-lived plasma cells can be generated. TFH cells are generated from naïve T cells during an immune response and are imprinted by their master transcription factor Bcl-6. It has been a long-standing question if TFH cells can persist in the body as long-term memory cells or if they die after the germinal center response has been terminated (2).

To follow the fate of TFH cells we sorted TCR-transgenic TFH and non-TFH effector cells from an ongoing germinal center response and transferred them into secondary hosts (Fig. 1). Upon transfer effector cells rapidly contracted with a small population of both TFH and non-TFH cells surviving as memory cells in peripheral lymphoid organs for at least 4 weeks in the absence of antigen (3). TFH cells strongly downregulated their signature genes Bcl-6, CXCR5, and PD-1 in the memory phase (Fig. 1). However, upon rechallenge with antigen they rapidly upregulated these markers again, and had a strongly enhanced potential to produce IL-4 and IL-21. A very high expression of CXCR5 and low expression of CCR7 equips re-activated TFH memory cells with a chemokine receptor profile favoring a quick migration to the B cell follicle. In contrast with non-TFH memory cells, re-activated TFH cells have a cytokine profile to efficiently support B cells in germinal center reactions once again (3).

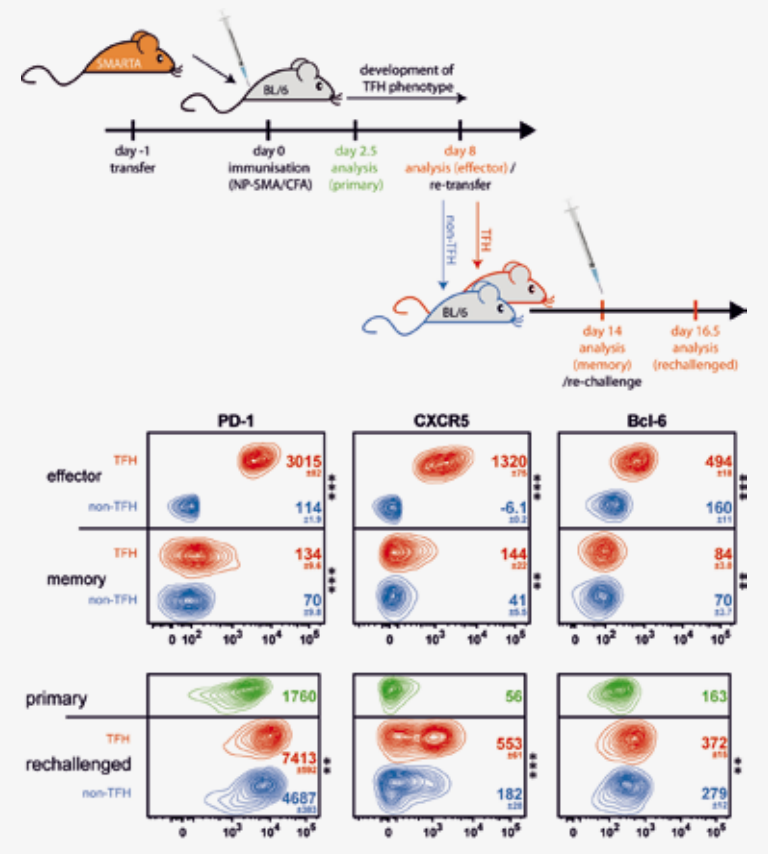

Figure 1: Analysis of TFH cells in different phases of the immune response.
Smarta-TCR transgenic T cells were transferred into C57BL/6 recipients which where subsequently immunized with the cognate antigen. At day 8 antigen-specific TFH and non-TFH cells were sorted and transferred into secondary hosts. Transgenic T cells were analyzed by flow cytometry for prototypic TFH markers at day 2.5 (primary response), day 8 (effector TFH cells), day 14 (memory TFH cells), and day 16.5 (2.5 days after rechallenge).

Immune reactions in inflamed tissues

Immune reactions in inflamed tissues are a complex reaction involving many different cell types. Beside cells of the innate immune system, infiltrating T and B cells are frequently found under these conditions. Compared to immune reactions in the lymph node, T/B interactions in inflamed tissue are still poorly understood. We are interested in the role of co-stimulatory molecules for T cell effector function and interaction with B cells. Another aspect is the function of B cells as antigen-presenting cells. Due to their high affinity antigen receptor B cells may gain an advantage over dendritic cells under conditions of chronic inflammation and limiting amounts of antigen.

Results and discussion

Most autoimmune diseases are characterized by massive lymphocytic infiltrates in tissues, e.g. the kidney in systemic lupus erythematosus patients (1). A better understanding of the pathophysiology of immune reactions in inflamed tissue will be helpful for the development of more specific treatment strategies.

As a general model for immune reactions in inflamed tissues we have adapted our in vivo T/B cooperation system to a mouse model of allergic airway inflammation (Fig. 1). In this model, antigen-specific T and B cells can be tracked simultaneously in the tracheobronchial lymph node and the inflamed lung tissue by flow cytometry and immunohistology. Cells are getting first activated in the lymph node. After repeated antigen challenge, large clusters of antigen-specific T and B cells can be observed in the lung tissue (Fig. 2). Interestingly, germinal center phenotype B cells can also be found in the lung tissue. At the same time, a large population of IL-21 producing effector T cells is present; however, they do not represent classical TFH cells.

ICOS is strongly expressed on antigen-specific T cells in lymph node and lung. We could show that ICOS/ICOS-L interaction influences the inflammation on several levels: i) ICOS costimulation regulates the pool size of effector T cells. ii) Thereby, also the expansion of antigen-specific B cells is influenced. iii) In the lymph node, ICOS has a special role for the maintenance of the TFH phenotype and thereby regulates the number of germinal center B cells. iv) In the lung, ICOS regulates the production of the effector cytokines IL-5 and IL-13. v) ICOS promotes the generation of IgA^+ plasma cells and memory B cells in the lung tissue.

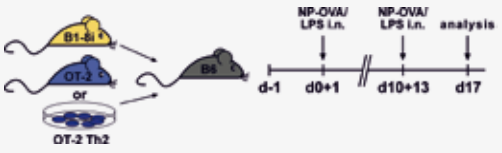

Figure 1: Mouse airway inflammation model.
A defined number of ovalbumin-specific T cells (from OT-2 mice, either naive or Th2-polarized) and nitrophenol-specific B cells (from B1-8i mice) are transferred into C57BL/6 recipient mice which are repeatedly challenged with an NP-OVA conjugate via the airways.

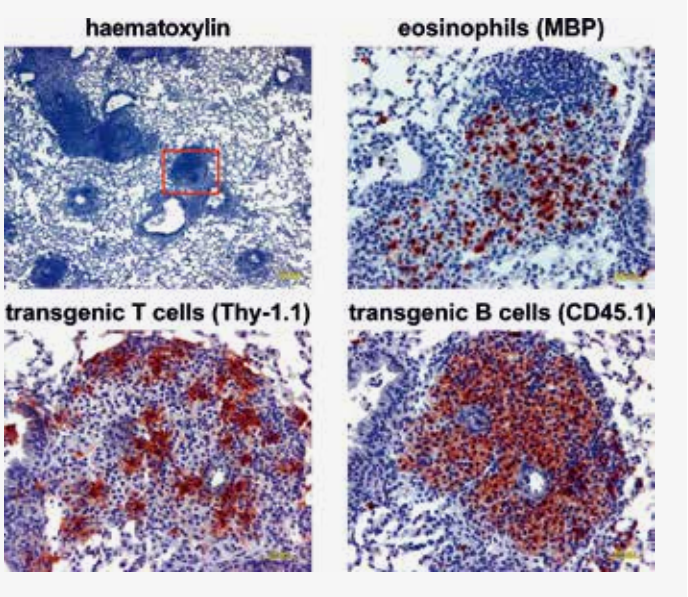

Figure 2: Infiltrates of antigen-specific T and B cells in the lung.

SCIENTISTS

M.S. Al Baz, A. Hutloff, D. Vu Van

COOPERATION PARTNERS

K.C. Beier, Charité University Medicine, Berlin; E. Hamelmann, Ruhr University, Bochum

PUBLICATIONS

1) Hutloff A, Büchner K, Reiter K, Baelde HJ, Odendahl M, Jacobi A, Dorner T, Kroczek RA: Involvement of inducible costimulator in the exaggerated memory B cell and plasma cell generation in systemic lupus erythematosus. Arthritis Rheum 2004;50:3211-3220.

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Max Löhning

Lichtenberg Professor of Experimental Immunology
Charité - University Medicine Berlin (CCM)
Department of Rheumatology and Clinical Immunology

Experimental Immunology

Balanced control of protective and pathogenic immunological memory in inflammation and infections

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Lymphocyte differentiation programs,
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GROUP MEMBERS

Group leader
Max Löhning, Prof. Dr. rer. nat.

Scientists (including their degrees)
Anja Fröhlich, Dr. sc. nat. ETH
Ahmed N. Hegazy, Dr. med. Dr. rer. nat.
Melba Muñoz, Dr. rer. nat.
Philippe Saikali, Ph.D.

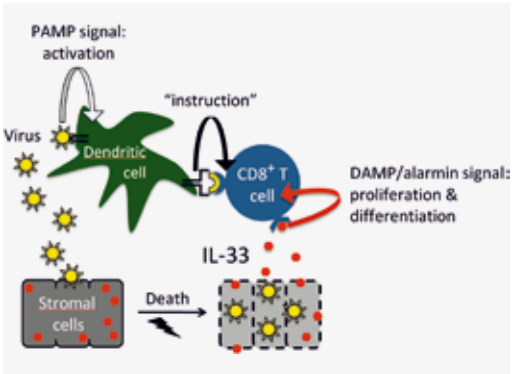
Ph.D. students
Claudia Baumann, MSc Biomed.
Caroline Helmstetter, Dipl.-Humanbiol.
Michael Peine, Dipl.-Biochem.
Elsa Pellet, MA Life Sciences
Julia Siede, MSc Mol. Med.
David Zimmel, Mag. rer. nat.

Technical assistance
Sabine Ebel, MTA, Vet.-Ing.
Vivien Holecska, BTA
Isabel Panse, BTA

Students
Ada Drobiazgiewicz

By virtue of faster and more vigorous antigen-specific immune responses, immunological memory can protect the organism from diseases of which it has recovered before. However, it also bears the risk to create immunopathology and autoimmunity. Immunological memory is maintained by long-lived antibody-secreting plasma cells and memory T and B cells that differentiate from naïve precursors upon antigen exposure. An in-depth understanding of immunological memory is crucial for the development of efficient vaccination strategies and for curative therapies of chronic immunopathology and autoimmunity, which are characterized by misdirected, pathogenic memory.

Key aim of this Lichtenberg group is a molecular understanding of the generation, maintenance and functional plasticity of immunological memory against pathogens and autoantigens. We study the cellular differentiation pathways and inductive signals for memory cell generation. In addition, we investigate the stability and flexibility of memory cells and their effector mechanisms as well as their functional capacity *in vivo* in the context of inflammation and viral or parasite infections. These studies are accompanied by the analysis of the molecular factors that regulate the longevity and functional quality of memory cells.



The alarmin IL-33 drives protective antiviral CD8⁺ T cell responses (Bonilla/Fröhlich et al., Science 2012, 335:984-9)

The concept of "pathogen-associated molecular patterns" ("PAMPs") as triggers of adaptive immunity is well established. In our recent work we provide first evidence that an endogenous "damage-associated molecular pattern" (DAMP) or "alarmin" is essential for orchestrating protective immune defense to viral infection. We show that interleukin-33 (IL-33), an alarmin released from necrotic cells, is necessary for potent cytotoxic CD8⁺ T cell (CTL) responses to replicating, prototypic RNA and DNA viruses in mice (Bonilla/Fröhlich et al., Science 2012). IL-33 signaled through its receptor ST2, which was found to be expressed on activated antiviral CTLs. IL-33 signaling enhanced clonal expansion in a CTL-intrinsic fashion, determined polyfunctional effector cell differentiation, and was necessary for immune control of viruses from diverse families. Moreover, the administration of recombinant IL-33 augmented vaccine-induced CTL responses. Our work establishes alarmins / DAMPs ("damage" as opposed to "danger") as a novel general principle how innate signals of viral infection orchestrate adaptive immune defense, thus opening new perspectives for targeted manipulations of CTL responses to viruses and vaccines.

COOPERATION PARTNERS

NIH, NIAID, Bethesda, MD, USA:
Dr. William E. Paul
Dr. Jinfang Zhu
Dr. Dragana Jankovic

University of Oxford, UK:
Prof. Dr. Fiona Powrie

University Hospital Zurich, Switzerland:
Prof. Dr. Rolf M. Zinkernagel
Prof. Dr. Hans Hengartner
PD Dr. Mike Recher

University of Geneva, Switzerland:
Prof. Dr. Daniel D. Pinschewer
Dr. Weldy Bonilla
Prof. Dr. Doron Merkler

University of Lausanne, Switzerland:
Dr. Lukas Flatz

University of Vienna, Austria:
Dr. Andreas Bergthaler

University of Essen:
Prof. Dr. Karl S. Lang

University of Düsseldorf:
Dr. Philipp Lang, Ph.D.

DKFZ, Heidelberg:
Prof. Dr. Thomas Höfer
Dr. Michael Floßdorf
Dr. Yevgeniy Vainshtein
Dr. Qin Zhang

University of Marburg:
Prof. Dr. Michael Lohoff

University of Giessen:
Prof. Dr. Christoph G. Grevelding

FU Berlin, Veterinary Medicine:
Prof. Dr. Susanne Hartmann
Dr. Sebastian Rausch

MPIIB Berlin:
Prof. Dr. Arturo Zychlinsky
Dr. Nikolaus Deigendesch

DRFZ Berlin:
Prof. Dr. Andreas Radbruch
PD Dr. Claudia Berek
Dr. Hyun-Dong Chang
Dr. Marc Ehlers
Dr. Andreas Hutloff
Dr. Farzin Mashreghi
Dr. Chiara Romagnani
Dr. Koji Tokoyoda

Charité, Berlin:
Prof. Dr. Gerd-Rüdiger Burmester
Prof. Dr. Frank Buttgereit
Prof. Dr. Alf Hamann
Dr. Ute Hoffmann
Dr. Anja A. Kühl
Prof. Dr. Marcus Maurer
Prof. Dr. Martin Metz
Prof. Dr. Gabriela Riemekasten
Prof. Dr. Alexander Scheffold
Prof. Dr. Hans-Dieter Volk
Prof. Dr. Margitta Worm

Miltenyi Biotec, Gergisch Gladbach:
Dr. Mario Assenmacher
Dr. Anne Richter

SELECTED PUBLICATIONS

Gaber, T., S. Schellmann, K.B. Erekul, M. Fangradt, K. Tykwinska, M. Hahne, P. Maschmeyer, M. Wagegg, C. Stahn, P. Kolar, R. Dziurla, M. Löhning, G.-R. Burmester and F. Buttgereit. 2011. MIF counter-regulates dexamethasone-mediated suppression of HIF-1κ function and differentially influences human CD4+ T cell proliferation under hypoxia. J. Immunol. 186, 764-774.

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The alarmin IL-33 drives protective anti-viral CD8 T cell responses

SCIENTISTS

Anja Fröhlich
Ahmed N. Hegazy
Max Löhning

COOPERATION PARTNERS

Prof. Dr. Daniel D. Pinschewer
Prof. Dr. Doron Merkler
Dr. Weldy Bonilla:

Department of Pathology and Immunology, and World Health Organization Collaborating Center for Vaccine Immunology, University of Geneva, Geneva, Switzerland

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Bonilla*, W.V., A. Fröhlich*, K. Senn, S. Kallert, M. Fernandez, S. Johnson, M. Kreutzfeldt, A.N. Hegazy, C. Schrick, P.G. Fallon, R. Klemenz, S. Nakae, H. Adler, D. Merkler, M. Löhning# and D.D. Pinschewer#. 2012. The alarmin interleukin-33 drives protective antiviral CD8+ T cell responses. Science 335, 984-989)

*Shared first authors; #shared last and corresponding authors.

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Cytotoxic CD8⁺ T cells (CTLs) are important key players in adaptive immune responses against viruses or tumors. Pathogen-associated molecular patterns (PAMPs) decisively influence antiviral immune responses, whereas the contribution of endogenous signals of tissue damage, so called alarmins, remain ill-defined. Interleukin-33 (IL-33) has been shown to be released upon cell damage or necrosis and its pro-inflammatory properties have been described in various infectious and inflammatory diseases. However, its inflammatory function has not been linked to anti-viral immune responses so far.

Using the well established model of lymphocytic choriomeningitis virus (LCMV) infection in mice, we could demonstrate that cytotoxic CD8 T cell (CTL) responses were severely impaired in the absence of IL-33 or its receptor ST2 (T1, IL-1RL1) resulting in reduced activation, expansion, cytokine production and, in consequence, impaired viral clearance. Thus, we define a so far unknown and unexpected role for IL-33 in the regulation of CTL responses to viral infections.

Expression of IL-33 and its receptor increase during LCMV infection

To identify novel factors regulating antiviral immune responses global gene arrays from splenocytes of LCMV-infected mice were performed and demonstrated that IL-33 and its receptor ST2 were prominently expressed during infection. Detailed kinetic analysis revealed a peak of IL-33 production on day 5 post infection (p.i., Figure 1A), while its receptor was most prominently expressed on a subset of activated, CD62L^{low} CD8 T cells at around day 6 p.i. (Figure 1B). These data clearly indicate an early release of IL-33 during LCMV infection and that activated CTLs are capable of responding to IL-33 by expression of ST2.

Cytotoxic T cell responses are strongly impaired in the absence of IL-33 signalling

To study the consequences of defective IL-33 signalling we infected wildtype (wt), *il33*- or *il1rl1* (ST2)-deficient mice with LCMV and evaluated the CTL response at its peak (day 9 p.i.). Surprisingly, total and virus-specific CTLs from both knockout strains were strongly compromised in their frequencies and total numbers

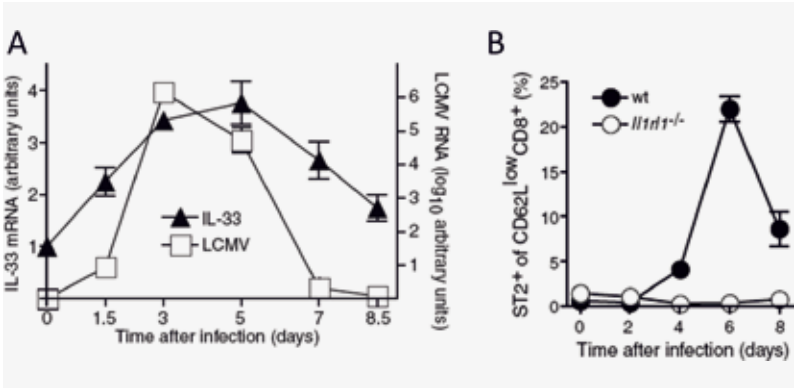

Figure 1: (A) Expression of IL-33 mRNA in context of viral RNA in spleens of LCMV-infected mice assessed by rtPCR. (B) Percentage of activated, CD62L^{low} CD8 T cells expressing ST2 after LCMV infection measured by flow cytometry.

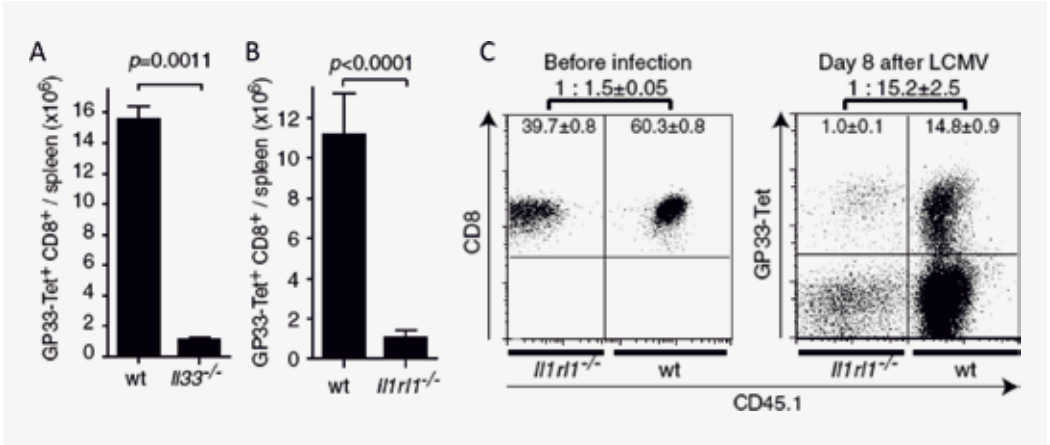

Figure 2: wt and (A) *il33* or (B) *il1rl1*-deficient mice were infected with LCMV and spleens were analyzed at day 9 post infection (p.i.). The frequency of GP33-specific CD8 T cells was determined by tetramer staining an flow cytometry. (C, left panel) Distribution of CD8 T cells in mixed bone marrow (BM) chimeras (CD45.1⁺ *wt*/CD45.1- *il-1rl1*$^{-/-}$). (C, right panel) Frequencies of LCMV-specific CD8 T cells in mixed BM chimeras at day 9 p.i. determined by tetramer staining.

(Figure 2A and B). Moreover, analysis of mixed *wt/il-1rl1*$^{-/-}$ bone marrow chimeras revealed that direct signalling of IL-33 to CTLs was required to mediate a full blown and robust CTL response to LCMV infection (Figure 2C). Consequently, this defect resulted in inefficient clearance of high dose LCMV infection (Figure 3A). Our finding was not restricted to infection with LCMV, an RNA virus, as *il1rl1*$^{-/-}$ mice showed similar impairment in CTL responses to thew DNA viruses murine-γ-herpes virus (MHV) and vaccinia virus (VV) (Figure 3B and C).

Cytotoxicity and plurifuctionality of CTLs is strongly compromised in the absence of IL-33 signaling

To gain further information about the functional quality of virus-specific CTLs in the absence of IL-33 signalling, we assessed their cytotoxicity *in vitro*. In line with increased viral titers, *il1rl1*$^{-/-}$ splenocytes revealed impaired killing capacities (Figure 4A). To exclude the influences of viral burden on the quality of CTL response and to enrich virus-specific CTL precursor frequencies, we transferred TCR transgenic, LCMV-specific P14 cells into WT recipients followed by LCMV infection. *il1rl1*$^{-/-}$ P14 cells produced less TNFα and IL-2 at the single cell level and showed lower expression of the degranulation marker LAMP1 (CD107a, Figure 4B). Taken together, our results demonstrate that lack of IL-33 signalling not only affects CTL frequencies, but also their functionality in respect to killing capacities and anti-viral cytokine production.

Activation and survival of CTLs is reduced in the absence of IL-33 signalling

To gain further insight into the impact of IL-33 signalling on CTLs we compared the expression of activation-associated surface molecules on wt and *il1rl1*$^{-/-}$ P14 cells. In the absence of IL-33 signalling, the frequencies of terminally differentiated virus-specific effector CTLs expressing KLRG-1 and NK1.1 were strongly reduced (Figure 5A and B). In line with these findings, CD62L was not efficiently down-regulated on total CD8 T cells. In addition, we found that anti-apoptotic BCL-2 levels were reduced in *il1rl1*$^{-/-}$ CTLs indicating that IL-33 is not only needed to drive the terminal differentiation of a potent and fully functional effector population but also to increase its survival capacity (Figure 5C).

Treatment with IL-33 augments vaccine-induced CTL- responses

As lack of IL-33 signalling results in defective CTL responses upon virus infection, we wondered whether treatment with recombinant IL-33 could on the other hand increase CTL responses induced by vaccination. Indeed, IL-33 boosted CTL responses to both recombinant vaccinia virus (Figure 6A) and virus-like particles (Figure 6B). Thus, IL-33 has the potential to increase CTL responses and might be suitable as adjuvant in treatment of tumors and chronic viral infections.

Conclusion and perspective

In this study, we could define a novel and completely unexpected role for IL-33 in the regulation of CTL responses to viral infections.

In the absence of IL-33 signalling, the induction of virus-specific CTL responses upon infection is dramatically impaired. Moreover, the remaining CTLs are poor killers and restricted in their plurifunctional cytokine profile. As a consequence, viral clearance from blood and organs is impaired. When administered together with a virus-based vaccine, IL-33 could potently augment the resulting CTL response.

CTL responses are not only essential for the control of viral infections, but also for the control of tumor growth. Thus, IL-33 administration might provide a novel therapeutic strategy to strengthen CTL responses and, in consequence, to combat these diseases. However, further studies in the human system are needed to evaluate the impact of IL-33 on CTL responses.

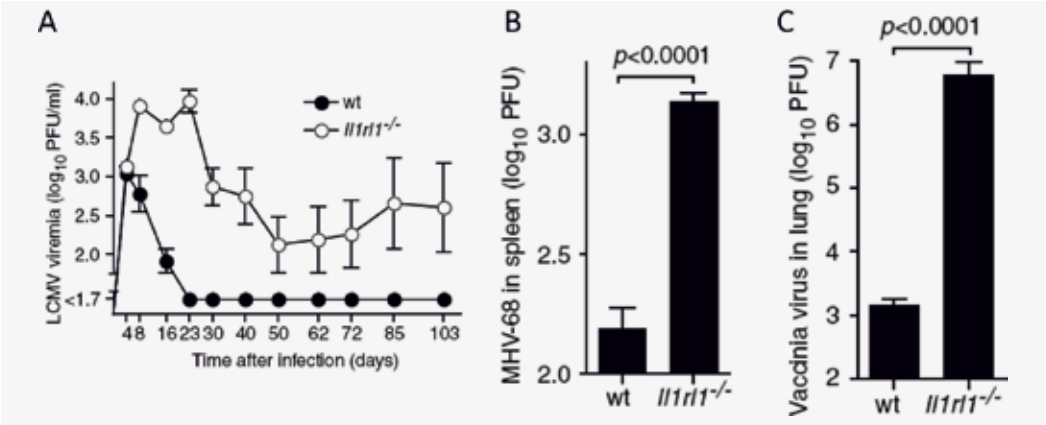

Figure 3: (A) *wt* and *il1rl1*$^{-/-}$ mice have been infected with 2x10^6 pfu LCMV, an RNA virus. At the time points indicated, viral titers were assessed in the blood. (B) Ten days after infection with murine-γ-herpes virus 68 (MHV-68, a DNA virus) viral titers in spleens of wt and *il1rl1*$^{-/-}$ mice were determined. (C) Titers of vaccinia virus (a DNA virus) in lungs of infected wt and *il1rl1*$^{-/-}$ mice at day 9 p.i.

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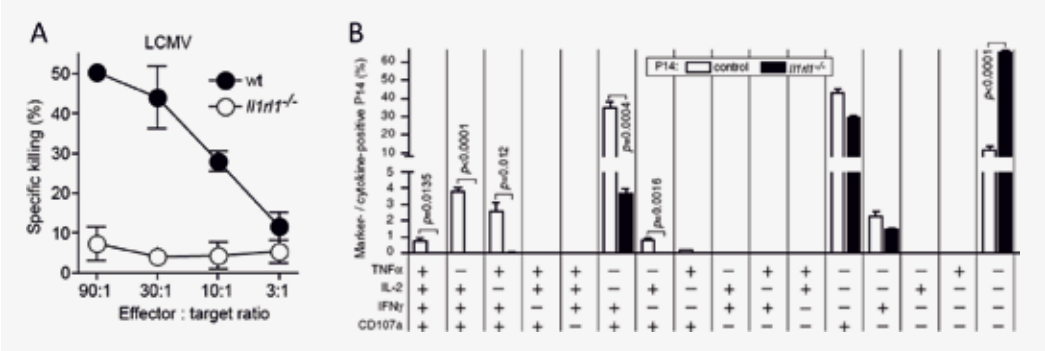

Figure 4: (A) Cytotoxicity of splenic *WT* and *IL-1Rl1*$^{-/-}$ CTLs were tested in a primary ex vivo Cr^{51} release assay at day 9 after LCMV infection. (B) Control (WT) or *IL-1Rl1*$^{-/-}$ P14 cells were adoptively transferred into WT hosts. Nine days after LCMV infection, cytokine production and CD107a expression by splenic P14 cells was assessed by peptide-mediated restimulation followed by intracellular staining and flow cytometric analysis.

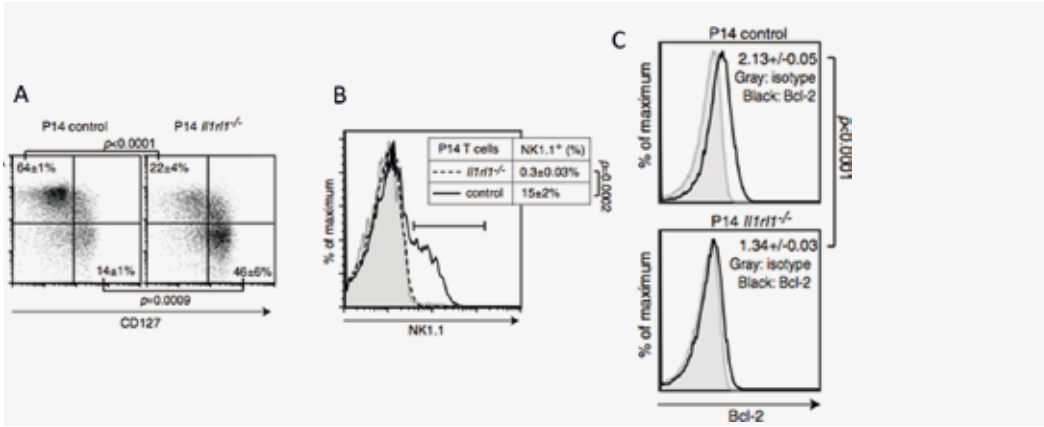

Figure 5: *wt* (control) or *il1rl1*$^{-/-}$ P14 cells were transferred into WT mice followed by LCMV infection. (A+B) Nine days post infection splenic P14 cells were isolated and analysed for expression of surface molecules. (C) Expression of Bcl-2 by wt and *il1rl1*$^{-/-}$ P14 cells was determined by intracellular staining and flow cytometry.

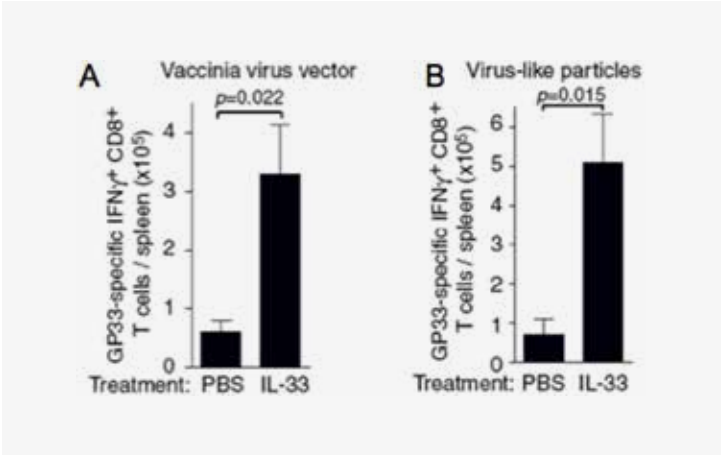

Figure 6: WT mice were vaccinated with (A) recombinant vaccinia virus or (B) virus-like particles (VLP) expressing the GP33 of LCMV. IL-33 was administered intra peritoneally (i.p.) from day 1-7 post immunization (p.i.). Frequencies of GP33-specific CTLs was determined at day 8 p.i. by flow cytometry.

Quantitative IFN-γ memory of CD4⁺ T helper cells

A major effector function of CD4+ T helper (Th) cells is the secretion of selected sets of cytokines. Upon stimulation, a certain percentage of antigen-experienced T cells within a population produces and secretes different types of cytokines[1]. Up to now, insight into the kinetics of cytokine production by individual T cells is lacking [2, 3, 4, 5]. The goal of this project is a mechanistic understanding of the cytokine memory of an individual T cell that is the basis for the cell's decision to express a certain cytokine in a recall response[6].

Introduction

Upon activation, only a fraction of stimulated Th cells produces effector cytokines. Moreover, there is an enormous variability in the per-cell amount of cytokine expression such that some cells produce a lot and others very little cytokine. Up to now the molecular basis for the decision of an individual cell to become a cytokine producer or non-producer is ill defined. It is also unclear whether a single cell quantitatively memorizes its cytokine expression in the context of a secondary stimulation.

Results and Discussion

Individual Th1 cells continuously produce IFN-γ during restimulation

We combined sequentially two techniques for the identification of individual cytokine producers: First, we labeled IFN-γ-producing cells alive by cytokine secretion assay technology[1], followed by intracellular staining of the respective cytokine after different culture periods (schematic overview, Fig. 1A). The majority of cells that was initially cytokine-positive stained also positive at later time points, indicating a stable population of cytokine producers that is continuously active for several hours during a recall stimulation (Fig. 1B). In addition, we observed a surprising stability of the IFN-γ expression level in individual cells over time.

Th1 cells have a quantitative IFN-γ memory

Next we addressed whether the amount of IFN-γ produced by a single cell is predictive of the future IFN-γ production probability and amount of this cell. We therefore sorted Th1 cells by FACS according to their IFN-γ production amounts into fractions with high, low or no IFN-γ production (Fig. 2A). These fractions were continuously cultured and restimulated in a daily kinetics to analyze their IFN-γ production behavior. We found that cells that were sorted for high IFN-γ production showed a higher probability to reexpress this cytokine and also produced more IFN-γ per cell (Fig. 2B). Thus, individual Th1 cells exhibit a quantitative memory for IFN-γ production probability and amount.

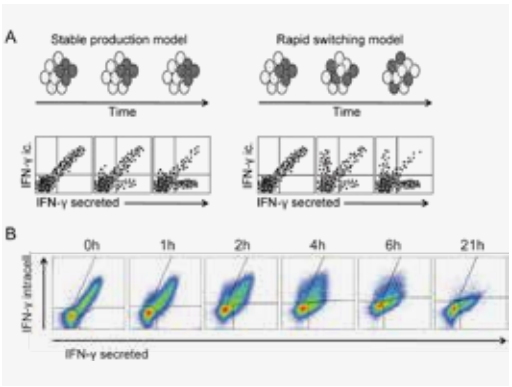

Figure 1
(A) Schematic illustration of possible distributions of cytokine-producing cells within a population and the two hypotheses based on stable production or rapid switching of all cells between cytokine-producing and -nonproducing states.
(B) Upon restimulation of Th1 cells, live IFN γ⁺ cells were labeled by IFN γ secretion assay and continuously cultured in the presence of the stimulus. Intracellular IFN γ counterstainings were performed at the indicated time points. Data are representative of three independent experiments.

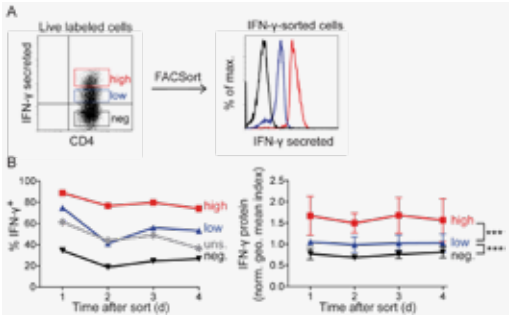

Figure 2
(A) Following IFN γ secretion assay, Th1 cells were sorted for IFN γ expression amounts by FACS into IFN γ high, -low, and -negative populations. Histogram overlays show post-sort purities.
(B) Sorted fractions were cultured in the presence of IL 2, and the frequency of IFN γ⁺ cells was measured after daily restimulation. Geometric mean of IFN γ expressed per IFN γ⁺ cell was determined and normalized to the geometric mean of IFN γ expressed by the unsorted IFN γ⁺ cells. Data are representative of (left) or pooled from (right) three independent experiments.

SCIENTISTS

Caroline Helmstetter, Michael Peine, Max Löhning

COOPERATION PARTNERS

Prof. Dr. T. Höfer, M. Floßdorf; Division of Theoretical Systems Biology, German Cancer Research Center (DKFZ)

Dr. A. Richter; Miltenyi Biotec GmbH, Bergisch Gladbach

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Dynamics and stability of T-bet and GATA-3 expression in T helper cells *in vitro* and *in vivo*

SCIENTISTS

Elsa Pellet, Michael Peine, Max Löhning

COOPERATION PARTNERS

Prof. Dr. Thomas Höfer, DKFZ Heidelberg

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Governed by differentiation programs under the control of lineage-specifying transcription factors, naive T helper (Th) cells can develop into various effector populations, e.g. Th1 or Th2, that secrete distinct sets of cytokines. In this project, we investigate whether and to which extent Th cells commit to and maintain the diverse differentiation programs in an exclusive manner. Specifically, we examine the impact of different key transcription factor expression levels or of the co-expression of opposing transcription factors on Th cell effector functions. The results are integrated into mathematical models of Th cell differentiation.

Introduction

The Th1 and Th2 differentiation programs are governed by the transcription factors T-bet and GATA-3 and endow the cells with the capacity to secrete IFN-γ and IL-4, IL-13, and IL-5, respectively [1,2]. T-bet is induced by IFN-γ and IL-12 signals whereas GATA-3 is induced by IL-4 signals. It has been thought previously that the expression of T-bet and GATA-3 in single cells was mutually exclusive due to mechanisms of autoactivation and reciprocal inhibition intrinsic to the Th1 and Th2 differentiation programs [3-5]. Particularly, the direct and indirect autoactivation of T-bet and GATA-3 suggests a bimodal expression behavior [6,7]. However, we have shown recently that Th2 cells can be reprogrammed by virus-induced cytokine signals (type I and type II interferons and IL-12) into a "Th2+1" cell subset that stably co-expresses T-bet and GATA-3 and secretes both IFN-γ and IL-4 [8]. Using different concentrations of polarizing cytokines, we now test the possibility of multiple stable T-bet and GATA-3 expression levels and their impact on Th cell effector functions.

Results and outlook

Increasing IL-12 concentrations during Th1 cell priming resulted in higher T-bet and lower GATA-3 protein amounts per cell (Fig. 1), correlating with higher frequencies of IFN-γ—producing cells (not shown). Similarly, increasing IL-4 concentrations during Th2 cell priming yielded increasing GATA-3 protein amounts per cell (Fig. 2), correlating with higher frequencies of IL-4—producing cells (not shown).

Using adoptive T-cell transfers we are currently addressing whether Th1 and Th2 cells stably maintain graded amounts of their lineage-specifying transcription factors in the presence and absence of perturbations. In another approach, we generate GATA-3$^+$T-bet$^+$ Th2+1 cells and modulate T-bet and GATA-3 expression by RNA interference (Fig. 3), thereby examining whether a transient reduction of the expression of one transcription factor in the presence of its molecular antagonist is sufficient to extinguish an otherwise stably adopted differentiation program.

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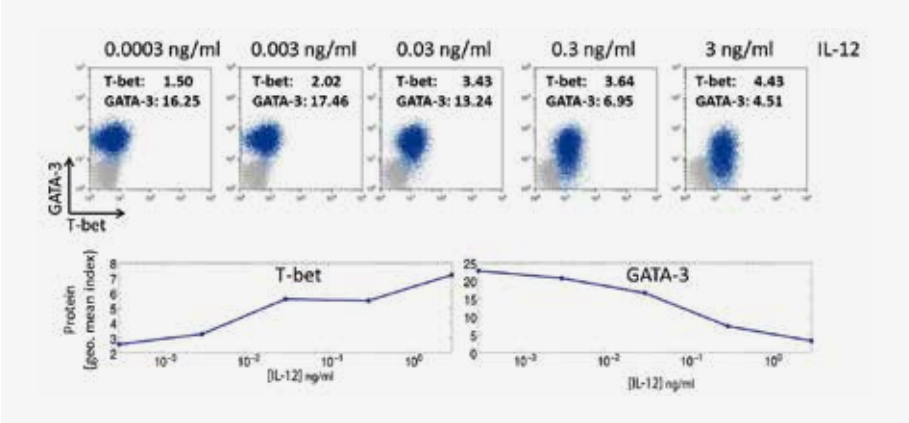

Fig. 1: Increasing IL-12 concentrations during Th1 cell differentiation result in increasing T-bet and decreasing GATA-3 protein amounts per cell. Naive $CD4^+CD62L^{hi}$ T cells from DO11.10 mice were activated with OVA peptide and APCs in the presence of anti—IL-4 and different concentrations of IL-12 to generate Th1 cells. On day 5 of culture, T-bet and GATA-3 protein amounts were determined by FACS.

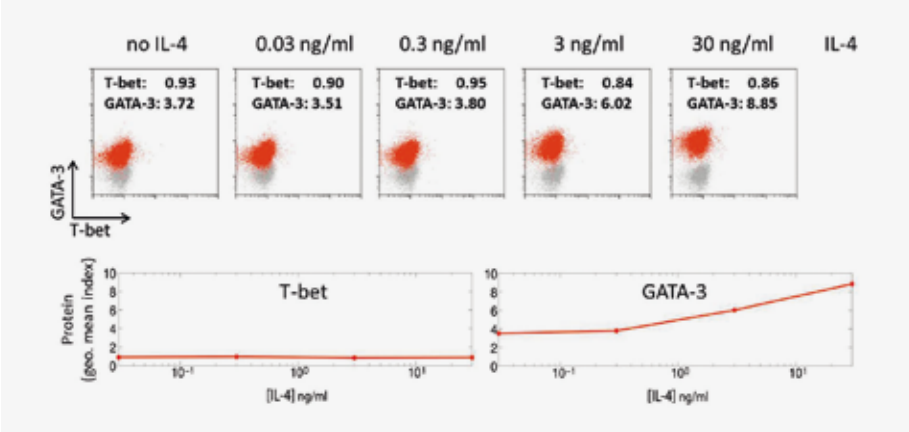

Fig. 2: Increasing IL-4 concentrations during Th2 cell differentiation result in increasing GATA-3 protein amounts per cell. Naive $CD4^+CD62L^{hi}$ T cells from Balb/c mice were activated with anti-CD3/anti-CD28 in the presence of anti—IL-12, anti—IFN-γ, and different concentrations of IL-4 to generate Th2 cells. On day 5 of culture, T-bet and GATA-3 protein amounts were determined by FACS.

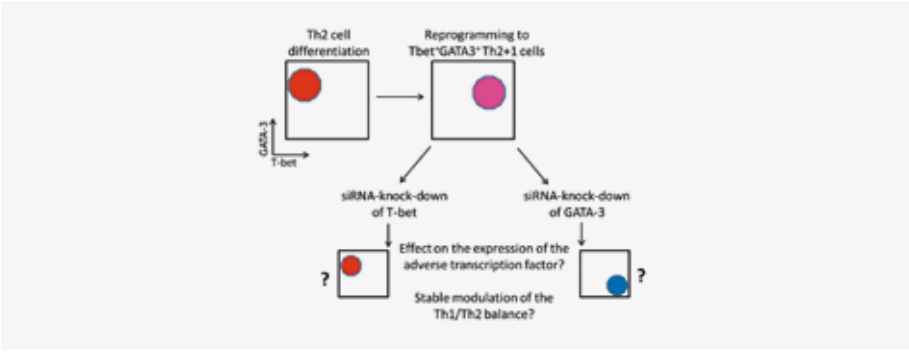

Fig. 3: Can GATA-3+T-bet+ Th2+1 cells be reverted to Th2 cells by interfering with T-bet expression? Can they be further reprogrammed to Th1 cells by interfering with GATA-3 expression? GATA-3+T-bet+ Th2+1 cells will be generated from Th2 cells *in vitro* or *in vivo*, and T-bet or GATA-3 expression will be reduced by RNA interference. The resulting transcription factor profile as well as functional properties such as cytokine production will be determined.

Gabriela Riemekasten

Charité - University Medicine Berlin (CCM)
Department of Rheumatology and Clinical Immunology

Cell Autoimmunity

Developing new therapies for treatment of SLE & Systemic Sclerosis

KEYWORDS

Lupus, Regulatory T cells, Systemic sclerosis, agonistic autoantibodies, fibrosis

GROUP MEMBERS

Group Leader:
Prof. Dr. med. Gabriela Riemekasten

Scientists:
Dr. med. Jens Humrich
M.Sc. Biotech. Jeannine Günther
Dr. med. Mike Oliver Becker
Dr. med. Philipp Enghard

PhD students:
Dipl. Biochem. Reinmar Undeutsch
Dipl. Ing. Angela Kill
M. Sc. Biol. Caroline von Spee-Mayer
Dipl. Biol. Angelika Rose

Technical Assistant:
BTA Anika Klaus

MD students:
Jan Klocke
Olivia Weigert
Katharina Kopetschke

Students:
Özlem Vural
Josephine Scholz

The focus of our research group is the pathogenesis of rheumatic connective tissue diseases, mainly of systemic lupus erythematosus (SLE) and systemic sclerosis (SSc), in order to develop new and better treatment strategies.

SLE is characterized by loss of self-tolerance of T and B cells towards nuclear antigens. We investigate the role of different T cell subsets in SLE pathogenesis in order to develop strategies to re-establish self-tolerance. One major goal is to enrich the pool of functional regulatory T cells (Treg) by cytokine therapy or adoptive Treg transfer. In addition, the identification of autoantigen-specific effector T cells by flow-cytometry allows us to develop antigen-specific approaches. Currently we aim to identify autoantigen-specific Treg to specifically modulate antigen-specific effector T cells. Murine lupus models and SLE patient samples are used to develop these strategies and to translate them to clinical application. Furthermore, we are exploring the potential of urinary T cells as biomarkers to monitor SLE activity.

In Systemic Sclerosis (SSc) we analyze the role of agonistic autoantibodies (Aabs) in the pathogenesis of the disease. To date we investigate *in vitro* and *in vivo* effects of Aabs directed to the angiotensin II receptor type 1 (AT1R) and the endothelin-1 receptor type A (ETAR). We hypothesize that a chronic activation of AT1R and ETAR by agonistic Aabs drives inflammation and fibrosis already in an early stage of disease. We study the effects of these functional Aabs in human vascular and immune cells as well as in murine organs. Cytometry, immunohistochemistry, real-time PCR, western blot, ELISA and chemotaxis assays are used for analyzing the induced effects and signal cascades to develop future treatment strategies in early disease. Additionally, our group evaluates these antibodies as biomarkers of SSc in collaboration with a company.

COOPERATION PARTNERS

Dr. Andreas Grützkau, DRFZ Berlin

Dr. Anja Kühl, Department of Gastroenterology, Infektiology and Rheumatology, Charité Berlin

Prof. Dr. Andreas Radbruch, DRFZ Berlin

Prof. Dr. Alf Hamann, DRFZ Berlin

Prof. Dr. Andreas Schwarting, University Mainz

Dr. Axel Roers, University Cologne

Dr. Christian Probst, EUROIMMUN AG, Lübeck

Dr. Claudia Berek, DRFZ Berlin

Prof. Dr. Duska Dragun, Department of Nephrology und Transplantation, Charité Berlin

Prof. Dr. Falk Hiepe, Department of Rheumatology and Clinical Immunology, Charité Berlin

Prof. Dr. Gerd-Rüdiger Burmester, Department of Rheumatology and Clinical Immunology, Charité Berlin

Prof. Dr. Hans-Jürgen Thiessen, University Rostock

Dr. Harald Heidecke, CellTrend GmbH, Luckenwalde

Dr. Hedda Wardemann, Max Planck Institute, Berlin

Prof. Dr. Jochen Hühn, DRFZ Berlin

Dr. Johan Van der Vlag, University Nijmegen, Netherlands

Dr. Kirsten Falk, Max-Delbrück-Centre, Berlin

Dr. Marc Ehlers, DRFZ Berlin

Prof. Dr. Marco Mattucci-Cerinic, University Florence, Italy

Prof. Dr. Margitta Worm, Department of Dermatology, Charité Berlin

PD Dr. Martin Witzenrath, Department of Infectiology and Pneumology, Charité Berlin

Dr. Olaf Rötzschke. Max-Delbrück-Centre, Berlin

Dr. Peter Klein Weigel, DRK Clinic Mark-Brandenburg

Dr. Rudi Manz, DRFZ Berlin

Dr. Susanne Hartmann, Freie Universität Berlin

Dr. Tim Sparwasser, Technical University Munich

Dr. Timothy Radstake, Universität Nijmegen, Netherlands

Dr. Wolfgang Schlumberger, EUROIMMUN AG, Lübeck

Prof. Dr. Wolfgang Kübler, Physiology Institute, Charité Berlin

SELECTED PUBLICATIONS

Engler JB, Undeutsch R, Kloke L, Rosenberger S, Backhaus M, Schneider U, Egerer K, Dragun D, Hofmann J, Huscher D, Burmester GR, Humrich JY, Enghard P, Riemekasten G. Unmasking of autoreactive CD4 T cells by depletion of CD25 regulatory T cells in systemic lupus erythematosus. Ann Rheum Dis. 2011 Sep 16.

Riemekasten G, Philippe A, Näther M, Slowinski T, Müller DN, Heidecke H, Matucci-Cerinic M, Czirják L, Lukitsch I, Becker M, Kill A, van Laar JM, Catar R, Luft FC, Burmester GR, Hegner B, Dragun D. Involvement of functional autoantibodies against vascular receptors in systemic sclerosis. Ann Rheum Dis. 2011 Mar;70(3):530-6

Becker MO, Brückner C, Scherer HU, Wassermann N, Humrich JY, Hanitsch LG, Schneider U, Kawald A, Hanke K, Burmester GR, Riemekasten G. The monoclonal anti-CD25 antibody basiliximab for the treatment of progressive systemic sclerosis: an open-label study. Ann Rheum Dis. 2011 Jul;70(7):1340-1

Systemic Lupus Erythematosus – Role of the Treg-IL-2 axis and antigen-specific T cells in the pathogenesis and treatment of SLE

SCIENTISTS

J. Humrich, C. von Spee-Mayer, A. Rose, P. Enghard, J. Kloke, A. Klaus, G. Riemekasten

COOPERATION PARTNERS

A. Radbruch, A. Hamann, A. Scheffold, AiCuris, F. Hiepe, A. Roers, A. Thiel, R. Baumgrass, B. Sawitzki, W. Uckert, Ch. Zielinski

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Humrich JY, Morbach H, Undeutsch R, Enghard P, Rosenberger S, Weigert O, Kloke L, Heimann J, Gaber T, Brandenburg S, Scheffold A, Huehn J, Radbruch A, Burmester GR, Riemekasten G. Homeostatic imbalance of regulatory and effector T cells due to L-2 deprivation amplifies murine lupus. Proc Natl Acad Sci USA. 2010; 107:204-9.

Engler JB, Undeutsch R, Kloke L, Rosenberger S, Backhaus M, Schneider U, Egerer K, Dragun D, Hofmann J, Huscher D, Burmester GR, Humrich JY, Enghard P, Riemekasten G. Unmasking of autoreactive CD4 T cells by depletion of CD25 regulatory T cells in systemic lupus erythematosus. Ann Rheum Dis. 2011 Sep 16.

Weigert O, von Spee C, Undeutsch R, Kloke L, Humrich JY, Riemekasten G. CD4+Foxp3+ regulatory T cells prolong drug-induced disease remission in (NZBxNZW) F1 lupus mice. Arthritis Res Ther. 2013 Feb 27;15(1)

Quantitative and qualitative abnormalities of CD4+Foxp3+ Treg have been associated with SLE. Furthermore, the production of IL-2, the essential cytokine for peripheral Treg homeostasis, is deficient in T cells in human and murine SLE (Humrich et. al. 2010). We now established the link between IL-2 deficiency and Treg abnormalities in SLE and developed an IL-2 treatment strategy as a novel therapeutic approach for SLE. In addition, we analyzed the potential of polyclonal Treg to prolong drug-induced remission in a murine lupus model. Furthermore, we established a method to investigate the role of antigen-specific T cells in SLE pathogenesis.

IL-2 restores Treg:Tcon balance and ameliorates established disease

The general importance of CD4+Foxp3+ Treg as physiologically relevant inhibitors of lupus could be demonstrated by several approaches in the (NZBxNZW) F1 model. A state of IL-2 deficiency was confirmed in lupus prone mice by the detection of a progressive deficiency of IL-2 and IL-2-producing CD4+ T cells in the lymphoid tissues (Humrich et al., 2010). The decline in IL-2 producing Tcon was associated with progressive Treg abnormalities and disease progression, suggesting that an acquired IL-2 deficiency contributes to disease development. Treatment of lupus mice with exogenous recombinant IL-2 (rIL-2) strongly promoted the expansion of endogenous CD4+Foxp3+ Treg in lymphoid organs and peripheral blood and the expression of CD25 on Treg. Taken together, an acquired and self-amplifying disruption of the Treg-IL-2 axis induced a progressive homeostatic imbalance between Treg and effector Tcon and promoted the development of disease. The reversibility of this homeostatic impairment therefore provides strong rationales for an IL-2 based immunotherapy of SLE (Humrich et al. 2010).

IL-2 deprivation of CD4+Foxp3+ Treg from SLE patients

To confirm the rationale for an IL-2 based therapy in SLE, we started to investigate whether Treg from SLE patients display phenotypic and homeostatic abnormalities associated with IL-2 deficiency by flow cytometry. In line with recent publications (Bonelli et al. 2009, Suen et al. 2008) we found that although the frequency of Foxp3+CD127- Treg among CD3+CD4+ T cells was increased in SLE patients compared to healthy subjects, the surface expression of CD25 was strongly reduced in Treg, which is considered as one of the phenotypic hallmarks of IL-2 deficiency (Figure 1). Furthermore, we found a strongly decreased ratio between proliferating Foxp3+CD127- Treg and Foxp3- Tcon indicating that a homeostatic imbalance between Treg and effector T cells is present also in SLE patients. In summary CD4+Foxp3+ Treg from human SLE patients display some phenotypic alterations that are similar to those observed in lupus and IL-2 deficient mice, suggesting an IL-2 deprivation of Treg also in human SLE patients. In vitro stimulation of SLE PBMCs with IL-2 can reverse these effects. (von Spee-Mayer et al., manuscript in preparation).

CD4+Foxp3+ regulatory T cells sustain drug-induced disease remission in (NZBxNZW) F1 lupus mice

In consideration to the clinical translation of a Treg-based immunotherapy of SLE, we explored the potential of CD4+Foxp3+ Treg to maintain disease remission after induction of remission with an established cyclophosphamide (CTX) regimen in lupus-prone (NZBxNZW) F1 mice. Additional adoptive transfer of Treg after the CTX regimen significantly increased the survival and prolonged the interval of remission compared to mice that received only the CTX regimen (Figure 2). This was associated with an increase in the Treg frequency in the peripheral blood indicating a compensation of CTX induced Treg deficiency by the Treg transfer. This knowledge may be important for the future design of a clinical study involving the transfer of autologous Treg (Weigert et al., 2013).

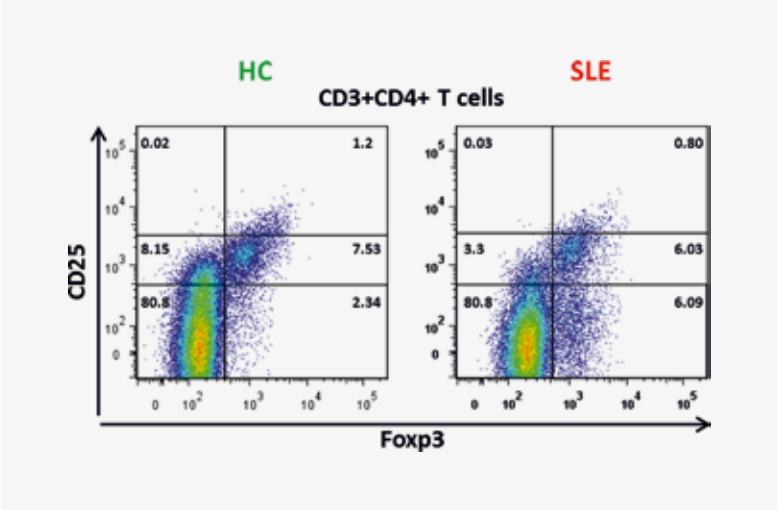

Figure 1: Representative flow-cytometric analysis of PBMCs from a healthy control (HC) and SLE patient gated on CD3+CD4+ T cells, showing the expression of Foxp3 (x-axis) and CD25 (y-axis)

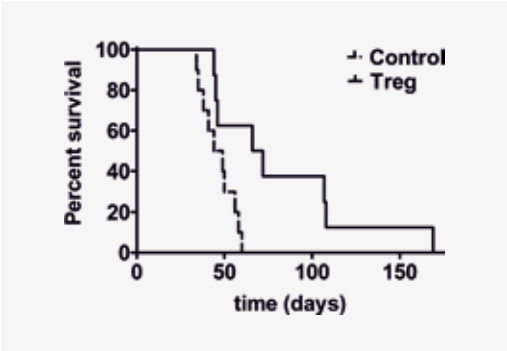

Figure 2: Survival of (NZB/W) F1 mice with active disease after induction of remission with GC/CTX and after an additional Treg transfer (Treg) compared to age-matched mice received only GC/CTX and PBS (Control)

PUBLICATIONS

Humrich JY, Morbach H, Undeutsch R, Enghard P, Rosenberger S, Weigert O, Kloke L, Heimann J, Gaber T, Brandenburg S, Scheffold A, Huehn J, Radbruch A, Burmester GR, Riemekasten G. Homeostatic imbalance of regulatory and effector T cells due to L-2 deprivation amplifies murine lupus. Proc Natl Acad Sci USA. 2010; 107:204-9.

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FUNDING

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Systemic Sclerosis – effects of agonistic autoantibodies directed against the angiotensin receptor type 1 and the endothelin receptor type A on various cells

SCIENTISTS

A. Kill, J. Günther, M. Becker, G. Riemekasten

COOPERATION PARTNERS

Dr. Anja Kühl, Charité Berlin

Prof. Dr. Duska Dragun, Charité Berlin

Prof. Dr. Gerd-Rüdiger Burmester, Charité Berlin

Dr. Harald Heidecke, CellTrend GmbH, Luckenwalde

PD Dr. Martin Witzenrath, Charité Berlin

Prof. Dr. Wolfgang Kübler, Charité Berlin

Functional autoantibodies (Aabs) against the angiotensin II receptor type 1 (AT1R) and the endothelin receptor type A (ETAR) have been identified in patients suffering from systemic sclerosis (SSc). Here we identified pathological effects of these Aabs on human peripheral blood mononuclear cells (PBMCs), neutrophils, endothelial cells and fibroblasts mediated through AT1R and ETAR.

Results:

Anti-AT1R and ETAR Aab positive IgG from SSc patients (SSc-IgG) induced in endothelial cells (HMEC-1) VCAM-1 and IL-8 expression, blockable with AT1R and ETAR/ETBR antagonists when compared to IgG from healthy donors serving as normal control (NC-IgG). IL-8 levels in the supernatants of HMEC-1 correlated with the antibody levels of the used SSc IgGs. In fibroblasts, collagen-I expression was induced, blockable with AT1R and ETAR/ETBR antagonists, and c-jun was upregulated.

SSc-IgG significantly increased the migration of neutrophils and PBMCs when compared to NC-IgG. SSc-IgG induced T cell migration was significantly reduced by AT1R and ETAR antagonists, but not so the migration induced by NC-IgG. In addition, T cells migrated towards SSc-IgG in an anti-AT1R and anti-ETAR Aab level-dependent manner. Stimulation of PBMCs by SSc-IgG resulted in a significantly increased secretion of IL-8 and an abundant, but due to a broad range not significant, secretion of CCL18 when compared to the stimulation by NC-IgG. Both, IL-8 and CCL18, were significantly reduced by AT1R and ETAR antagonists. Correlation analysis of cytokine levels in supernatants of PBMCs with clinical data of SSc-IgG donors revealed a negative correlation of IL-8 expression with the time since onset of SSc features like Raynaud's phenomenon, and an association of CCL18 expression with the incidence of vascular complications.

Discussion: Here we could show that anti-AT1R and anti-ETAR Aabs from SSc patients create inflammatory and profibrotic conditions via the induction of IL-8, VCAM-1, collagen I and CCL18. All these effects could be significantly abrogated by the application of selective receptor antagonists and depended, at least in part, on the anti-AT1R and ETAR levels of the used SSc-IgG fractions. IgG from patients in the first years of disease induced *in vitro* a significantly higher IL-8 release from PBMCs, thus they may play an important role particularly in the early stage of SSc contributing to disease pathogenesis.

Perspectives: The inflammatory and profibrotic effects upon Aab stimulation of PBMCs, neutrophils, endothelial cells and fibroblasts *in vitro*, and their associations with clinical findings suggest a role for autoantibody-mediated activation of these cells mediated through the AT1R and ETAR in the pathogenesis or even the onset of the disease. These findings may provide new treatment strategies.

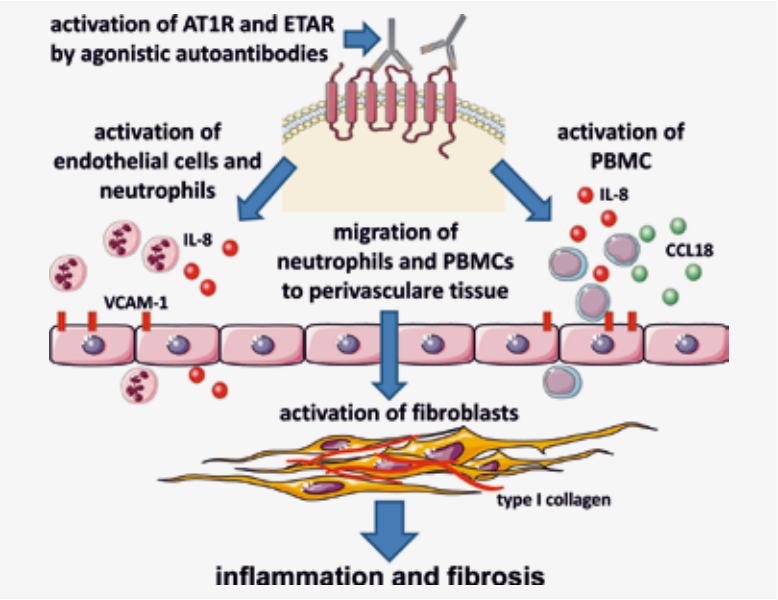

Figure 3: Schematic overview of inflammatory and profibrotic effects induced by Aabs against AT1R and ETAR

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Riemekasten G, Philippe A, Näther M, Slowinski T, Müller DN, Heidecke H, Matucci-Cerinic M, Czirják L, Lukitsch I, Becker M, Kill A, van Laar JM, Catar R, Luft FC, Burmester GR, Hegner B, Dragun D. Involvement of functional autoantibodies against vascular receptors in systemic sclerosis. Ann Rheum Dis. 2011 Mar;70:530-6.

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Photo: K.Zimmermann

Alexander Scheffold

Charité - University Medicine Berlin (CCM)
Department of Rheumatology and Clinical Immunology

Cellular Immunology

From suppression to self-regulation:how T cells protect us from our immune system

KEYWORDS

Immunomodulation, Interleukin-10, Notch, regulatory T cells, cell therapy, liver

GROUP MEMBERS

Group leader
Prof. Alexander Scheffold, PhD

Scientists
Katrin Neumann, PhD

PhD Students
Christian Neumann
Christine Rudolph
Frederik Heinrich
Andreij Mantei
Stefanie Gamradt
Petra Bacher
Ulla Knackmuss

Master students
Jonas Ahlers
Franziska Hahn

Technical assistant
Thordis Hohnstein

Students
Ruth Seikowski
Viktoria Junghans

Secretary
Dirk Rossbach

We try to understand how T lymphocytes can control or prevent immunopathology resulting from autoimmunity, allergy or from excessive inflammatory responses. We analyze, which mechanisms inflammatory T cells employ to prevent collateral damage induced by their own effector functions against pathogens and we try to define the physiological environment for this modulation. Another major project is the characterization of the rare T cells in the human body, which react to autoantigens or harmless environmental antigens.

We have developed a sensitive technology which allows to visualize autoreactive T cells directly from the blood. The sensitivity is high enough to access autoreactive T cells even in the naive as well as in the regulatory T cell (Treg) repertoire. Using gene expression profiling and functional *in vitro* and *in vivo* assays, we are characterizing the inducing signals and the transcriptional network regulating the expression of the anti-inflammatory or tissue-protective cytokines IL-10 and IL-22.

We have identified Notch as a switch to activate IL-10 as well as IL-22 in inflammatory T cells and further defined critical transcription factors. We are also trying to define the physiological niche for T cell modulation by Notch and additional environmental signals, which shall be translated into *in vitro* T cell modulation strategies. Monitoring of autoreactive effector and regulatory T cell populations in healthy persons and patients will be used to define the effect of modulation strategies directly on disease-relevant T cell targets or to use autoantigen-specific natural occurring Treg for therapeutic application.

Understanding the molecular and cellular basis of physiological immune regulation or suppression will help to identify disease-associated alterations and specific intervention points for targeted therapies.

COOPERATION PARTNERS

Prof. Charlotte Esser, Leibniz-Institut für umweltmedizinische Forschung, Universität Düsseldorf

Prof. Stephan Bereswil, Dr. Markus Heimesaat, Institut für Mikrobiologie und Hygiene, Charité – Universitätsmedizin Berlin

Prof. Kathryn Calame, Institute of Medicine, Columbia University, New York, USA

Dr. Sascha Rutz, Genentech, San Francisco, USA

Petra Bacher, Dr. Mario Assenmacher, Miltenyi Biotec GmbH, Bergisch Gladbach

Prof. Oliver Cornely und Angela Steinbach, Department of Internal Medicine, Universität zu Köln

Prof. Axel Brakhage und Dr. Olaf Kniemeyer, Hans Knöll Institut Jena

Dr. Jochen Maul, Gastroenterology, Charité, Berlin

Dr. Berit Rosche, Department of Neurology, Charité, Berlin

Prof. Frank Buttgereit, Clinics for Rheumatology and Clinical Immunology, Charité Berlin

Dr. Eugen Feist, Clinics for Rheumatology and Clinical Immunology, Charité Berlin

Prof. Margitta Worm, Dr. Guido Heine, Dermatology, Charité, Berlin

Prof. Christina Zielinski, Dermatology, Charité, Berlin

Prof. Joachim Spranger, Department of Endokrinology, Charité, Berlin

Dr. Anja Kühl, Gastroenterology, Charité, Berlin,

Prof. Sin-Hyeog Im, School of Life Sciences and Immune Synapse Research Center, Gwangju Institute of Science and Technology, Republic of Korea

Prof. Dr. Charlotte Esser, Leibniz Research Institute for Environmental Medicine, Düsseldorf

SELECTED PUBLICATIONS

Bacher P and Scheffold A. Flow-cytometric analysis of rare antigen-specific T cells. Cytometry 2013 (in press)

Bacher P, Schink C, Teutschbein J, Kniemeyer O, Assenmacher M, Brakhage AA, Scheffold A. Antigen-Reactive T Cell Enrichment for Direct, High-Resolution Analysis of the Human Naive and Memory Th Cell Repertoire. J Immunol. 2013 Mar 11. [Epub ahead of print]

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Analysis of the human T cell repertoire direct against disease-relevant antigens

SCIENTISTS

Stefanie Gamradt, Franziska Hahn, Petra Bacher

COOPERATION PARTNERS

Mario Assenmacher, Miltenyi Biotec GmbH, Bergisch Gladbach

Oliver Cornely und Angela Steinbach, Department of Internal Medicine, Universität zu Köln

Axel Brakhage und Olaf Kniemeyer, Hans Knöll Institut Jena

Jochen Maul, Gastroenterology, Charité, Berlin

Berit Rosche, Department of Neurology, Charité, Berlin

Eugen Feist, Clinics for Rheumatology and Clinical Immunology, Charité Berlin

Margitta Worm, Dr. Guido Heine, Dermatology, Charité, Berlin

Christina Zielinski, Dermatology, Charité, Berlin

Joachim Spranger, Department of Endokrinology, Charité, Berlin

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PUBLICATIONS

Bacher P and Scheffold A. Flow-cytometric analysis of rare antigen-specific T cells. Cytometry 2013 (in press)

Bacher P, Schink C, Teutschbein J, Kniemeyer O, Assenmacher M, Brakhage AA, Scheffold A. Antigen-Reactive T Cell Enrichment for Direct, High-Resolution Analysis of the Human Naive and Memory Th Cell Repertoire. J Immunol. 2013 Mar 11. [Epub ahead of print]

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For therapeutic application, the modulation of inflammatory T cells is particularly relevant for T cells involved in "pathologic" immune reaction, e.g. autoimmunity, graft rejection or allergies. In patients these T cells are often extremely rare and therefore difficult to analyze. We have developed a sensitive technology which allows for the first time to visualize and rapidly isolate autoreactive T cells directly from the blood. The sensitivity is high enough to access autoreactive T cells even in the naive T cell repertoire of healthy persons. We are currently using this technology for the monitoring of disease-relevant T cell populations in healthy persons and patients with immune-mediated diseases and for the isolation and *in vitro* modulation of pathologic T cell populations for potential therapeutic application.

Identification of human naive and memory T cells reactive to autoantigens

We have developed a sensitive flow-cytometric assay based on magnetic pre-enrichment of CD154+ T cells to visualize rare antigen-reactive naive and memory T helper cells directly from human peripheral blood (Antigen-Reactive T cell Enrichment, ARTE). The detection limit of about 1 cell out of 105 to 106 permitted the direct enumeration and characterization of auto-, tumor- or neo-antigen reactive T cells. Interestingly, we can show that autoreactive T cells and even T cells reacting against neo-antigens can be detected not only in the naive but also in the memory CD4+ T cell compartment of healthy donors. We are currently analyzing whether such memory T cells against neo- or autoantigens are induced by crossreactivity.

Pathogen-related cytokine micro-signatures in the human T cell repertoire

Furthermore, the analysis of high target cell numbers after pre-enrichment of rare antigen-specific T cells from large blood samples, dramatically improved the identification of small subpopulations. For example, the dissection of the antigen-specific memory responses into small cytokine producing subsets revealed great heterogeneity between pathogens, but also pathogen-related "micro-signatures" refining T helper cell subset classification.

Perspectives:

The possibility to directly analyze CD4+ T cells reactive against basically any antigen of interest at high resolution within the naive and memory repertoire will open up new avenues to investigate CD4+ T cell-mediated immune reactions and their use for clinical diagnostics. Our data also indicate that crossreactivity might significantly contribute to autoreactive T cell memory formation in humans as it has recently been demonstrated for memory T cells against pathogens (1). We are currently using this technology to get a comprehensive overview on T cells reacting against various auto- or environmental antigens within the naive, memory and regulatory T cell compartment from one individual donor or patient.

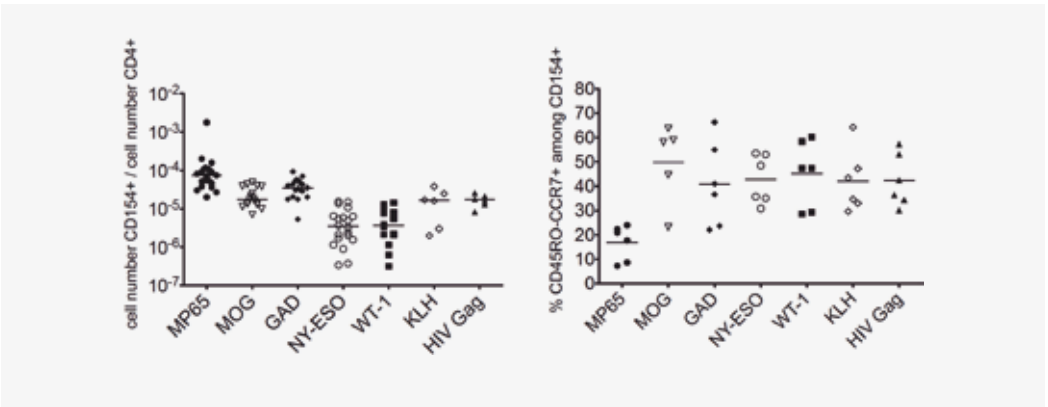

Figure 1: Frequencies and naive/memory distribution of T cells reactive to auto- or neo-antigens: T cells reactive towards the indicated antigens were analyzed from 108 PBMC using ARTE. Memory/naive classification is based on expression of CD45Ro and CCR7. Absolute cell numbers are determined after subtraction of background from a non-stimulated sample.

Molecular regulation of T helper cell self-control

Inflammatory T cells possess intrinsic control mechanisms which contain inflammatory reactions and limit inflammation associated damage, e.g. via the production of cytokines with anti-inflammatory (IL-10) or tissue protective (IL-22) capacity (1). We have identified the Notch-signalling pathway and the Notch ligand Dll4, expressed by plasmacytoid dendritic cells (pDC) and vascular endothelial cells, as a potent inducer of IL-10 and IL-22 in inflammatory T cells. However the underlying transcriptional machinery that regulates IL-10 and IL-22 in these T cells is still insufficiently understood. Using gene expression profiling of cytokine secreting versus non secreting cells we identified candidate transcription factors, which we are currently analyzing for the role in inflammatory cell self-control.

Molecular regulation of IL-10 expression

Among the most differentially expressed transcription factors, we identified Prdm1 (codes for Blimp-1), and Maf (Fig.1A) to be strongly associated with IL-10 production. Prdm1 was highly restricted to the Th1 differentiation program, whereas Maf was expressed also by other T helper cell subsets. We could demonstrate that IL-10 induction by c-Maf critically depends on Blimp-1 in Th1 cells (Fig.1B). Further in vivo studies revealed that Blimp-1 also limits Th1-mediated immunopathology (Fig.1C). Notch activation does not change the overall IL-10 associated expression pattern but probably acts via upregulating c-Maf and Blimp-1. Thus, we have identified a novel mechanism by which c-Maf regulates IL-10 expression in Th1 cells, further supporting its role as a master regulator of IL-10.

Molecular regulation of IL-22 expression

Another target of the Notch pathway is IL-22, which has been shown to play a protective role for instances in models of autoimmune hepatitis and IBD. IL-22 is preferentially induced under Th17/22 polarizing conditions suggesting a Notch-dependent but subset specific transcriptional regulation. Gene expression analysis of sorted IL-22 secreting versus non-secreting T cells identified a number of promising candidate transcription factors that are currently under investigation.

Perspectives:

The Notch pathway displays a powerful molecular switch to induce the expression of the highly anti-inflammatory and tissue-protective cytokines IL-10 and IL-22 in pro-inflammatory Th1 and Th17 cells, respectively. Since Notch is implicated in many cell differentiation processes, deciphering the underlying cell-type specific transcriptional network will be important to develop specific therapeutic intervention strategies.

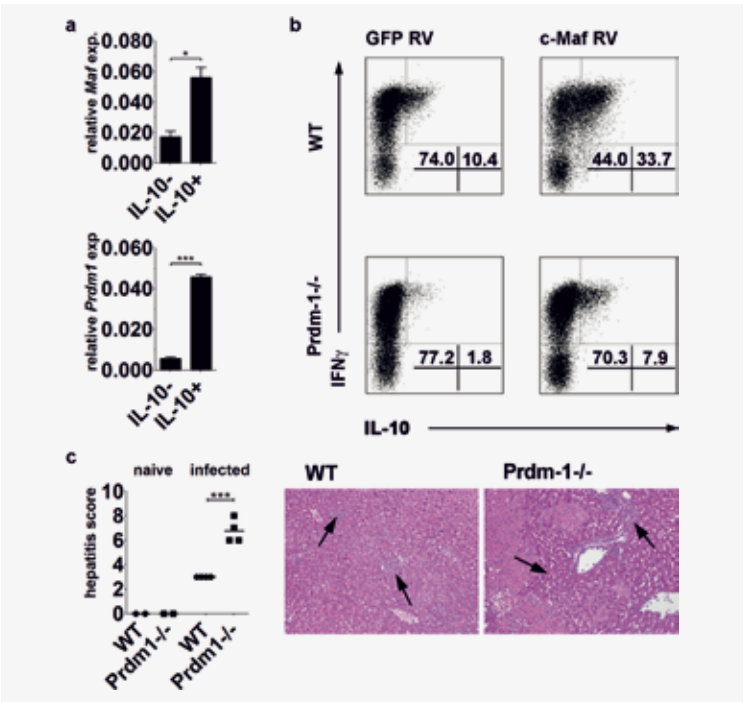

Figure 1: (A) Prdm1 and Maf expression segregates with IL10 secretion in Th1 cells. (B) Ectopic c-Maf expression can not rescue IL-10 production in the absence of Blimp-1. (C) Blimp-1 limits Th1 mediated immunopathology after Toxoplasma gondii infection.

SCIENTISTS

Christian Neumann, Frederik Heinrich, Katrin Neumann, Jonas Ahlers, Victoria Junghans, Ulla Knackmuss

COOPERATION PARTNERS

Dr. Sascha Rutz, Genentech, San Fransisco, USA

Dr. M. Heimesaat, Dr. A. Kühl, Charité – Universitätsmedizin Berlin

Prof. Dr. A. Radbruch, Dr. Mir-Farzin Mashreghi, DRFZ, Berlin

Prof. Dr. Charlotte Esser, Leibniz Research Institute for Environmental Medicine Düsseldorf

Prof. Kathryn Calame, Columbia University, New York, USA

Prof. Sin-Hyeog Im, Gwangju Institute of Science and Technology, Republic of Korea

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The physiological niche for inflammatory T cell modulation

SCIENTISTS

Katrin Neumann, Christine Rudolph, Jonas Ahlers

COOPERATION PARTNERS

A. Hamann, M. Heimesaat, A. Kühl, E. Schott, Charité – Universitätsmedizin Berlin; E. Esplugues, A. Hauser, A. Radbruch, C. Romagnani, Deutsches Rheuma-Forschungszentrum Berlin; A. Thiel, Berlin-Brandenburg School for Regenerative Therapies; A. Beilhack, Universitätsklinikum Würzburg; R. Adams, MPI für molekulare Biomedizin Münster

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FUNDING

SFB633, Teilprojekt A1 & A8; DFG Einzelprojekt

The gastrointestinal organs including the liver are a major site for immunomodulation of inflammatory responses to face the continuous confrontation with gastrointestinal microbiota. Especially the liver has a unique potential for tolerance induction, e.g. upon liver transplantation. We showed that liver sinusoidal endothelial cells (LSEC) induce anti-inflammatory properties in naive and effector/memory CD4⁺ T cells. LSEC and endothelial cells in general are the major source of the Notch ligand Dll4, which we identified as a major immunomodulator of inflammatory T cells. This introduces endothelial cell/T cell interaction sites such as the liver as a physiological niche for inflammatory T cell modulation. We are now analyzing the molecular mechanisms of modulation of T cell responses within the liver and their contribution to maintenance of tolerance and regulation of inflammation.

LSEC modulate naive CD4⁺ T cells

We have shown that LSEC, a population of MHCI^{int}B7^{low} non-professional hepatic APC, can prime antigen-specific naive CD4⁺ T cells. LSEC-activated CD25^{int}IFNγ^{low} CD4⁺ T cells, called T_{LSEC}, have regulatory activity and suppressed proliferation of naive CD4⁺ T cells *in vitro* and hepatic inflammation *in vivo* (1). We demonstrated that the low co-stimulatory signal provided by LSEC during priming is responsible for the tolerogenic phenotype of T_{LSEC}.

LSEC modulate effector/memory CD4+ T cells: Induction of IL-10 expression in Th1 cells by notch signaling

We identified LSEC as a hepatic APC population that constitutively express the Notch ligand Dll4. Stimulation of naive CD4⁺ T cells by LSEC under Th1-polarizing conditions induced IL-10 expression in IFNγ⁺ Th1 cells that was significantly reduced after inhibition of the Notch pathway. Interestingly, despite high levels of Dll4, LSEC induced only low expression of the liver-protective cytokine IL-22 in Th17 cells suggesting additional suppressive mechanisms that need to be clarified.

Perspectives:

We are currently analyzing how LSEC-derived factors, besides Dll4, influence the modulation of T cells *in vitro* and *in vivo*. We also examine how this modulation is influenced in response to inflammation. The specific role of Dll4/notch will be addressed, using *in vivo* Notch blockade or Dll4 ko or transgenic animals (2, 3). Via whole organ or *in vivo* imaging we also want to visualize and localize Dll4/notch signaling in healthy and diseased mice, to identify the physiological niches for T cell modulation.

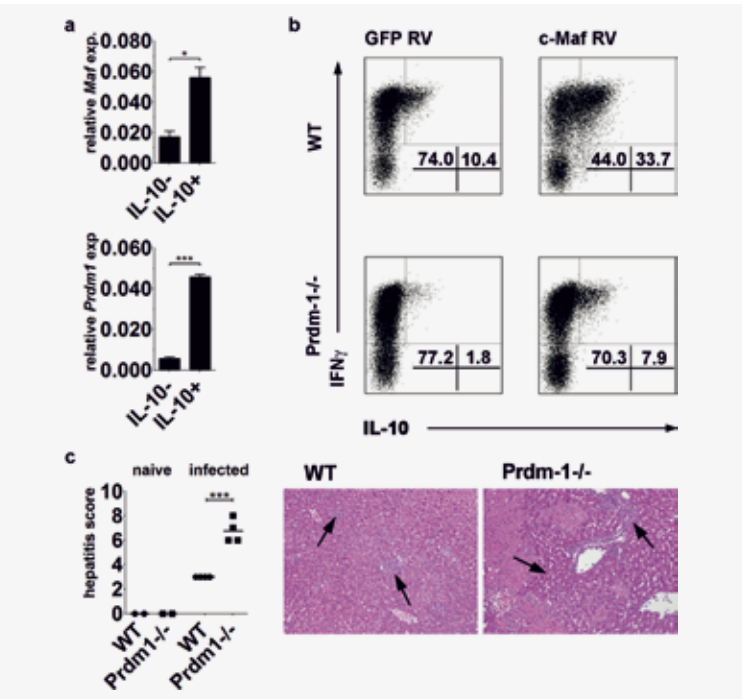

Figure 1: LSEC induce strong IL-10 expression in Th1 cells by notch signaling. (A) Frozen liver samples were stained with LSEC-specific anti-CD146 and anti-Dll4 antibody. Nuclei were stained with DAPI. (B) OVA-specific naive CD4⁺ T cells were cocultured with LSEC under Th1-polarizing conditions in the presence or absence of γ-secretase inhibitor (GSI) for 4 days. Spleen-derived APC (SAPC) served as control. IL-10 mRNA expression and IL-10 production was quantified. IL-10 expression by IFNγ⁺ Th1 cells was determined by intracellular cytokine staining.

Photo: K.Zimmermann

Joachim Sieper

Charité - University Medicine Berlin
Department of Gastroenterology, Infectiology, Rheumatology

Spondyloarthritides

Sooner diagnosis and more effecient treatment of spine diseases

KEYWORDS

T cell response
Antigen-specific response
Immunohistology
Early diagnosis
Targeted therapy

GROUP MEMBERS

Group leader

Prof. Dr. Joachim Sieper

Scientists

Dr. Uta Syrbe
Dr. Hildrun Haibel
Inge Spiller
Dr. Sandra Hermann
Dr. Henning Brandt
PD Dr. In-Ho Song
Dr. Dennis Poddubnyy

Ph.D. students

Janine Bleil
Christopher Sichau
Kristina Conradt

Technical assistance

Peihua Wu
Rene Maier
Beate Buss
Renate Lies
Renate Pauli
Annegret Langdon
Petra Tietz

Students

Esther Abt
Sebastian Leidig

Our main research interest is the pathogenesis, epidemiology, diagnosis and treatment of a group of chronic inflammatory rheumatic diseases termed spondyloarthritides (SpA). This can best be separated according to recent new classification criteria into predominant axial SpA (including patients with ankylosing spondylitis) and into predominant peripheral SpA.

Regarding pathogenesis we focus on the investigation of the immune response, especially on the T cell response. Another major interest is the interaction between inflammation and new bone formation, two major features of this disease. These investigations are based on a unique combination of the analysis of clinical and imaging (x-rays and MRI) data, of histological studies of bone material obtained from the spine of affected patients and on the analysis of biomarkers in the peripheral blood of patients. Our aim is to find a curative treatment for this chronic inflammatory disease and to identify therapeutic options targeting both inflammation and new bone formation.

Another focus is the early diagnosis of patients with SpA. Here we made a major contribution to the development of new classification criteria for both axial and peripheral SpA and to the development of a diagnostic algorithm including MRI investigations. Furthermore, we have developed and tested screening parameters for primary care physicians for patients with potential SpA, a crucial step for making a diagnosis earlier.

Based on the analysis of data from the nationwide GErman SPondyloarthritis Inception Cohort (GESPIC) we contributed to a better understanding to the course of early forms of this disease, including radiographic progression in the sacroiliac joints and the spine.

Finally, we have been involved in the designs and conductions of several crucial studies with biologics in patients with SpA with a special focus on axial SpA. As a result of this work the first TNF-blocker was approved in Europe for the indication of non-radiographic axial SpA in 2012.

COOPERATION PARTNERS

J. Braun, Rheumazentrum Ruhrgebiet, Herne, Germany

G.-R. Burmester, Med. Klinik mit S Rheuma, Charité Mitte, Berlin

J. Listing, A. Zink, Klinische Forschung, Klinische Epidemiologie, Charité Universitätsmedizin Berlin Mitte

A. Grützkau, A. Radbruch, P. Wu, Immunologie, DRFZ Berlin

A. Thiel, Immunologie im BCRT, Charité CVK, Berlin

R. Lories, Universität Leuven, Belgien

G. Schett, Universität Erlangen

D. van der Heijde, Leiden, Niederlande

SELECTED PUBLICATIONS

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GErman SPondyloarthritis Inception Cohort (GESPIC)

SCIENTISTS

Denis Poddubnyy, Hildrun Haibel, Beate Buß, Anja Weiß, Jochanna Callhoff, Joachim Listing, Kristina Conrad, Uta Syrbe, Martin Rudwaleit, Joachim Sieper

COOPERATION PARTNERS

J. Brandt, Rheumatologische Praxis, Berlin

J. Braun, X. Baraliakos, Rheumazentrum Ruhrgebiet, Herne

G.-R. Burmester, E. Feist, Charité Universitätsmedizin Berlin

K. Karberg, Rheumatologische Praxis, Berlin

E. Märker-Hermann, Städtisches Klinikum Wiesbaden

A. Krause, E. Gromnica-Ihle, Rheumaklinik Berlin-Buch

D. Pick, Rheumatologische Gemeinschaftspraxis, Bad Neuenahr

G. Schett, Universität Erlangen-Nürnberg

R. Schmidt, E. Riechers, T. Witte, H. Zeidler, Med. Hochschule Hannover

S. Zinke, Praxis für Innere Medizin/ Rheumatologie, Berlin

PUBLICATIONS

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GESPIC is an ongoing, prospective, longitudinal study on the clinical, functional, and structural outcome of spondyloarthritis (SpA) of short duration (inception cohort). GESPIC was set up in 2000 as part of the German Competence Network Rheumatology program and, as a national multicenter study, involves university hospitals, community hospitals, and private practices. The main focus of GESPIC is on natural course and outcomes in axial SpA (both, ankylosing spondylitis – AS and non-radiographic axial SpA). The first of more than 800 included patients achieved in 2012 the observation duration of 12 years. The baseline characteristics of GESPIC were reported already in 2009 [1], later on we reported data on progression of radiographic sacroiliitis [2]. Here we summarised data concerning prediction of radiographic progression in the spine in patients with axial SpA using clinical parameters and biomarkers, which were obtained and published in 2011-2012.

Prediction of radiographic spinal progression in axial spondyloarthritis

Two hundred and ten patients with axial SpA from GESPIC have been selected for this analysis based on availability of radiographs at baseline and after two years of follow-up. Spinal radiographs were scored by two trained readers in a concealed, randomly selected order according to the modified Stoke Ankylosing Spondylitis Spine Score (mSASSS) scoring system. Radiographic spinal progression was defined as a worsening of the mean mSASSS score by ≥2 units over two years. Altogether, 14.3% of the patients with axial SpA (20% with AS and 7.4% with non-radiographic axial SpA) showed radiographic spinal progression after 2 years. The following parameters were independently associated with radiographic spinal progression: presence of syndesmophytes at baseline (OR = 6.29, $p < 0.001$), elevated markers of systemic inflammation (CRP: OR = 3.81, p=0.001 or erythrocyte sedimentation rate: OR = 4.04, p = 0.001), and cigarette smoking (OR = 2.75, p = 0.012), that was confirmed in the multivariate logistic regression analysis. A predictive matrix model was constructed in order to demonstrate an additive value of the risk factors and in order to define an individual risk of radiographic spinal progression based on the combination of the risk factors – figure 1 [3].

Thus, activity of systemic inflammation seems to be relevant for radiographic progression in the sacroiliac joints and in the spine, while baseline syndesmophytes and cigarette smoking predict radiographic progression in the spine only.

Role of biomarkers in prediction of radiographic spinal progression in axial spondyloarthritis

Despite an overall good performance, the developed matrix model does not predict radiographic spinal progression in axial SpA with a 100% sensitivity and specificity. Over the last years we attempted to find biomarkers, which could have an added value in prediction of such a progression.

So called Wingless (Wnt) pathway has been recognized in the past years as possible link between inflammation and new bone formation in axial SpA. We investigated serum levels of Wnt antagonists Dikkopf-1 and Sclerostin in patients with AS from GESPIC. Both, sclerostin [4] and DKK-1 [5] serum levels were significantly lower in patients with new syndesmophytes formation in comparison to patients without new syndesmophytes after 2 years of follow up, indicating a protective value of Wnt-antagonists in the process of structural damage development in the spine in patients with axial SpA.

Most recently, we investigated a panel of biomarkers reflecting activity of systemic inflammation, bone destruction and new bone formation in patients with axial SpA, who were at high risk for radiographic spinal progression due to the presence of syndesmophytes at baseline and elevated CRP. Baseline serum levels of matrix metalloproteinase 3, bone morphogenetic protein 2, procollagen type II N-propeptide, and vascular endothelial growth factor (VEGF) were significantly higher and osteoprotegerin serum level was significantly lower in patients with new syndesmophyte formation after two years as compared to patients without radiographic progression [6]. Of these, VEGF demonstrated the strongest association with radiographic spinal progression: in patients with syndesmophytes at baseline, VEGF serum level of >600 pg/ml had a sensitivity of 53%, a specificity of 97%, and an OR = 36.6, $p < 0.05$ as a predictor of mSASSS worsening by ≥2 units over 2 years (after adjustment for elevated CRP and smoking OR was 37.2, $p < 0.05$). The same serum level of VEGF demonstrated a sensitivity of 47%, a specificity of 94%, and an OR = 13.6, $p < 0.05$ as a predictor of syndesmophyte formation (after adjustment for CRP and smoking OR = 14.0, $p < 0.05$) [7]. Thus, VEGF is a biomarker, which might be useful for prediction of radiographic spinal progression and following therapeutic decision in patients who are at

risk for such a progression due to the presence of baseline syndesmophytes with or without further risk factors.

Non-steroidal anti-inflammatory drugs (NSAIDs) and prevention of radiographic spinal progression in axial spondyloarthritis

Prevention of radiographic spinal progression in axial SpA is an important therapeutic goal, since development of structural damage in the spine is associated with limitation of spinal mobility and impaired function. We analyzed in GESPIC an association between NSAIDs intake and radiographic spinal progression over two years in axial SpA. In total, 164 patients with axial SpA (88 with AS and 76 with non-radiographic axial SpA) were selected for this analysis based on availability of spinal radiographs at baseline and after 2 years of follow-up and of the data on NSAIDs intake. An index of the NSAID intake (ASAS NSAIDs intake score) counting both dose and duration of drug intake was calculated. High NSAIDs intake (NSAIDs intake score ≥50 meaning that patients took at least 50% of the maximal recommended dose of the NSAID over 2 years) in AS was associated with lower likelihood of significant radiographic progression defined as an mSASSS worsening by ≥2 units: OR = 0.15, p = 0.045 (adjusted for baseline structural damage, elevated C-reactive protein and smoking status) in comparison to patients with low NSAIDs intake (NSAID index <50). This effect was most pronounced in patients with baseline syndesmophytes plus elevated CRP: mean mSASSS progression was 4.36 ± 4.53 in patients with low NSAIDs intake vs 0.14 ± 1.80 with high intake, p = 0.02 – figure 2 [8].

Thus, data from GESPIC provided important evidences in support of the disease-modifying efficacy of the NSAIDs in axial SpA. Furthermore, we identified a group of patients (patients with risk factors for radiographic spinal progression – syndesmophytes and elevated CRP) who might especially benefit from continuous NSAIDs therapy.

Perspectives:

The project is ongoing and included patients will be continuously evaluated. An analysis of the rates and predictors of radiographic progression after 4 years (spine and sacroiliac joints) is ongoing now. A comprehensive analysis of the biomarker data and a development of an improved predictive model are underway. Magnetic resonance imaging of the sacroiliac joints and of the spine is included in the investigational plan for patients who remain in the stage of the non-radiographic axial SpA for more than 4 years starting from baseline. Clinical outcomes of non-radiographic axial SpA and AS over time, clinical relevance of radiographic progression, patients who demonstrate especially rapid radiographic spinal progression, evolution of the disease and therapy are further research aims.

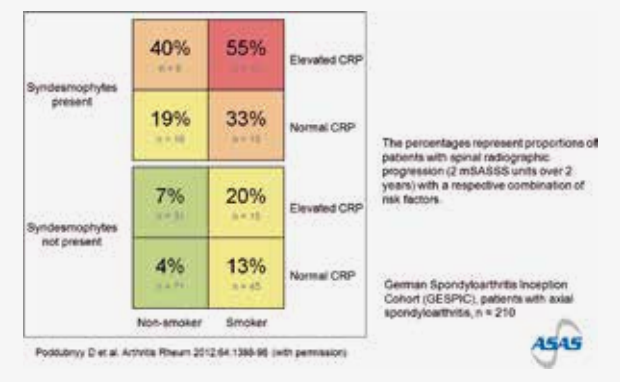

Figure 1: A matrix model for prediction of radiographic spinal progression in axial spondyloarthritis.

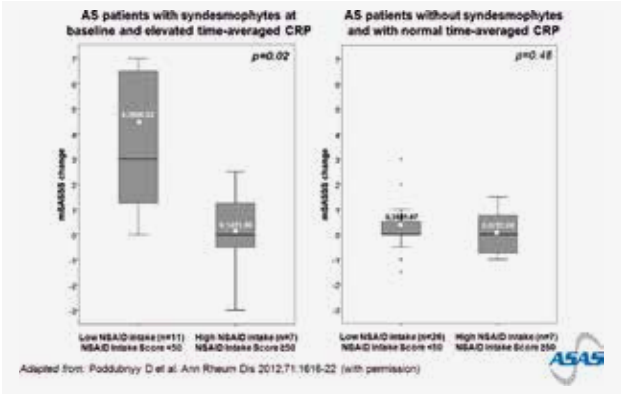

Figure 2: Effect of NSAIDs on radiographic spinal progression in AS patients with and without risk factors for such a progression.

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FUNDING

As part of the German competence network in rheumatology (Kompetenznetz Rheuma), GESPIC has been financially supported in 2000-2007 by the German Federal Ministry of Education and Research (Bundesministerium für Bildung und Forschung – BMBF). After 2007 financial support has been obtained also from Abbott, Amgen, Centocor, Schering-Plough, and Wyeth. Since 2010 additional support has being obtained also from ANCYLOSS (grant number FKZ 01EC1002D) and ArthroMark (grant number FKZ 01EC1009A) projects funded by BMBF.

Prospective clinical drug trials in axial Spondyloarthritis

SCIENTISTS

In-Ho Song [1]
Joachim Listing [2]
Anja Weiß [1]
Hildrun Haibel [1]
Denis Poddubnyy [1]
Martin Rudwaleit [1]
Joachim Sieper [1;2]

[1] Charité Universitätsmedizin Berlin, Campus Benjamin-Franklin, Med. Klinik I / Rheumatologie

[2] German Rheumatism Research Centre

ESTHER trial ("Effects of Etanercept versus Sulfasalazine in early axial spondyloarTHritis on active inflammatory lEsions as detected by whole body MRI") [1]

The ESTHER trial is a prospective randomised controlled trial in which patients with early axial spondyloarthritis (SpA) with a disease duration of less than 5 years were included. 40 patients were randomised to treatment with the tumor necrosis factor alpha (TNFα)-inhibitor etanercept (ETA, enbrel® 25mg s.c. twice weekly) vs. 36 patients who were randomised to treatment with sulfasalazine (SSZ, 2-3g p.o.). At screening all patients needed to have active inflammatory lesions on magnetic resonance imaging (MRI) in the sacroiliac joints (SI-joints) and / or the spine. The primary endpoint of this study was the reduction of active inflammation on MRI. This was significantly more often reached by ETA-treated patients compared to SSZ-treated patients [3]: the SI-joint MRI score in the ETA-group decreased from 7.7 at baseline to 2.0 at week 48 (p=0.02) compared to a decrease from 5.4 to 3.5 in the SSZ-group. Similar changes could be found in the spine (change from 2.2 to 1.0 in the ETA-group vs. 1.4 to 1.3 in the SSZ-group, p=0.01). Also the reduction of enthesitic sites on MRI was significantly more pronounced in the ETA-group (from 26 to 11) compared to the SSZ-group (24 to 26) (p=0.04 for the difference). Furthermore 50% of ETA-treated patients went into remission compared to only 19% in the SSZ-group at week 48.

Similar response rates in patients with ankylosing spondylitis and non-radiogrpahic axial SpA after one year of treatement with etanercept- results from the ESTHER study [2]

In a subgroup analysis we analysed whether there is a difference in the response between patients with AS compared to patients with non-radiographic axial SpA (nr-axSpA) [3] during treatment with ETA [4]. Clinical, laboratory and MRI data were analysed.

At baseline there were no significant differences between the 20 patients with AS and 20 patients with nr-axSpA regarding age (34.8 (SD 8.2) vs. 34.3 (SD 9.1 years), gender (60% vs. 55% male), HLA-B27 positivity (90% vs. 80%) and parameters of disease activity. Only MRI scores were slightly but not significantly higher in the AS group. After 48 weeks of ETA treatment there was a small but not significant benefit in in favour of the nr-axSpA group in nearly all outcome parameters (Figure 1).

Thus, a similar response was found in patients with early axial SpA who were treated with ETA. Thus, the response rate to TNF-blockers does not differ between AS and nr-axSpA if the baseline data regarding symptom duration and disease activity are similar for the two groups.

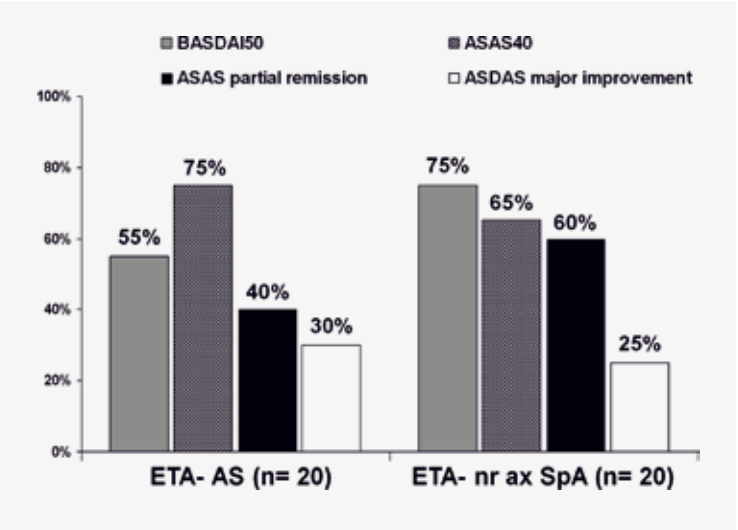

Figure 1: Comparison of clinical response in the group with ankylosing spondylitis (AS, n= 20) versus non-radiographic axial spondyloarthritis (nr-axSpA, n=20) who were treated with etanercept for one year.

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Frequency and duration of drug-free remission after 1 year of treatment with etanercept versus sulfasalazine in early axial spondyloarthritis: 2 year data of the ESTHER trial [4]

In the 2-year data of the ESTHER trial we assessed:(1) the frequency and duration of drug-free remission and efficacy ETA treatment after flare in patients with early active axial SpA who were treated with ETA (n=40) versus SSZ n=36) for 48 weeks and (2) the efficacy of ETA treatment in patients in year 2 who did not reach remission at week 48.

At week 48, patients who reached study remission (Assessment of Spondyloarthritis international Society (ASAS) plus MRI remission) were followed up without active treatment up to 1 year. In case of a flare, patients were treated with ETA for another year. All patients who were not in ASAS plus MRI remission at week 48 were treated with ETA in year 2.

ASAS plus MRI remission at week 48 was reached significantly more often in ETA-treated compared to SSZ-treated patients (33% vs. 11%, p=0.03) (Figure 2). However, the flare rate was not different between these two groups: 69% in the ETA group versus 75% in the SSZ group. Only 8% of patients initially treated with ETA versus 3% of those initially treated with SSZ reached permanent drug-free remission (not significant) (Figure 2). After treatment with ETA over 1 year, patients with flare showed an improvement in all clinical and imaging variables.

Thus, patients with axial spondyloarthritis treated with ETA over 1 year did not reach drug-free remission in a higher percentage compared to patients from a control group treated with SSZ.

In another prospective clinical trial patients with AS who had received a first course of rituximab treatment were followed [5].

Some patients with active AS had shown a good clinical response at week 24 after a first course of rituximab treatment [6].The aim of this study was to assess the efficacy of rituximab during a follow-up period.

Patients (n= 9) who had shown a response to a first course of rituximab were observed for 24 weeks. In case of a flare patients received a second course of rituximab (two infusions with each 1000mg of rituximab) and were followed for another 48 weeks.

4 patients showed a continuous and stable response until week 48 without re-treatment with a mean BASDAI (Bath AS Disease Activity Index) of 2.7 (±1.7) and a mean ASDAS (AS Disease Activity Score) of 1.9 (±0.5) at week 48. In 5 patients who received a second course of rituximab after a flare a significant clinical response could be observed with a reduction of the BASDAI from 4.2 (±1.6) to 1.7 (±1.5) and of the ASDAS from 2.9 (±1.0) to 1.3 (±0.2).

Thus, active AS patients who showed an initial response to rituximab treatment showed a good and sustained clinical response with or without a second course of rituximab. These data seem to confirm that rituximab could be a treatment alternative in TNF-blocker-naïve patients.

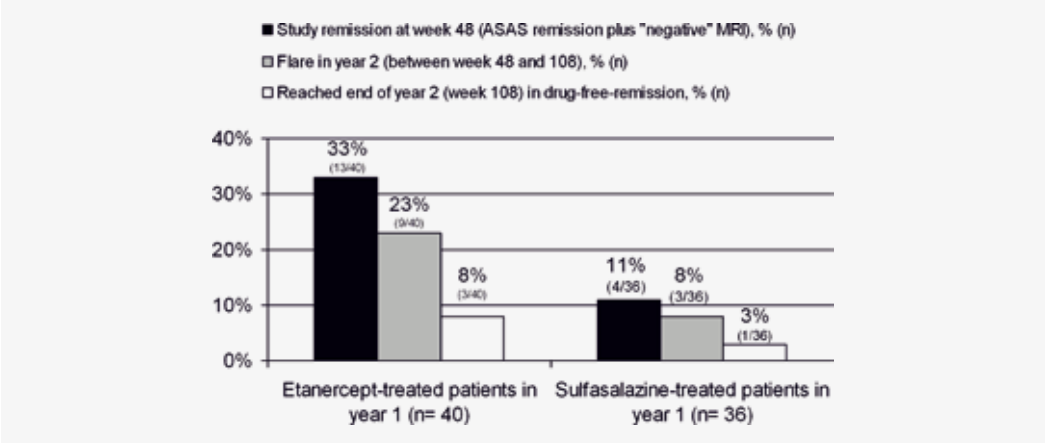

Figure 2: The percentage (number) of patients reaching ASAS plus MRI remission at week 48 in ETA-treated patients (n=40) versus SSZ treated patients (n=36) is shown, as well as the percentage (number) of patients who experienced a flare during year 2 or who stayed in drugfree remission until the end of year 2 (permanent drug-free remission).

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FUNDING

The ESTHER trial was financially supported by Pfizer (ehemals Wyeth). Pfizer also provided the study drug etanercept.

The rituximab trial was financially supported by Roche Roche Pharma AG who also provided the study drug rituximab.

Pathogenesis of spondyloarthritis: Mechanisms of inflammation and new bone formation

SCIENTISTS

H. Appel, J. Bleil, K. Conrad, R. Maier, C. Sichau, J. Sieper, U. Syrbe, P. Wu

COOPERATION PARTNERS

U. Schlichting – Charité Universitätsmedizin

G. Schett – Universität Erlangen

A. Hempfing - Werner-Wicker-Klinik, Bad Wildungen

U. Müller-Ladner/E.Neumann - Justus-Liebig-Universität Gießen

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FUNDING

Immunobone SPP1468

SFB 633 TPA4

In this project we investigate the mechanisms of inflammation and new bone formation involved in spondyloarthritis. The aims are I) to identify the triggers of inflammation, the involved immune cells and crucial cytokines and II) to investigate the mechanisms which lead to new bone formation, a hallmark of ankylosing spondylitis (AS), the prototypic form of spondyloarthritis. With this work we hope to identify new disease-specific therapeutic targets.

Triggers and mechanisms of inflammation

Despite spinal inflammation a subclinical inflammation within the intestine is observed in about 50 % of the patients with AS. This suggests that mucosal antigens might act as inflammatory triggers in AS (1). Therefore, we analyzed the frequency of $CD4^+$ effector cells, which react to commensal mucosal bacteria (Escherichia (E.) coli) in AS patients. We observed an enrichment of E. coli-reactive $CD4^+$ effector cells within inflamed joints of patients with AS (and peripheral joint involvement) but not in patients with rheumatoid arthritis (Figure 1) [1.2]. This indeed points to a potential role of mucosal antigens in triggering inflammation in AS.

Since genome-wide association studies suggested a pathogenic role of the IL-23-IL-17-pathway in AS (2) we analyzed the frequency of $IL\text{-}17^+$ and $IL\text{-}23^+$ cells in facet joints of AS patients. We found a higher numbers than in controls of $IL\text{-}17^+$ cells but also of $IL\text{-}23^+$ cells within the subchondral bone marrow of AS patients (Figure 2) [3.4]. Interestingly, most of the $IL\text{-}17^+$ cells belonged to the myeloid lineage and only a very minor part was $CD3^+$.

Mechanisms of new bone formation

We performed histomorphometric analysis and determined the expression of cartilage and bone differentiation markers to identify pathways involved in new bone formation in AS. We found no clear indication for the involvement of enchondral new bone formation in joint remodeling in AS; our data rather point to a dominant role of subchondral bone marrow fibrosis in driving this process.

Perspectives:

Our data support the view that the IL-23-IL-17 pathway is involved in the pathogenesis of ankylosing spondylitis making it an attractive therapeutic target in AS.

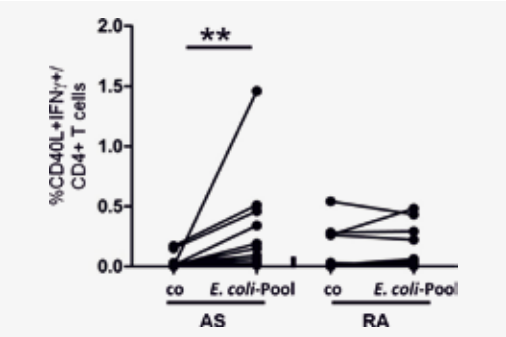


Figure 1: Frequency of *E. coli*-reactive $CD4^+$ T cells determined according to CD40L and IFNg expression in stimulations without (co) or with a pool of *E. coli*-specific proteins (*E.coli*-Pool) in synovial fluid of patients with ankylosing spondylitis (AS) or rheumatoid arthritis (RA). ** p<0.01, Mann Whitney U Test

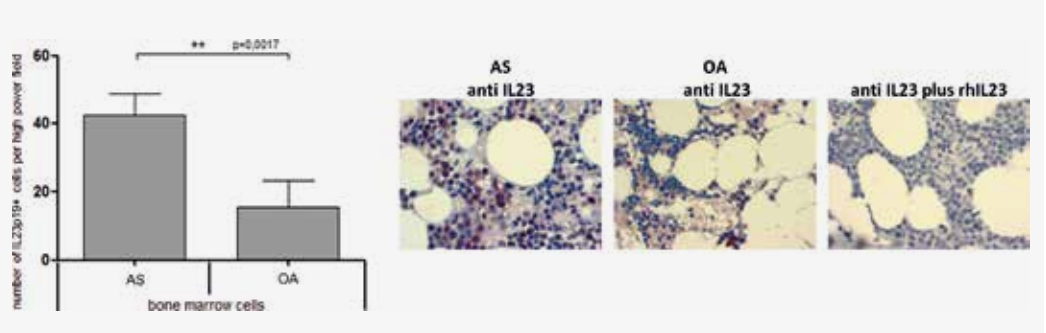


Figure 2: Left panel: Number (mean+ SD) of $IL23p19^+$ cells in facet joints of patients with ankylosing spondylitis (AS) or osteoarthritis (OA). Right panel: representative examples of IL-23p19-stainings of facet joints of AS and OA patients and a control staining after blockade with recombinant human IL-23. .

Photo: K.Zimmermann

Margitta Worm

Charité - University Medicine Berlin (CCM)
Allergie-Center, Department of Dermatology, Venerology and Allergology

Allergology

Novel therapeutic approaches of type-I allergy by immunomodulation

KEYWORDS

Allergy
IgE
vitamin D
fatty acids
plasma cells

GROUP MEMBERS

Group leader
Prof. Dr. med. Margitta Worm (Allergie-Centrum-Charité. Klinik für Dermatologie, Venerologie und Allergologie)

Prof. Dr. rer. nat. Andreas Radbruch (DRFZ)

Scientists
Dr. med. Guido Heine
Dr. rer. nat. Magda Babina
Dr. rer. nat. Christin Weise
Dr. rer. medic. Sabine Dölle

PhD. Students
Dipl. biol. Genndiy Drozdenko
Dipl. biol. Friederike Thiele
Dipl. biol. Juliane Lindner
Kiran Kumar, M.Sc.
Vandana Kumari, M.Sc.

Technical assistance, Student apprentices and others
Dennis Ernst (MTA)
Ariane Lungwitz (Study Nurse)
Tim Hollstein (M.D. student)

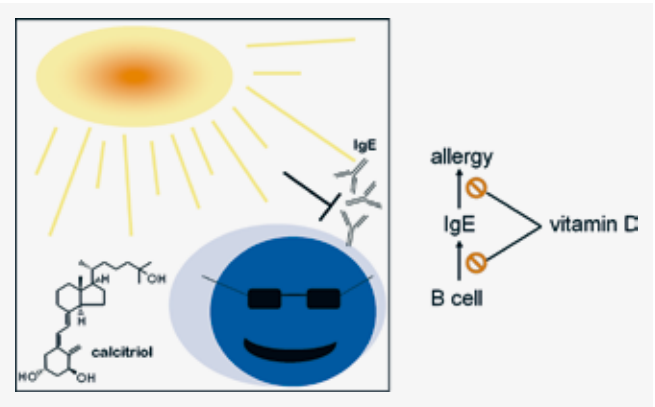
„Allergology" group is focussed on immunomodulation of IgE-dependent type I allergies. Currently, approx. 20% of the German population are affected by hay fever, allergic asthma or atopic dermatitis. Specific immunotherapy is the only established causal treatment, but limited by a long treatment time, suboptimal responder rates and a potential risk of relapse. Our research is focussed on the understanding of molecular and cellular key events for the development and maintenance of IgE production. Special interests among our group include immunomodulatory functions of nuclear receptor ligands and bioactive lipids, but also the biology of long-lived plasma cells in allergy.

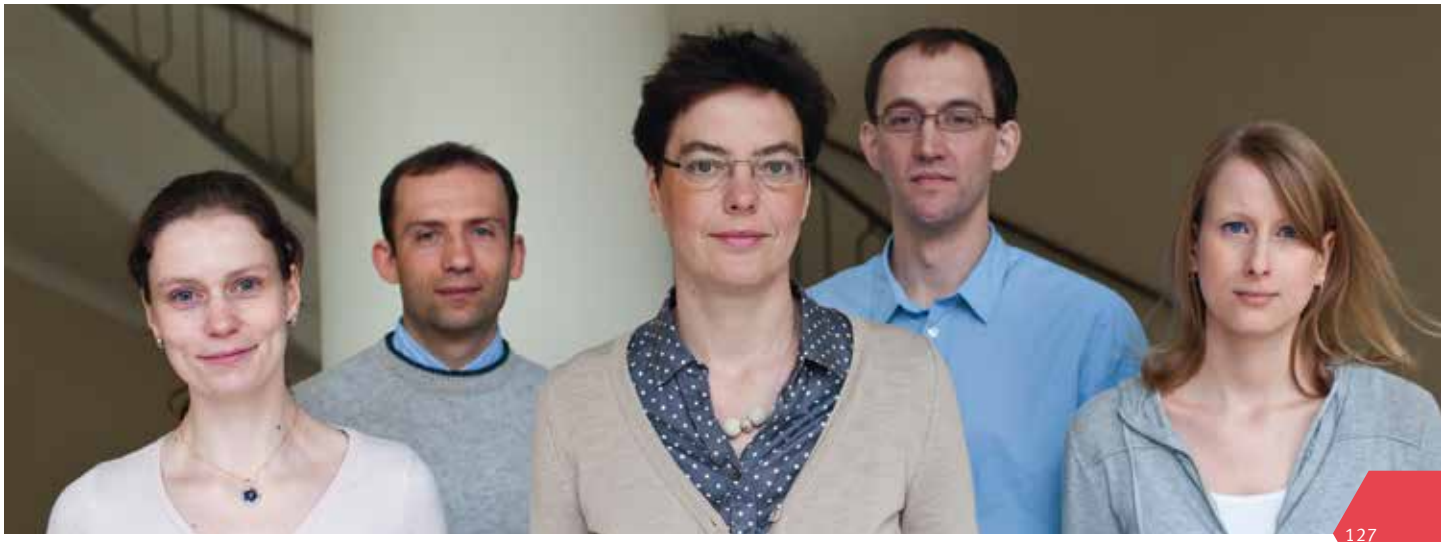
Nuclear receptor ligands include molecules like vitamin D and retinoids. We have shown that vitamin D modulates profoundly the initial B cell activation and hampers the IgE response. Currently, we study in detail molecular and cellular signalling and also initiated a clinical translation program. In these studies, we target vitamin D receptors in immune cells *in vivo* and determine the impact on specific allergic symptoms. Our overall aim is to achieve long-term tolerance to allergens.

Bioactive lipids display immune modulatory features and currently we investigate their cellular localization and binding partners in relevant target cells.

The biology of plasma cells in allergy is important as long lived plasma cells may be a reason for promoting a life long disease. We have shown that mucosal challenge triggers the generation of long-lived IgE secreting plasma cells. Currently we study B cell deleting strategies on outcome of allergies.

The overall perspective of our research is to develop novel strategies for innovative treatment protocols in allergy. We transfer our experimental data into clinical trials (ad hoc translation).





COOPERATION PARTNERS

Prof. René St. Arnaud, Shriners Hospital for Children, Genetics Unit, Montréal, Canada

Prof. Dr. rer. nat. Ria Baumgrass, Deutsches Rheuma-Forschungszentrum Berlin, Cell signaling group, Berlin

Prof. Dr. med. Thomas Dörner, Med. Klinik mit Schw. Rheumatologie und Klinische Immunologie, Charité – Universitätsmedizin Berlin

Prof. Dr. vet. Reinhold Erben, Universität für Tiermedizin, Institut für Pathophysiologie, Wien, Österreich

PD Dr. rer. nat. Lutz Hamann, Charité – Universitätsmedizin Berlin, Institut für Mikrobiologie und Hygiene CCM, Germany

Prof. Dr. rer. nat. Susanne Hartmann, Institut für Immunologie, FB Veterinärmedizin, Freie Universität Berlin Berlin, Germany

Prof. Dr. med. Bastian Opitz, Prof. Dr. med. Martin Witzenrath, Christoph Tabeling, Charité – Universitätsmedizin Berlin, Med. Klinik mit Schw. Infektiologie und Pneumologie, Germany

Prof. Dr. med. Harald Renz, Philipps-University of Marburg, Dept of Clinical Chemistry and Molecular Diagnostics, Marburg, Germany

Prof. Dr. rer. nat. Michael Reth, MPI Immunbiology and Epigenetics, Freiburg, Germany

Prof. Dr. med. Gabriele Riemekasten, Charité – Universitätsmedizin Berlin, Med. Klinik mit Schw. Rheumatologie und Klinische Immunologie, Germany

Dr. rer. nat. R. Rühl, University of Debrecen, Medical and Health Science Centre, Dept. Biochemistry and Molecular Biology, Debrecen, Hungary

Prof. Dr. med. Birgit Sawitzki, Charité – Universitätsmedizin Berlin, Institut für Medizinische Immunologie, Germany.

Prof. Dr. med. Ralf Schumann, Charité – Universitätsmedizin Berlin, Institut für Mikrobiologie und Hygiene CCM, Germany

Prof. Dr. med. Hans-Dieter Volk, Charité – Universitätsmedizin Berlin, Klinik für Medizinische Immunologie, Germany

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Baumgrass, R., C. Brandt, F. Wegner, M. Abdollahnia, and M. Worm. 2010. Low-dose, but not high-dose, cyclosporin A promotes regulatory T-cell induction, expansion, or both. J Allergy Clin Immunol 126:183-184.

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Immunmodulation by nuclear receptors

SCIENTISTS

G. Heine, G. Drozdenko, F. Feutlinske, J. Lindner, A. Radbruch, M. Worm

COOPERATION PARTNERS

R. St. Arnaud, Shriners Hospital for Children, Montréal, Canada

R. Baumgrass, A. Hutloff, Rheuma-Forschungszentrum Berlin.

T. Dörner, G. Riemekasten, Lutz Hamann, B. Opitz, M. Witzenrath, C.Tabeling, B. Sawitzski, H-D Volk, Charité – Universitätsmedizin Berlin

R.G. Erben, Institut für Pathophysiologie, Wien Österreich

S. Hartmann, Institut für Immunologie, Fachbereich Veterinärmedizin Freie Universität Berlin

H. Renz, Philipps-University of Marburg

M. Reth, MPI Immunbiology and Epigenetics, Freiburg

The nuclear receptor ligand vitamin D mediates tolerogenic functions on adaptive immunity, which are relevant in type-I allergy. We demonstrated profound and long-lasting action of vitamin D on B cells including inhibition of IgE- and enhancing IL-10 expression. Currently, we investigate the molecular and cellular basis for the development of allergen-specific tolerance and the functional outcome in preclinical models. We initiated clinical pilot trials and established a protocol to detect vitamin D-responsive immune cells ex vivo. Aim of our research is the development of novel anti-allergic treatment protocols that include the targeted application of vitamin D metabolites and the direct translation of experimental data to clinical protocols.

The active metabolite of vitamin D, calcitriol (chem. 1,25-dihydroxyvitamin D), mediates tolerogenic functions on adaptive immune reactions [1] and is involved in the regulation of type-I allergy. Most calcitriol-functions are mediated after binding to its nuclear receptor and regulation of target gene transcription. We demonstrated that vitamin D receptor expression is inducible in human and murine B cells by specific activation that is dependent on T cell help and the cytokine IL-4. Interestingly, these cells can also synthesize active calcitriol from its inert precursor (Heine et al. 2008). These findings provide the molecular basis for the hypothesis that B cell activation is controlled in a vitamin D-dependent manner, e.g. in type-I allergy.

Type-I allergic symptoms are elicited after binding of an allergen to surface-bound specific IgE on effector cells [2]. Hence, IgE is an essential effector molecule in type-I allergy and the control mechanisms that lead IgE production are of major interest. An initial event is the immunoglobulin isotype class switch recombination to IgE in B cells, which depends on IL-4 from specific Th2-cells [3]. Still, the only causal treatment of type-I allergies is allergen-specific immunotherapy [4] that induces allergen-specific tolerance, but it is limited by long-treatment time, responder rates and risk of relapse. Our group is focussed to investigate novel molecular and cellular mechanisms that mediate long-term allergen-specific tolerance considering vitamin D.

Genomic interaction of the vitamin D receptor with target genes control type-I allergy.

We previously reported that ***ex vivo*** induced IgE expression is blocked by activated vitamin D receptors (VDR); either using the natural ligand calcitriol, but also synthetic low-calcemic derivates (Hartmann et al. 2011). We identified a VDR-binding site in the IgE-switch promoter region (sε) and proved binding by chromatin immunoprecipitation (ChIP), Fig. 1 and (Milovanovic et al. 2010). In consequence, the IgE switch transcript expression, which essential for IgE class switch recombination, is blocked by a transrepressive VDR-DNA complex including SMRT (*silencing mediator of retinoid and thyroid receptors*) and histone deacylases (HDAC), Fig.1. Accordingly, the humoral allergen-specific IgE response was strongly

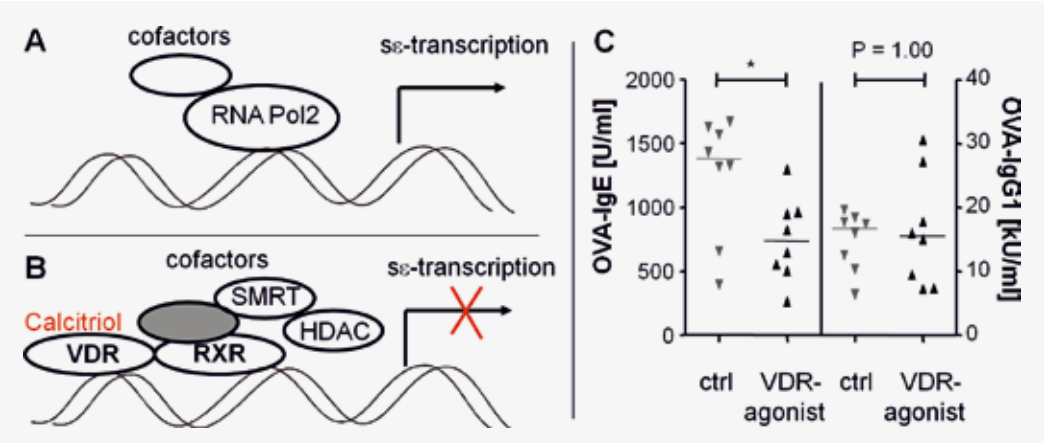

Figure 1: Vitamin D-mediated inhibiton of IgE.
(A) Transcription of sε initiates IgE class switching (B). Active vitamin D (calcitriol) recruits a transrepressive complex to sε. (C) Proof-of-principle: a synthetic VDR agonist inhibits the OVA-specific humoral IgE, but not IgG1 response in BALB/c mice.

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reduced upon VDR activation using a synthetic agonist in mice in a proof-of-principle experiment (Hartmann et al. 2011). However, therapeutic use of VDR-agonists is limited by side effects and short half-life (approx. 2 h).

We described that activated B cells can synthesize active vitamin D (calcitriol) from its inert precursor upon activation (Heine et al. 2008), which was reproduced by others in the meantime [5]. Thus, these activated B cells become independent of exogenous calcitriol in the presence of sufficient concentrations of provitamin D. We observed that provitamin D deficiency is frequent and pronounced during the winter months, s. Fig.2A (Heine et al. 2010), as provitamin D-synthesis is UV-dependent. An initial pilot trial demonstrated that daily vitamin D-intake improves the provitamin D status and was well tolerated (Heine et al. 2011), but the exact provitamin D serum concentration for immunological function was not clear. Therefore, we performed a dose-escalation study in which vitamin D-responsive immune cells were monitored, Fig.2B. Indeed, the frequencies of B cells that express vitamin D-inducible surface antigens (as surrogate markers for vitamin D-activity) were increased in the peripheral blood in the vitamin D group, but not in controls, Fig.3C.

The data unravelled to us the immunological provitamin D threshold concentration, which is an essential step for further clinical trials. Currently, we investigate the impact of provitamin D on the allergen-specific immune response in a controlled clinical pilot trial (www.clinicaltrials.gov; ProGIT, end in Autumn 2014).

Aim of our work is to determine the immunomodulatory potential of activated vitamin D receptors on type-I allergy, to understand the mechanism and to develop novel clinical protocols. In perspective we want to determine the cellular and molecular mechanisms involved in vitamin D-regulation of allergies, enhance long-term allergen-specific tolerance induction and thus clinical course of type-I allergies.

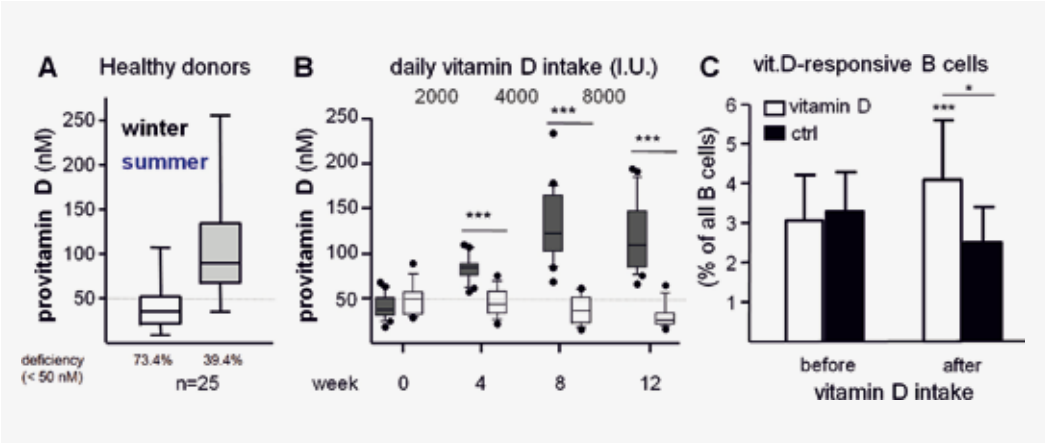

Figure 2: Targeting immune cells by exogenous provitamin D.
(A) Provitamin D deficiency is frequent and pronounced during winter. (B) Vitamin D intake improves the provitamin D status. (C) Increased frequencies of vitamin D-B cells upon vitamin D intake.

PUBLICATIONS

Milovanovic, M., G. Heine, W. Hallatschek, B. Opitz, A. Radbruch, and M. Worm. 2010b. Vitamin D receptor binds to the epsilon germline gene promoter and exhibits transrepressive activity. J Allergy Clin Immunol 126:1016-1023.

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FUNDING

DFG, Investitionsbank Berlin

Fatty acids and allergy

SCIENTISTS

C. Weise, S. Dölle, A. Radbruch, M. Worm

COOPERATION PARTNERS

R. Rühl, University of Debrecen, Hungary

Western lifestyle and dietary habits contribute to the globally growing incidence of type-I allergy. Thereby, the increased consumption of vegetable oils containing high omega-6 (n-6) polyunsaturated fatty acids (PUFA) and the decrease of n-3-PUFA-rich fish is characteristic resulting in a dietary n-6:n-3-PUFA-ratio which is tady 15- to 20-times higher than in the traditional diet (1). This n-6:n-3-PUFA-ratio may promote allergic diseases including atopic dermatitis by proinflammatory n-6-PUFA-mediators (2). In this project, we investigate the mechanisms of bioactive fatty acids to develop novel preventive and therapeutic approaches in allergic diseases.

Introduction:

Atopic dermatitis is a frequent chronic-remittent inflammatory skin disease with polygenetic and environmental pathophysiology (3). Breast-milk contains an optimal combination of the PUFA arachidonic acid (AA; C20:4n-6) und docosahexaenoic acid (DHA; C22:6n-3), which are important for the maturation of the immune system (4, 5). Interestingly, the severity of atopic dermatitis was associated to a disturbed n-6-PUFA-metabolism (6). This suggests that a dietary supplementation of anti-inflammatory n-3-PUFA should be combined with n-6-PUFA for optimal efficacy.

Results and discussion:

Accordingly, the combination of DHA with AA, but not each PUFA alone, reduced significantly the allergen-induced dermatitis in our established mouse model (Fig. 1A) (12). This amelioration was associated with increased Foxp3$^+$ regulatory T cell infiltration, anti-inflammatory IL-10 expression and reduced keratinocyte proliferation in skin lesions. Detailed *in vitro* studies identified direct effects of DHA/AA on keratinocyte function leading to an enhanced IL-10 expression (Fig.1B). However, *in vivo* indirect mechanisms are likely as well, e.g. by regulatory T cells, as the *in vitro* keratinocyte proliferation was unaffected by these PUFA.

Perspectives:

These data demonstrate the immunomodulatory potential of PUFA and the dependency on time point, dose and PUFA-composition of supplementation. Currently, we are investigating additional relevant PUFA and their mediators for optimization of preventive and therapeutic concepts.

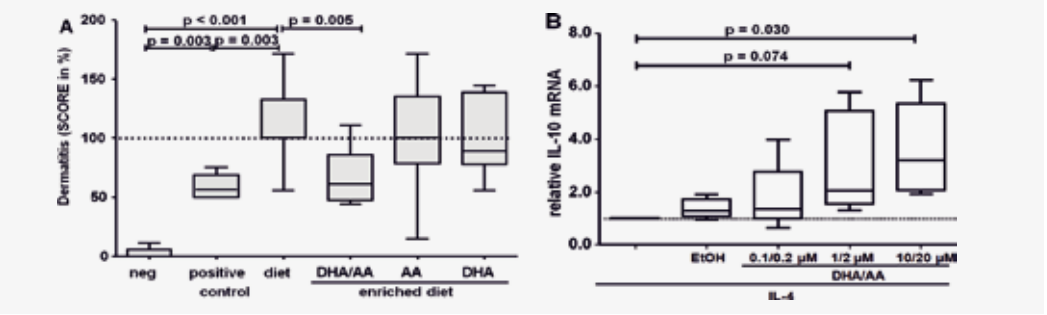

Figure 1: Fatty acids control allergen-induced dermatitis.
A) DHA/AA–enriched food reduced allergen-induced dermatitis in Balb/c mice (n≥8, normalized to diet control). B) Fatty acids induce IL-10 expression in keratinocytes (n=5, normalized to PUFA-negative control).

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FUNDING

Charité – Universitätsmedizin Berlin

IgE secreting plasma cells in type-I allergy

The central molecule of type-I allergy is immunoglobulin E (IgE) results from interaction of allergen-specific Th2 cells with B cells and their isotype class switch recombination to IgE. After differentiation to plasmablasts, IgE is secreted in the serum and binds to the surface receptors of effector cells, e.g. mast cells. During allergen-exposition, e.g. pollen season, specific IgE serum concentrations are transiently increased. This is most likely related to the antigen-dependent generation of novel short lived plasma blasts from memory B cells and the short IgE-half-life of 12 h. Thus, other cells maintain specific IgE concentrations and allergy in the off-season, namely long-lived plasma cells.

Long-lived IgE plasma cells were discovered in 1983[1] and are considered therapy resistant until now towards conventional approaches such as allergen-specific immunotherapy, cyclophosphamide, cyclosporine or anti-CD20 B cell depletion. A lack of proliferation and migration as well as surface CD20 down regulation may be responsible. To date, the existence of a plasma cell survival niche is discussed which provides specific cytokines and survival signals[2]. We could show that long-lived allergen-specific IgE plasma cells are generated by repeated allergen-inhalation (Luger et al. 2010). These cells reside in the bone marrow and also spleen and were resistant to chemotherapeutic drugs.

Recently was discovered that pharmacological inhibition of immune proteasomes, e.g. using bortezomib, eliminates pathogenic plasma cells leading to clinical remission in mice[3]. We established a protocol of plasma cell depletion in a murine allergy model, Fig.1A. The data show that allergen-specific and total IgE serum concentrations are strongly decreased following bortezomib treatment in type-I sensitized mice Fig.1B. In ongoing experiments we further investigate the impact of B cell depletion on the clinical course of allergy in preclinical models. We further aim to improve our understanding of the physiologic role of plasma cells for the maintenance of allergies with the overall goal to develop novel anti-allergic treatment strategies.

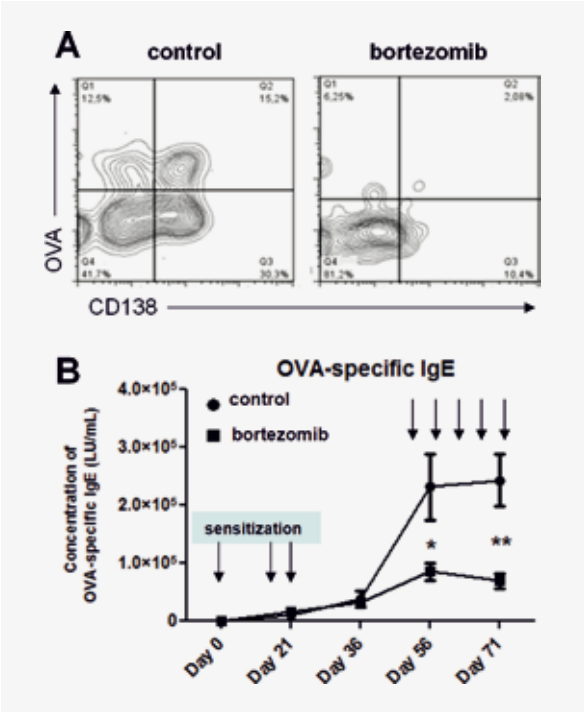

Figure 1: Impact of plasma cell depletion on IgE serum concentrations. A) Plasma cells (OVA+CD138+) were largely depleted by bortezomib in the spleen. Flow-cytometric plots are gated on live single B220-IgGhi cells. B) Serum IgE concentrations after Bortezomib treatment (5 times).

SCIENTISTS

K. Kumar, E.O. Luger, A. Radbruch, M. Worm

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FUNDING

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Program Area 2

Epidemiology of rheumatic diseases

Listing, Statistics & Clinical Studies 134
Minden, Pediatric Rheumatology 138
Strangfeld, Pharmacoepidemiology 142
Westhoff, Prognostic Studies in Early Arthritis 146
Zink, Health Services Research 150

Joachim Listing

Statistics & Clinical Studies

What does a study tell us?

KEYWORDS
clinical studies
statistical methods

GROUP MEMBERS

Group leader
Dr. Joachim Listing

Scientist
Anja Weiß
Johanna Callhoff
Dörte Huscher

Medical data manager
Claudia Fritz

Research assistent
Sabine Achtelstetter

Student
Christoph Thomann

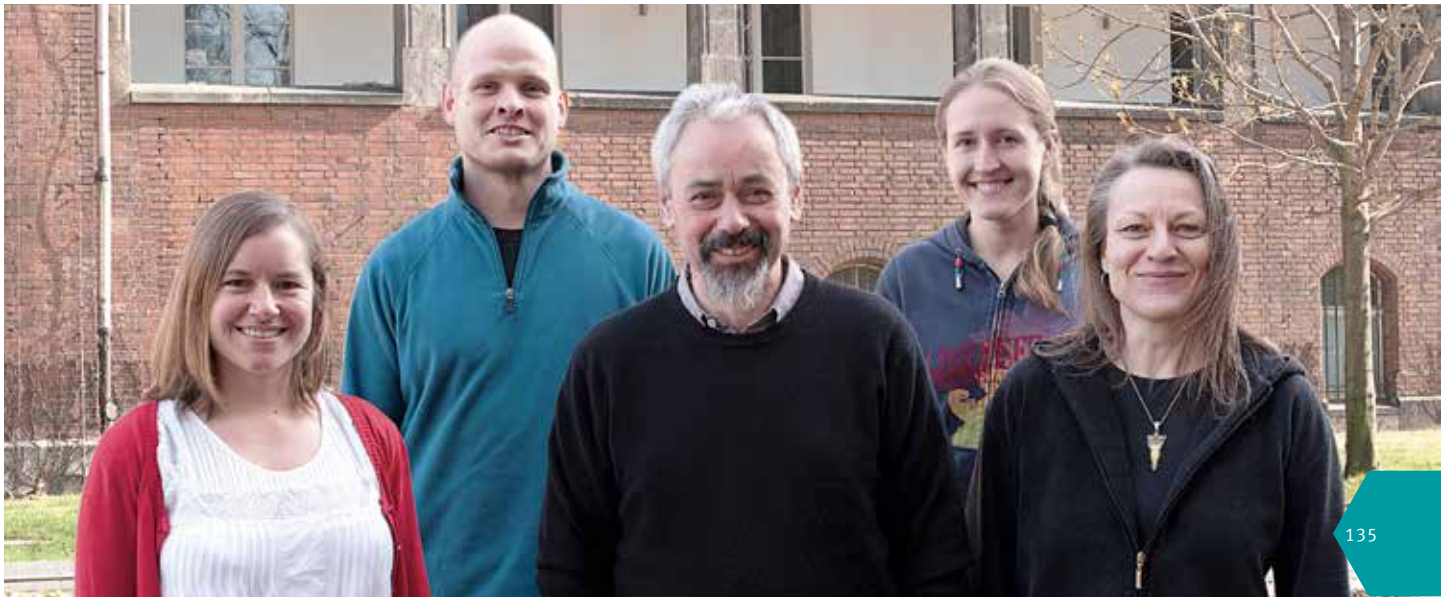
In some studies the results are not as clear as the authors thought they are. Considering all factors which may lead to biased results or wrong conclusions in an appropriate and suffcient manner is an important issue. Misspecified hypotheses are another and frequently overlooked reason for invalid conclusions. At the end simple interpretation is needed although the design of the experiment, the design of the study or the analysis of the data were possibly rather complex. Statisticians are not able to pull rabbits out of a hat. However, our group tries to support scientists from the institute as well as cooperation partners in the planning and conduction of studies as well as in the analysis and interpretation of the data.

Our work includes:

- Evaluation of safety and efficacy of new drugs
- Evaluation of new therapy strategies
- Assessment of the prognostic value of biomarkers and of risk factors for serious adverse events
- Diagnostic trials
- Meta-analysis of RCT data
- Biometric responsibility for trials on animals and ethical applications by DRFZ scientists
- Supervision of all statisticians in the Epidemiology unit.

We are currently taking the biometrical part in two double blinded randomized placebo controlled clinical trials [RCTs] (in osteoarthritis of the hands and in adult Still's disease) and in two phase II trials (efficacy of ustekinumab in ankylosing spondylitis (AS) (TOPAS) and efficacy of different treatment regimes of NSAIDs on radiographic damage in AS (ENRADAS)). For TOPAS, we expect results in May 2013. Statistical analysis and interpretation of the results was provided for a BMBF funded diagnostic study and for another RCT investigating the efficacy of glucocorticoids in ankylosing spondylitis. We were further involved in long term extension studies of randomized clinical trials, cohort studies managed by researches of the epidemiology unit as well as those managed by Joachim Sieper's group. We continued to provide statistical support for basic scientists of the institute. We were involved in applications of EU and German government grants and industry sponsored projects. Furthermore, Dörte Huscher who works in Angela Zink's group supported our work by giving biometrical advice to rheumatologists and basic scientists. She collaborates closely with Frank Buttgereit's and Gabriela Riemekasten's group, with Jörg Distler (Erlangen) and Oliver Distler (Zürich), and collaborates as a statistician with the ANZAC research institute, the Expert Panel on Outcome Measures in Pulmonary Arterial Hypertension related to Systemic Sclerosis and with the Connective Tissue Disease – Interstitial Lung Disease study group.

Last year we extended our research agenda. Johanna Callhoff performed a systematic review and meta-analysis of randomized controlled trials in rheumatoid arthritis. A clear impact of biologic agents on the improvement in function was found. However, there was a substancial heterogenity in the results of the individual RCTs which did not allow to draw firm conclusions regarding differences in the efficacy of the drugs. Moreover this finding suggests that the results of other meta-analyses suggesting differences in the efficacy of biologic agents need to be interpretated with caution. Johanna Callhoff also put a question mark behind a conclusion frequently drawn from two large RCTs that combination therapy of a biologic agent plus methotrexate is more effective than monotherapy with the biologic agent. This conclusion is not supported by a more complete consideration of RCT results.



COOPERATION PARTNER

Joachim Sieper, Gerd-Rüdiger Burmester, Frank Buttgereit, Jaqueline Detert, Hildrun Haibel, In-Ho Song, Denis Poddubnyy, Gabriela Riemekasten, Martin Rudwaleit; Charité, Universitätsmedizin Berlin

Jürgen Braun, Xenofon Baraliakos; Rheumazentrum Ruhrgebiet Herne

Georg Schett, Jörg H.W. Distler; Universität Erlangen-Nürnberg

Oliver Distler; Universitäts-Spital Zürich, Schweiz

Markus.J. Seibel; ANZAC Research Institute, Sidney, Australia

NETWORKS

ASAS, EPOSS, RODS

SELECTED PUBLICATIONS

Althoff CE, Sieper J, Song IH, Haibel H, Weiß A, Diekhoff T, Rudwaleit M, Freundlich B, Hamm B, Hermann KG. Active inflammation and structural change in early active axial spondyloarthritis as detected by whole-body MRI.Ann Rheum Dis. 2012 Jun 26. [Epub ahead of print]

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SCIENTISTS
Johanna Callhoff
Anja Weiß
Joachim Listing

The Impact of Biologics on the Improvement in Functional Capacity- a Meta-Analysis

We conducted a meta-analysis of randomised controlled trials on patients with rheumatoid arthritis (RA) to evaluate the impact of biologic agents on functional capacity. It was investigated whether biologics relevantly improve functional capacity compared to conventional disease modifying antirheumatic drugs (DMARDs) and whether there are differences between recent onset RA and longstanding RA or between biologic agents. The analysis showed that biologics do improve functional capacity clinically relevant compared to DMARD control groups for both DMARD-naive patients and DMARD-nonresponders. No significant differences between the biologics were discovered.

Introduction

A variety of biologic and non-biologic treatment options is available for RA patients today. All biologic agents with market authorisation have been studied with respect to their clinical effectiveness in several randomised controlled clinical trials (RCTs). They have been shown to have a great impact on disease activity and patient reported outcomes.

Besides clinical effectiveness, physical function is an important patient reported outcome in trials on RA. Physical function has not only a clear impact on quality of life, low functional capacity is also a risk factor of premature mortality. To combine the existing research on the influence of biologics on functional capacity, we used the technique of a meta-analysis. By this way all published data on eligible RCTs could be included in our investigation.

Results

The aim of this analysis was to investigate the impact of biologics on physical function of RA patients and to compare the different biologic agents abatacept, adalimumab, certolizumab, etanercept, golimumab, infliximab and rituximab. Studies had to be double blind, DMARD/placebo controlled and randomised; the study duration had to be at least 12 weeks. To identify all relevant studies for the meta-analysis, an extensive literature search (in which 1307 references were evaluated) was conducted by two investigators independently, resulting in 35 studies which were included in the analysis.

Most RCTs measure physical function with the health assessment questionnaire (HAQ). Therefore, our primary outcome measure was the standardized mean difference (SMD) between change from baseline in HAQ-score in treatment and control groups.

$$SMD = \frac{(HAQ_{T1} - HAQ_{T2}) - (HAQ_{C1} - HAQ_{C2})}{SD_{pooled}}$$

(T = Treatment, C = Control, 1 = Baseline, 2 = Study end, SDpooled = pooled standard deviation of HAQ in treatment and control group).

This standardized outcome measure allows us to compare results from different studies and it shows how much of the improvement on physical function is added by biologics comparing them to a placebo or DMARD control treatment.

Tab. 1: Baseline characteristics of the RCTs for the different biologics.

Biologic	Number of studies (treatment arms)	Number of patients per study	Weeks of study duration	Years of disease duration	Studies with MTX naive patients (treatment arms)	HAQ at Baseline
Abatacept Control group	5 (5)	349 193	41	8.0 7.5	1 (1)	1.6 1.6
Adalimumab Control group	11 (19)	137 133	30	7.8 7.8	2 (3)	1.6 1.6
Certolizumab Control group	3 (3)	497 179	16	7.1 6.9	0 (0)	1.6 1.6
Etanercept Control group	5 (7)	151 133	36	7.4 7.2	1 (1)	1.7 1.6
Golimumab Control group	4 (4)	122 134	17	6.6 7.0	1 (1)	1.4 1.4
Infliximab Control group	3 (5)	181 150	54	4.3 4.9	2 (3)	1.6 1.5
Rituximab Control group	4 (4)	195 160	31	9.0 8.3	1 (2)	1.8 1.9

The baseline characteristics of the included studies are shown in Table 1. In total there were 9066 participants in treatment groups and 4801 in control groups.

As expected, the improvement in the HAQ score was larger in recent onset RA patients who were DMARD naïve (Fig1.). The mean improvement was lower in longstanding RA patients who had inadequately responded to DMARDs.

On the standardized SMD scale biologics improve functional capacity to a significantly higher magnitude (~half a standard deviation of the HAQ; SMD of 0.5 corresponds to 0.3 on the HAQ scale) than patients treated with placebo and MTX or other DMARDs. Furthermore the superiority was higher in DMARD-IR than in DMARD naïve RA (Fig. 2). But nevertheless the difference between the treatment with biologics and DMARDs/Placebo was also in DMARD naïve RA clinically relevant and statistically significant.

For each biologic agent we observed an impact on the improvement in functional capacity (Fig. 3). Since there was still a substantial heterogenety in the results of the RCTs the differences between them did not achieve statistical significance (p=0.29).

Perspectives

Another meta-analysis on the impact of biologics on functional capacity in patients with spondyloarthritis is planned.

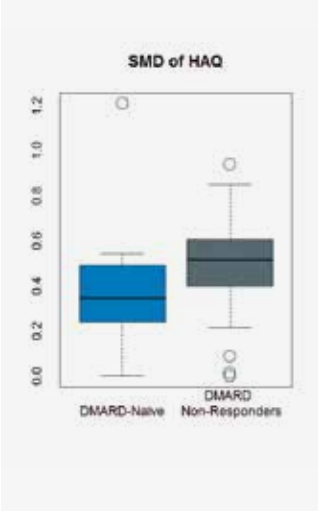


Fig. 2: Standardized mean differences (SMD) by DMARD-response status

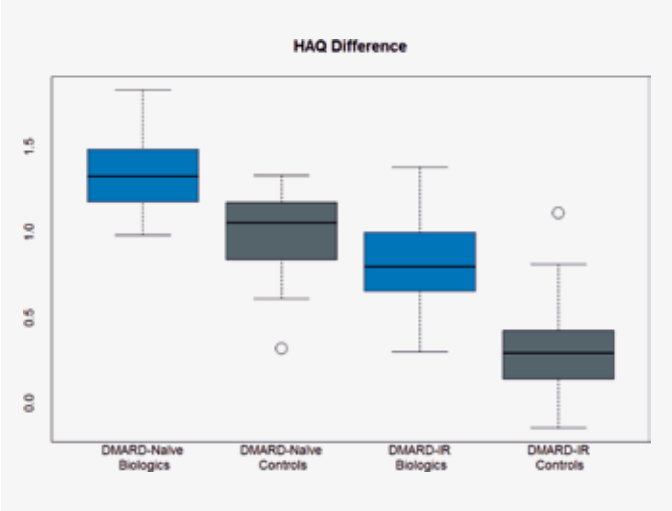


Fig. 1: Mean improvement in the HAQ score by type of treatment in recent onset DMARD naive RA and longstanding RA (DMARD inadequate responders (IR))

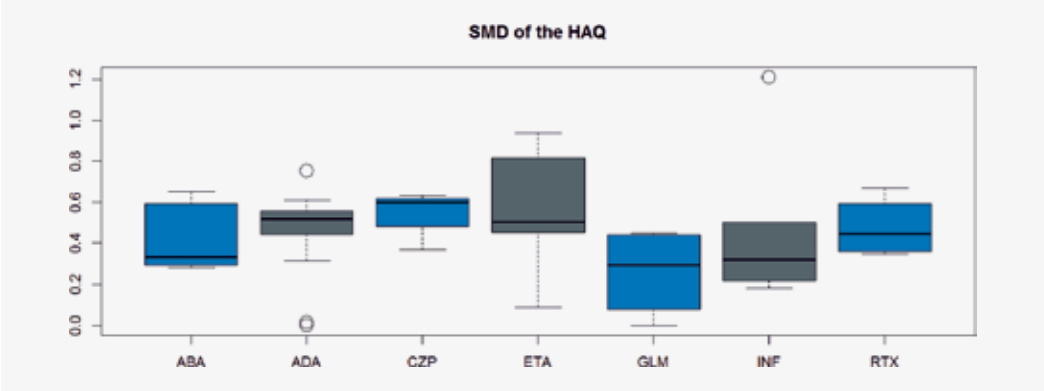


Fig. 3: Standardized mean difference of the HAQ for each biologic agent. ABA = abatacept, ADA = adalimumab, CZP = certolizumab, ETA = etanercept, GLM = golimumab, INF = infliximab, RTX = rituximab.

Kirsten Minden

Pediatric Rheumatology

Juvenile rheumatic diseases – presence and future

KEYWORDS
Juvenile idiopathic arthritis
Outcome, Drug safety
Transition, Cost of illness

GROUP MEMBERS

Group leader
PD Dr. med. Kirsten Minden

Scientists
Dr. rer. nat. Jens Klotsche
Martina Niewerth (MPH)
Dr. med. Anna Raab
Dr. med. Claudia Sengler

Medical data manager
Ina Liedmann
Karin Weber

Students
Stephanie Everts
Nils Geisemeyer
Céline Heinermann
Jana Hörstermann
Anke Leder
Florian Milatz
Anneli Rieck
Yamina Saran
Teresa Steinhof
Annemarie Uhlmann

Doctoral students
Anja Fröhlich
Meike Storms
Katrin Stüdemann

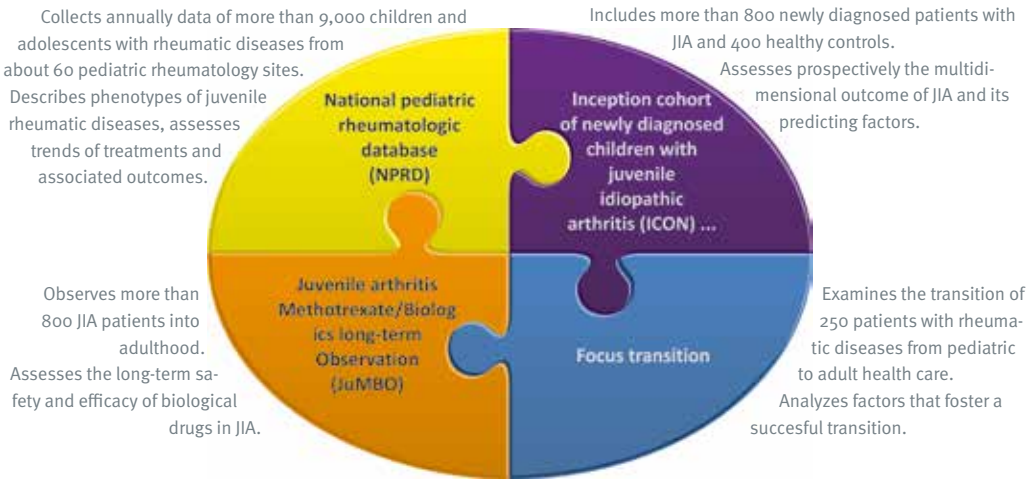
Rheumatic disorders are among the most common chronic conditions in children and adolescents. These illnesses are important causes of morbidity and disability, and they burden individuals and their families, as well as society, through enormous health care cost. Even though therapies have improved, rheumatic disorders often take a relapsing-remitting course and persist into adulthood, with a long-term outcome that is not easy to predict. There has been evidence that effective treatment during the early phases of the disease may prevent disability and morbidity lateron. Despite significant advances in drug treatment, evidence on best practice is still lacking for most of childhood onset rheumatic diseases.

Our group investigates processes and outcomes of pediatric rheumatology care. We describe phenotypes, course, and prognosis of juvenile rheumatic diseases, with a main focus on juvenile idiopathic arthritis (JIA).

In four prospective observational cohort studies (see figure) we deal with the following issues:

- What is the health care situation of children, adolescents and young adults with juvenile rheumatic diseases, and how does it change over time?
- What is the impact of juvenile rheumatic diseases on patients and their families?
- How effective and safe are biologic therapies in children, adolescents and young adults with JIA?
- What factors determine the course and outcome of juvenile rheumatic diseases?

All our projects are based on close cooperation with the entire pediatric rheumatology community in Germany, and networking with experimental groups.



COOPERATION PARTNER

Dr. Ivan Foeldvari, Kinderrheumatologische Praxis am AK Eilbek, Hamburg

Prof. Dr. Dirk Foell, Universitätskinderklinik Münster

Dr. Gerd Ganser, St. Joseph-Stift, Sendenhorst

Prof. Dr. Johannes-Peter Hass, Deutsches Zentrum für Kinder- und Jugendrheumatologie, Garmisch Partenkirchen

Prof. Dr. Gerd Horneff, Asklepios Kinderklinik Sankt Augustin

Dr. Anton Hospach, Olgahospital Stuttgart

Prof. Dr. Hans-Iko Huppertz, Prof.-Hess-Kinderklinik, Bremen

PD Dr. Tilmann Kallinich, Kinderklinik der Charité, Berlin

PD Dr. Jasmin Kümmerle-Deschner, Universitätskinderklinik Tübingen

Dr. Kirsten Mönkemöller, Kinderkrankenhaus der Stadt Köln

Dr. Angelika Thon, Medizinische Hochschule Hannover

Dr. Nikolay Tzaribachev, Rheumaklinik Bad Bramstedt

And 50 other pediatric rheumatologic and more than 100 adult rheumatologic sites

Prof. Dr. Arnd Heiligenhaus, Augenabteilung am St. Franziskus Hospital, Münster

and more than 100 opthalmologic sites

Prof. Dr. Min Ae Lee-Kirsch, Universitätsklinik Dresden

Dr. Heinrike Schmeling, Alberta Children's Hospital, University of Calgary, Kanada

Prof. Dr. Nico Wulffraat, Wilhelmina Ziekenhuis, Utrecht, Niederlande

PHARMACHILD | Pharmacovigilance in juvenile idiopathic arthritis patients treated with biologic agents and/or methotrexate

UCAN | Understanding Childhood Arthritis Network

SHARE | Single Hub and Access point for paediatric Rheumatology in Europe

SELECTED PUBLICATIONS

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Juvenile idiopathic arthritis – trends in treatment and outcomes.

SCIENTISTS

M. Niewerth, J. Klotsche, C. Sengler, A. Raab, K. Minden

COOPERATION PARTNERS

60 pediatric rheumatology and about 100 ophthalmology sites

A Heiligenhaus, St. Franziskus-Hospital, Münster

PUBLICATIONS

Minden K, Niewerth M. Rheumakranke Kinder und Jugendliche: Kerndokumentation und Prognose. Monatsschr Kinderheilkd 2012;160:237-43.

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FUNDING

The PRD has been supported by the child arthritis foundation.

The pediatric rheumatologic database (PRD) has recorded patients with juvenile idiopathic arthritis (JIA) nationwide since 2000. It therefore allows the description of trends of treatment and associated outcomes in JIA. Over the period from 2000 to 2011, more than 20,000 cases with JIA were registered. It could be shown that patients of all JIA categories have been treated more often and earlier with DMARDs, especially with biologics, over the past 12 years. Along with the changes in treatment, the outcome of patients with polyarticular course and systemic onset of JIA has improved. In patients with oligoarthritis, early DMARD treatment was associated with a rarer occurrence of uveitis. It is however still unclear which patients would benefit most from early DMARD treatment. Thus, further research toward a more personalized treatment of JIA is needed.

JIA is a heterogeneous disease, currently classified in seven categories according to the ILAR criteria. Among these categories, subtle variations exist with regards to clinical phenotypes (e.g., the number of affected joints), underlying pathogenesis, treatment approaches, and disease outcomes.

Over the past 12 years, more than 24,187 JIA cases were recorded: 2,065 in the year 2,000, 5,994 in the year 2011. The majority of them had oligoarthritis (OA), about 20% had rheumatoid factor (RF)-negative or RF-positive polyarthritis (PA). Patients with systemic onset of JIA (soJIA) accounted for approximately 5% of the patients recorded each year. The JIA spectrum of patients registered at 55 rheumatology sites (figure 1) in the year 2011 is shown in figure 2.

Fig. 1: Pediatric rheumatology sites which participated in the PRD in 2011

Oligoarticular juvenile idiopathic arthritis

Patients with OA have a maximum of 4 affected joints within the first 6 months of disease. However, in a subset of patients the disease progresses and takes a polyarticular course, referred to as extended OA. OA has very variable outcomes, ranging from complete remission to the development of an intractable polyarticular disease or of visual loss due to uveitis complications.

Out of the 544 patients with early OA (disease duration ≤12 months) who were recorded in the PRD between 2000 and 2005 and followed for at least 4 years, 15% experienced a polyarticular course and 16% an uveitis. At the 4-year-follow-up, 39% of the patients had an inactive or minimally active disease, and 75% reported no functional limitations (Childhood Health Assessment Questionnaire [CHAQ]=0). Predictors for an active disease (juvenile arthritis disease activity score [JADAS] >2) at the follow-up were not found. In contrast, lower disease activity (JADAS ≤10) at baseline predicted a better functional outcome after four years (p=0.012).

The JADAS at first documentation in the PRD was also a predictor for the development of uveitis (see table 1). Patients with a JADAS of >10 had a twofold higher chance to experience uveitis until the follow-up than those with a lower JADAS score (p=0.012).

Moreover, MTX use within the first 12 months of disease was negatively associated with the development of uveitis (p=0.019).

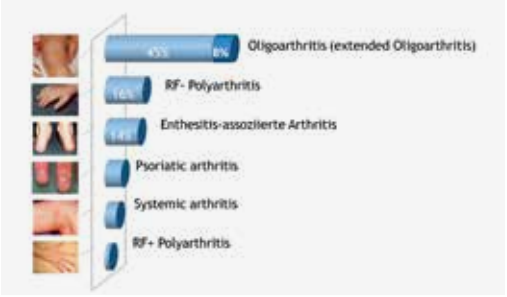

Figure 2: Relative frequencies of JIA categories of patients recorded in the PRD in 2011

Table 1: Predictors for uveitis in OA patients, based on longitudinal data of 544 OA patients.

Outcome at 4-year-follow-up	Parameter (at baseline)	Patients, in %	OR
Uveitis (ever)	JADAS ≤ 10	11.6%	
	JADAS › 10	21.7%	2.14
	ANA negative	10.6%	
	ANA positive	18.6%	1.96
	Age at onset (› 4 years)	10.9%	
	Age at onset (≤ 4 years)	20.9%	2.16
	No early MTX use	23.0%	
	Early MTX use	13.7%	0.53

Polyarticular disease

There are two onset-types and one course-type of polyarticular JIA (pJIA): RF-negative polyarticular JIA, RF-positive polyarticular JIA, and extended OA. It is known, that patients with pJIA have a worse prognosis than those with OA; they are therefore treated more often with conventional and/or biological disease modifying antirheumatic drugs (DMARDs). The data of the PRD show that during the past 12 years patients with pJIA have been treated increasingly more often with DMARDs in general and with biologics in particular. While in the year 2000, 70% and 2% of patients were treated with DMARDs and biologics, these were 79% and 32% in the year 2011, respectively. Moreover, patients with pJIA have been treated earlier in the disease course (figure 3).

Changes in treatment were coincided by changes in the health status of patients. Over the years, the adjusted mean annual JADAS-10 score significantly decreased from 10.3 to 8.1 (p=0.042), the CHAQ-score almost halved from 0.45 to 0.26 (p<0.001). A significant correlation was found between DMARD and biologic use and the decrease of disease activity (beta=-0.12) and functional restrictions over time (beta=-0.02, see figure 4), respectively.

Systemic juvenile idiopathic arthritis (sJIA)

Given the changes in treatments and outcomes in polyarticular JIA, those with respect to systemic-onset of JIA were even more pronounced: The use of biologicals increased more than tenfold from 2000 to 2011. On the other hand, disease activity, measured by the JADAS-10, of patients with sJIA almost halved over this period of time (p=0.031).

A positive trend over time was also noted for other health parameters, such as overweight. We detected that patients with soJIA suffered significantly more often from overweight (20%) than non-systemic JIA patients (14%). No participation in sport activities at school, functional impairment, and glucocorticoid treatment were significantly associated with overweight. However, the prevalence of overweight decreased from 15.3% in the year 2003 to 10.4% in the year 2011. The decreasing prevalence of overweight over time was significantly associated with the improvement of the patients´ functional capacity (beta=-0.1) and the more frequent use of biologic agents (beta=-0.2).

Perspectives

Using the PRD comprising several thousands of patients, we could demonstrate that tremendous changes in treatment and outcomes in JIA have occurred over the past 12 years, and that these changes significantly correlated. We also noted a large practice variation in drug treatment in JIA among the various pediatric rheumatology sites participating in the PRD. This reflects the lack of standards of care due to the inadequate empirical evidence about the best treatment strategy.

The evaluation of specific treatment strategies and detection of predictors of treatment response or intolerance is therefore required. By analyzing data of the inception cohort of newly diagnosed patients with JIA (ICON) and of the biologic register JuMBO (Juvenile arthritis Methotrexate/Biologics long-term Observation) we will address this need.

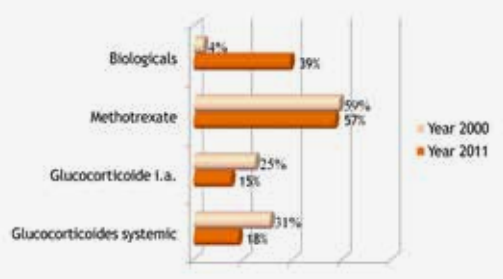

Figure 3: Treatment of patients with pJIA during the first 12 months of disease in the years 2011 and 2000.

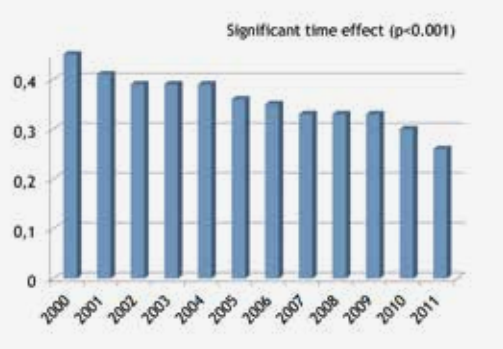

Figure 4: Mean functional status*, measured by the CHAQ, of pJIA patients from 2000 to 2011 (*adjusted for age, sex, disease duration).

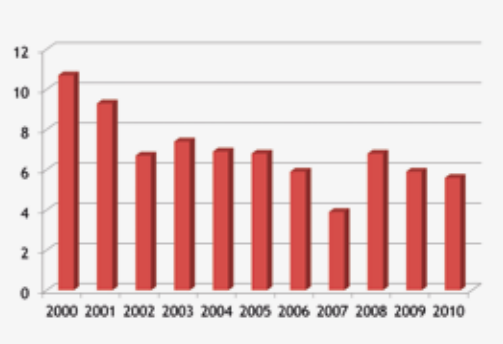

Figure 5: Mean disease activity*, measured by JADAS-10 (Score 0-40), of sJIA patients from 2000 to 2011 (*adjusted for age, sex, disease duration).

Anja Strangfeld

Pharmacoepidemiology

Drug safety in real world practice

KEYWORDS

Drug safety
Biologics
Epidemiologic drug registers

GROUP MEMBERS

Group Leader
Dr. med. Anja Strangfeld

Scientists
Dr. rer. nat. Joachim Listing
Dipl.-Stat. Maria Eveslage
PD Dr. med. MSc Kerstin Gerhold
Dipl.-Wi.Ing. Dörthe Kühl-Habich
Dipl.-Stat. Diana Pantigoso
DrPH MA Dagmar Pattloch
MSc Adrian Richter

Medical Data Managers
Claudia Fritz, Ulrike Kamenz, Bettina Marsmann, Steffen Meixner, Katja Willenberg, Susanna Zernicke

Students
Doreen Bontke, Peter Britz, Irina Dammenhayn, Ursula Hemetek, Juliana Hoffmann, Ina Kammermeier, Friederike Liepold, Alik Müller, Kristina Müller, Juliane Reinke, Jasmin Sandor, Anne Weichelt, Carina Wittkopp

Doctoral students
Katharina Höhne, Yvette Meißner, Madlen Spilka

The aim of pharmacoepidemiology in general is to examine the use of drugs and their effects in large groups of people. Safety and effectiveness are the most important factors to be evaluated. Our specific research interest are newly approved drugs to treat rheumatoid arthritis (RA).

During the last decade a number of new drugs (so called biologics) have been developed which directly interfere with cytokines that play important roles in the inflammatory process. Although all new drugs are intensively studied in randomized clinical trials (RCTs), it is not sure how safe they will be when applied in everyday rheumatological care. Two third of the patients treated with these drugs in routine care would not have fulfilled the inclusion criteria of RCTs, due to their age, number of comorbidities or low functional ability. Moreover, RCTs are performed for a short-time period only (usually about 6-12 months), therefore leaving open questions about the long-term safety and effectiveness of therapies which are of major importance for life-long treatment of chronic diseases.

To monitor the long-term safety and efficacy of biologics we run an epidemiologic drug register. RABBIT (Rheumatoid Arthritis: oBservation of Biologics Therapy) is a longitudinal observational cohort study. Patients are included with the start of a biologic agent or, as controls, with the start of a new DMARD treatment after failure of at least one other treatment. Once enrolled, patients are observed for up to 10 years irrespective of further treatment changes. Until the end of December 2012 we have enrolled almost 11000 patients, 2/3 of them under treatment with biologics.

RABBIT is among the largest biologics registers worldwide. Since the initiation of RABBIT in 2001, cooperation with other registers has been developed and extended. In 2010 a concerted analysis including three registers was performed to evaluate a safety signal discovered in our register.

In addition, RABBIT is also a member of ENCePP – the European Network of Centers of Pharmacovigilance and Pharmacoepidemiology. This network was founded by the EMA (European Medicines Agency for Evaluation of Medical Products) in order to be able to react to urgent safety inquiries on a European basis.

During the last years we published important results regarding the safety of particular agents, concerning the risk of infection, of malignant disease or of heart failure under treatment with TNF-alpha inhibitors. With our growing knowledge about the strengths and pitfalls in analysing observational cohort data we continuously develop our statistical methods to obtain valid results.

Our vision is to find ways of predicting outcome and safety of treatment, for various groups of patients to conclude the best individual therapy from a patient's characteristic profile.

COOPERATION PARTNERS

More than 300 rheumatologists in Germany, who enrolled patients

International: ARTIS (S), BIOBADASER (E), BSRBR (GB), DANBIO (DK),

SELECTED PUBLICATIONS

Strangfeld A, Eveslage M, Schneider M, Bergerhausen HJ, Klopsch T, Zink A, Listing J. Treatment benefit or survival of the fittest: what drives the time-dependent decrease in serious infection rates under TNF inhibition and what does this imply for the individual patient? Ann Rheum Dis 2011; 70:1914-1920.

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RABBIT Risk Score for serious infections

SCIENTISTS

Strangfeld A, Listing J, Eveslage M, Gerhold K, Kühl-Habich D, Pantigoso D, Pattloch D Richter A

COOPERATION PARTNERS

More than 300 rheumatologists from all parts of Germany who enrolled patients

PUBLICATIONS

Strangfeld A, Eveslage M, Schneider M, Bergerhausen HJ, Klopsch T, Zink A, Listing J. Treatment benefit or survival of the fittest: what drives the time-dependent decrease in serious infection rates under TNF inhibition and what does this imply for the individual patient? Ann Rheum Dis 2011; 70: 1914-1920.

Zink A, Manger B, Kaufmann J, Eisterhues C, Krause A, Listing J, Strangfeld A. Evaluation of the RABBIT Risk Score for Serious Infections. Ann Rheum Dis 2013 (under review).

FUNDING

RABBIT is supported by a joint, unconditional grant from all manufacturers of biologics approved for the treatment of rheumatoid arthritis: Abbott (since 2003), Amgen (2003-2008)/Swedish Orphan Biovitrum (2009-2012), Bristol-Myers Squibb (since 2007), Essex pharma/MSD (since 2001), Wyeth/Pfizer (since 2001), Roche (since 2007), and UCB (since 2009).

Role of the funding source: The principal investigators and their team have full academic freedom in study design and conduct, data analysis and publication of results.

The RABBIT Risk Score for serious infections was developed in 2011 on a cohort of patients with rheumatoid arthritis (RA) enrolled in the German biologics register RABBIT between 2001 and 2007. To evaluate this score data with an independent patient set, we used patients enrolled in RABBIT after 1st of January 2009. We first calculated the numbers of serious infections expected according to the RABBIT Risk Score and then compared them with the observed numbers in the evaluation sample. A high agreement between observed and expected rates of serious infections was found. The score was highly predictive in groups of patients with low as well as with high risk for serious infections. The RABBIT Risk Score is a reliable instrument to calculate the risk for serious infections in individual patients based upon clinical and treatment information. It therefore helps rheumatologists to balance benefits and risks of treatment, to avoid high-risk treatment combinations and thus to make informed clinical decisions.

Meta-analyses of randomised controlled trials as well as observational cohort studies found evidence for an increased risk of serious infections in patients treated with TNFα inhibitors. However, treatment with TNFα inhibitors is not the only risk factor of serious infections. In patients with RA the severity of the disease, comorbid conditions as well as comedication with glucocorticoids are likely to contribute to that risk. Using data from the German biologics register RABBIT we therefore determined the impact of different factors on the risk for serious infections. To allow this, we took changes in the risk in individual patients during the observation time as well as changes in the composition of the cohort into account, i.e. we differentiated between the overall risk observed in the cohort and the individual risk of each patient. In daily care, the individual risk of serious infection is modified by changes in treatment, changes in the activity of the disease, changes in functional capacity and by comorbid conditions. On the cohort level the risk declines over time, an effect which is mainly caused by (high-risk) patients lost to follow-up. Using data of RA patients enrolled in the German biologics register RABBIT between 2001 and 2007 and taking statistically into account the above mentioned processes on the cohort and individual level, we were able to identify the contribution of different risk factors on the serious infection risk of individual patients. This analysis resulted in the RABBIT risk score.

By means of this score it is possible to estimate the risk of individual patients by taking their disease characteristics and treatment details into account (Fig. 1). How is this figure to be read? If we take a patient aged 65 with RA and chronic obstructive pulmonary disease (COPD) as an example: if this patient is treated with MTX and 7.5 mg/d prednisone, his risk for developing a serious infection during the next 12 months is 4.7 %. If the treatment of this patient is insufficient to control the activity of the RA and therefore the prednisone dose is increased to 15 mg/d, his risk increases to 8.2%Adding a TNF inhibitor would increase the risk further to 14.3 %, but if the TNF inhibition is effective

Risk factors		Version 1: Number of serious infections per 100 PYs	Version 2: Percent of patients with at least one infection per year
Intercept	Always add	-3.996	-4.191
Age	If age › 60 add	0.479	0.470
Function (FFbH)	Add	-0.01014*FFbH	-0.00865*FFbH
Alternatively: HAQ	Add	-0.362(HAQ-3.16)	-0.309(HAQ-3.16)
Chronic lung disease	If yes add	0.522	0.484
Chronic renal disease	If yes add	0.441	0.415
Previous serious infection	If yes add	0.748	0.992
Number of treatment failures	If › 5 add	0.443	0.397
Mean glucocorticoid dose	If 7.5-14mg/d add	0.756	0.782
Mean glucocorticoid dose	If >= 15mg/d add	1.554	1.355
Treatment with TNF inhibitor	If yes (last 3 months) add	0.593	0.589
Calculate the sum of the corresponding values		Sum1	Sum2
Rabbit Risk Score Calculate		e^{sum1}	$1\text{-}e^{(-e^{sum2})}$

Table 1 Calculation of the RABBIT Risk Score. PY = patient years

and the prednisone dose can be tapered down below 7.5 mg/d, the risk is reduced to 3.9 %. However, this risk calculation was based upon one single cohort. The aim of our second analysis was to evaluate the score with an independent cohort of patients not included in its development.

Results

For the evaluation of the risk score, we used patients who were enrolled in RABBIT between January 2009 and January 2012 at start of treatment with a TNFα inhibitor or a non-biologic DMARD (nbDMARD). Data of 1,522 RA patients treated with TNFα inhibitors and of 1,468 patients treated with nbDMARDs were available. The median observation time was 1.6 years.

Table 1 shows the risk factors included in the score, their weights and the formula for the calculation of both versions of the score (expected numbers of serious infections and expected numbers of patients with at least one infection).

We applied the original RABBIT Risk Score based upon the number of infections per 100 patient-years to the evaluation cohort and found a high agreement between the observed and the expected rates (Fig. 2, Table 2).

A strength of our risk score is that it includes time-varying parameters like disease activity and disability which results in more precise risk estimates on the individual level than treatment and sociodemographic factors only.

The RABBIT Risk Score which was originally developed with 5,044 patients could be confirmed in an independent cohort of 2,603 patients. The score supports "personalized medicine". It helps rheumatologists to balance benefits and risks of treatment, to avoid high-risk treatment combinations and thus to make informed clinical decisions.

Access to the online score calculator is possible via the RABBIT website www.biologika-register.de.

Perspectives

Our next project about serious infections will analyze the risk for serious infections under other biologics treatments like rituximab, tocilizumab and abatacept.

We will further search for other individual factors that may help rheumatologists to find the best possible treatment for patients based upon their individual profile.

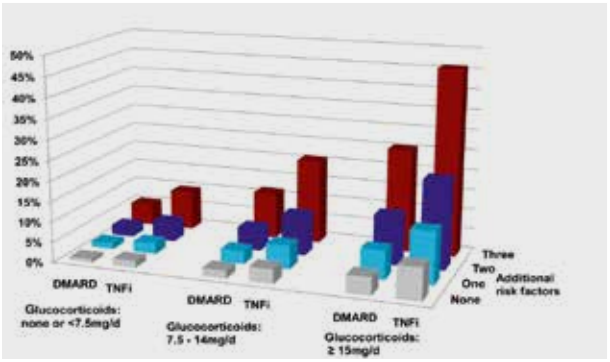

Fig.1 : The RABBIT Risk Score

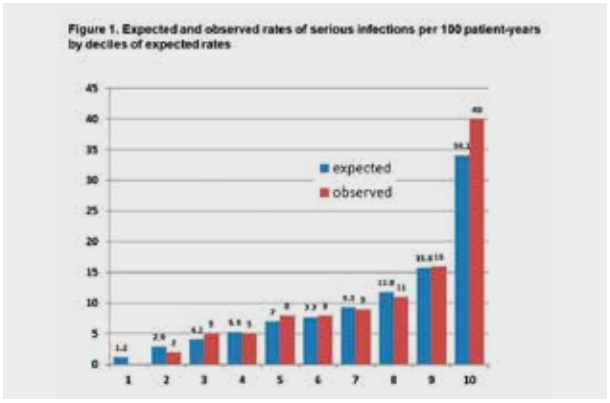

Fig.2: Expected and observed rates of serious infections per 100 patient-years by deciles of expected rates

			Number of serious infections per 100 PY [CI]			
	n	PY	Exp. n	Obs. n	Exp. rate / 100 PY	Obs. rate / 100 PY
TNFα inhibitor, no risk factor	764	1059	16.8	16	1.6	1.5 [0.9 – 2.5]
nbDMARD, no risk factor	632	816	7.0	6	0.9	0.7 [0.3 – 1.6]
TNFα inhibitor, ≥ 1 risk factor*, no GC	635	871	28.9	37	3.3	4.3 [3.0 – 5.9]
nbDMARD, ≥ 1 risk factor*, no GC	674	939	15.5	19	1.7	2.0 [1.2 – 3.2]
TNFα inhibitor + GC, no other risk factor	225	196	8.6	5	4.4	2.6 [0.8 – 6.0]
nbDMARD + GC, no other risk factor	128	86	1.9	2	2.2	2.3 [0.3 – 8.4]
TNFα inhibitor, ≥ 1 risk factor* + GC	206	160	15.3	11	9.6	6.9 [3.4 – 12.3]
nbDMARD, ≥ 1 risk factor* + GC	141	96	5.1	8	5.3	8.3 [3.6 – 16.4]
TNFα inhibitor total	**1830**	**2286**	**69.6**	**69**	**3.0**	**3.0 [2.3 – 3.8]**
nbDMARD total	**1575**	**1937**	**29.6**	**35**	**1.5**	**1.8 [1.2 – 2.5]**

Table 2 Expected and observed numbers and rates of serious infections in the evaluation cohort stratified by risk factors. n=Numbers of patients, observed (obs), expected (exp), patient-years (PY), confidence interval (CI), GC = glucocorticoids, nbDMARD=synthetic disease modifying anti-rheumatic drug.
* at least one out of: chronic lung disease, chronic renal disease, age above 60 years, previous serious infection, high number of DMARD failures. GC: treatment with glucocorticoids ≥ 7.5mg/d prednisolone equivalent.

Gisela Westhoff

Prognostic Studies in Early Arthritis

Clinical features, biomarkers and onset of intervention – cohort studies identifying predictors of dis-ease course

KEYWORDS
early arthritis
rheumatoid arthritis
predictors
prognosis
oral health

GROUP MEMBERS

Group leader
Dipl.-Psych. Gisela Westhoff

Statistician
Johanna Callhoff

Students
Katja Molina
Mariya Chubrieva

Ph.D. student
Katrin Cobarrubias (until 3/2012)

The focus of our research is on course and prognosis of inflammatory arthropathies. In recent years, we have established several large cohorts which have given important insights and inspired further research. All studies are performed in collaboration with a large network of German rheumatologists.

Main results of our previous studies were the identification of the protective role of body fat on joint damage, the influence of smoking on need for anti-rheumatic drugs, or the predictive importance of morning stiffness for work disability. Fatigue at disease onset was shown to be a significant long-term indicator of morbidity and mortality.

We contributed to the program area's interest in health services research with a study on health care utilization and unmet needs of RA patients in the general population. We investigated referral patterns of patients with early arthritis from primary care physicians to rheumatologists. Both studies showed improvement concerning access of patients with arthritis to rheumatologic care. We also identified subgroups of patients with insufficient treatment and considerable delay in access.

Since 2009 we have been running the inception cohort study "Course And Prognosis of Early Arthritis (CAPEA)" with more than 100 rheumatologists and more than 1000 patients. First results from this cohort show the strong predictive value of periodontal disease for the course of early arthritis and the treatment requirements (see report). We further showed that depression is a stronger predictor of work disability than disease activity or response to treatment. Together with fatigue and obesity, depression is also a strong predictor of limitations in walking abilities. Finally, current and even past use of oral contraceptives resulted in significantly better patient-reported outcomes, independent of inflammation (see report).

In the future, we will analyse the predictive value of semi-quantitative ultrasonography in collaboration with Marina Backhaus, Charité. Together with Matthias Schneider, University of Duesseldorf, and Georg Schett, University of Erlangen, we will investigate the prognostic value of different biomarkers for the course of early arthritis. Together with Thomas Dietrich, University of Birmingham, we are currently developing a patient-reported periodontitis questionnaire for epidemiological studies, using dentists' reports and periodontal x-rays for validation.

COOPERATIONS PARTNER

Prof. Thomas Dietrich, The School of Dentistry University of Birmingham, Birmingham, UK;

Prof. Thomas Dörner, Charité - Universitätsmedizin Berlin;

Dr. Edmund Edelmann, Rheumapraxis Bad Aibling;

Prof. Georg Schett, Medizinische Klinik 3 - Immunologie und Rheumatologie, Erlangen;

Prof. Matthias Schneider, Klinikum H.-Heine-Universität Düsseldorf und ca. 150 Rheumatologen bundesweit.

Technische Kooperation: Auftragsforschungs-Institut Winicker Norimed, Nürnberg

SELECTED PUBLIKATIONS

Westhoff G, Zink A [Epidemiology of primary Sjögren's syndrome]. Z Rheumatol. 2010;69:41-9.

Westhoff G, Westhoff G et al. Diagnostic spectrum, treatment indication and symptom duration in initial referrals to the rheumatologist. Z Rheumatol 2010;69(10):910-918.

Westhoff G, Dorner T, Zink A Fatigue and depression predict physician visits and work disability in women with primary Sjogren's syndrome: results from a cohort study. Rheumatology (Oxford) 2012;51(2):262-269.

SCIENTISTS
Gisela Westhoff
Johanna Callhoff

COOPERATION PARTNERS
Thomas Dietrich, Birmingham

REFERENCES

Westhoff G, Dietrich T, Schett G, de Pablo P, Zink A. The impact of periodontal disease on early inflammatory arthritis persists even after all teeth are lost. EULAR, Berlin, 6 - 9 June 2012. Ann Rheum Dis 73[Suppl 3], 500.

Pre-clinical tooth loss at arthritis onset is associated with higher disease activity, higher use of glucocorticoids and worse treatment response in a large early arthritis cohort study

Growing evidence suggests an association between periodontitis (PD) and rheumatoid arthritis (RA). Both diseases are associated with considerable bone loss (Fig. 1). Chronic PD is a major cause of tooth loss. The number of teeth may therefore, to some extent, reflect history of PD. Previous data suggest that patients with RA have fewer teeth, and that those who have experienced more tooth loss may be more likely to develop RA. Whether tooth loss is associated with increased disease activity or response to treatment in RA is unknown.

Objective

To evaluate the association between pre-arthritis tooth loss and disease activity as well as treatment response among patients with early arthritis in a longitudinal study.

Methods

The study sample included 540 patients with early arthritis (<6 months) enrolled in the CAPEA cohort study who were followed for at least 6 months. Data collection included tender and swollen joint counts (TJC28/SJC28), disease activity (DAS28), ESR, rheumatoid factor (RF), ACPA and the 2010 ACR-EULAR RA classification criteria. Study participants were categorized by self-reported number of teeth (excluding wisdom teeth; 0-10, 11-20, 21-27, 28). The association between pre-arthritic tooth loss and treatment response at 6 months according to the EULAR response criteria was analysed with bi- and multivariate analyses. Multivariate logistic regression models with 'moderate or no response' as the outcome were performed, adjusted for age, sex, education level, body mass index (BMI <25, 25-30, >30), smoking at symptom onset (yes/no), RF and/or ACPA positivity, and the fulfillment of the 2010 ACR-EULAR RA criteria.

Fig. 1: Bone loss in periodontitis and rheumatoid arthritis

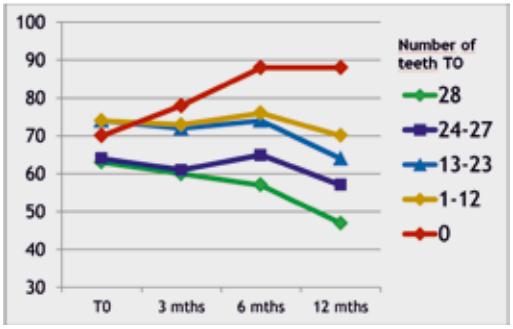

Fig. 2: Glucocorticoid use (%) in patients with inflammatory arthritis, by number of teeth

Results

Patients (65% female) were 56±14 years old and had mean symptom duration of 13 ±7 weeks. 59% were RF and/or ACPA positive, 67% fulfilled the new criteria for RA and 87% received a DMARD therapy. At 6 months, 52% of the patients achieved 'good', 32% 'moderate' and 16% 'no' response according to the EULAR Response Criteria. The mean number of teeth reported at study entry was 19±9 (23.5% 0-10, 14.9% 11-20, 39.9% 21-27, and 21.6% 28 teeth). Tooth loss was associated with older age, higher BMI and smoking. Patients with ≤10 teeth also had significantly higher ESR, T/SJCs and a higher DAS28 at 6 months compared with those with >10 teeth. However, this was at least partially explained by older age. Multivariate analysis adjusting for age and the above mentioned parameters confirmed smoking and severe tooth loss as predictors of insufficient EULAR response at 6 months (≤10 vs. 28 teeth: adj. OR 3.8, 95% CI 2.0-7.1; P = <0.001; smoking vs. non-smoking: adj. OR 1.7, CI 1.1-2.4; P = 0.009). After 12 months, there was a strong correlation between number of teeth and glucocorticoid use (Fig. 1), which was maintained after adjustment for other risk factors (Tab. 1).

Conclusion

Our results show that patients who had lost many teeth already before arthritis onset had significantly higher disease activity, higher need of glucocorticoids and poorer treatment response compared with those who had lost few or no teeth. These results suggest that prearthritic tooth loss is a predictor of disease activity and treatment response in patients with early arthritis.

Perspectives

With a grant from the German League Against Rheumatism we are currently evaluating an illustrated patient-reported periodontitis questionnaire. This instrument will enable comparative epidemiological studies into the possibly differential association between periodontitis and all kinds of inflammatory rheumatic diseases.

Baseline Parameters	Reference	adj. OR	95% CI	P
ACPA low positive	negative	1,8	1,1– 3,1	0,012
ACPA high positive	negative	1,9	1,3-3,0	‹0,001
Morning stiffness ≥1 h	‹ 1 h	1,6	1,1 – 2,2	0,006
Edentulous	25-28 teeth	7,4	2,7-20,2	‹0,001
1-12 teeth	25-28 teeth	2,6	1,6-4,3	‹0,001
13-24 teeth	25-28 teeth	2,1	1,3-3,3	0,003

Table 1 Baseline predictors of glucocorticoid use at 12 months. Multivariate adjusted for age, sex, BMI, education, smoking and joint counts

Significantly better patient-reported outcomes in women with early inflammatory arthritis using oral contraceptives compared to never users

SCIENTISTS
Gisela Westhoff, Johanna Callhoff, Angela Zink

COOPERATION PARTNERS
Georg Schett

REFERENCES
Westhoff G, Callhoff J, Schett G, Zink A

Significantly better patient-reported outcomes in women with early inflammatory arthritis using oral contraceptives compared to never users EULAR 2013 abstract

FUNDING
Pfizer Germany and German League Against Rheumatism

Data on the effects of oral contraceptives (OC) on the course of inflammatory arthritis (IA) are controversial. While some observations suggested a beneficial effect on disease activity, others did not. However, a recent analysis of data from the Norfolk Arthritis Register (NOAR) showed that the use of OC at symptom onset, and even years before, is associated with beneficial functional outcomes.

Objective

To investigate the association between the use of OC and arthritis outcomes in women with early IA within the first 12 months of rheumatologic care.

Methods

311 women with early IA (<6 months), who were 18 - 55 years old and did not use hormone replacement therapy, were followed with respect to acute phase reactants (CRP, ESR), swollen and tender joint counts (S/TJC28), duration of morning stiffness and physician-reported global health (GH 0-10), as well as to the patient-reported dimensions pain, morning stiffness, fatigue, global health (VAS 0-10), functional capacity (FFbH 0-100), and the sum score of the Patient Health Questionnaire Depression Measurement (PHQ9, 0-27). The use of OC was reported as never, past or currently. Outcomes were adjusted for age, body mass index (BMI), number of children and years of education by generalized linear models.

Results

Women were 44 ±9 years old and had symptom duration of 12 ±7 weeks at study entry. At baseline, 61% fulfilled the new RA classification criteria and 82% were clinically diagnosed with RA at 12 months. 85% took DMARDs at that point in time. 22% had never used OC (mean age 45), 57% had used them in the past (mean age 47) and 21% used them currently (mean age 35 years). The mean intake lasted 14 ±8 years in past and 16 ±8 years in current users. The current use of OC was at no time associated with acute phase reactants, joint counts, duration of morning stiffness or antirheumatic therapies. It was, however, significantly associated with almost all considered patient-reported outcomes (PROs) (Table). The effect of OC on PROs was, notably, almost the same in past and current users, although the past users were older and might not have used OC for several years before arthritis onset.

Conclusion

Our findings are in accordance with previous observations that the use of OC has a beneficial effect on IA symptoms. They confirm the NOAR data that women with past or current OC use have better functional outcomes without having less signs of inflammation. Our data suggest that the 'stimulating hormone estrogen' may affect catabolic or anabolic bioenergetic processes, resulting in less fatigue, and a better function in daily life, without affecting inflammation itself. The long-lasting effect of previous OC use needs to be explained. The use of hormones should be routinely reported in studies observing inflammatory arthritis.

Table Patient-reported outcomes at 12 months of women with early IA by use of oral contraceptives

OC use	n (%)	Morning stiffness severity§*	Fatigue severity§*	Problems getting started§*	Problems to focus§*	Problems with daily chores§*	Global health§*	Depression (PHQ9 0-27)*	Physical function (0-100)*
never	68 (21.9)	3.6	4.5	4.4	3.1	4.1	4.4	6.8	80.1
past	176 (56.6)	2.8	3.3	3.1	2.4	2.6	3.3	5.2	87.5
current	67 (21.5)	2.6	3.4	2.9	1.5	2.6	3.3	4.4	87.7
P#		0.021	0.034	0.007	0.002	0.002	0.013	0.012	0.013

*Adjusted means; §numerical rating scales 0-10; #P value: never versus current users

Foto: G.Rottmann

Angela Zink

Health Services Research

Improvement of health care by continous outcome measurement

KEYWORDS
Health care research
Routine care
Prescription patterns
Practice variation
Burden of illness

GROUP MEMBERS

Group leader
Prof. Angela Zink, Prof., PhD

Scientists
Dörte Huscher, Dipl.Math(FH)

Medical data managers
Sascha Bischoff, BA

Katja Thiele

Students
Gregor Förster; Andrea Pfäfflin; Benjamin Lorenz

Therapeutic innovations which have become available during the past decade have tremendously changed daily rheumatologic care. Treatment aims in rheumatoid arthritis are now remission or low disease activity in order to prevent structural damage and enable the patient to lead a normal life. Numerous strategic clinical studies have proven the benefit of early intervention and continuous disease control. Our group, in collaboration with a network of German rheumatologists, investigates how successful new treatments and treatment strategies have been implemented in routine care. We analyse to what extent results from clinical studies apply to unselected rheumatological patients which differ from trial patients e.g. by age or co-morbidity.

Therefore we are interested in the broad picture of current care including its deficits and costs. In addition, we are involved in national and international projects to develop instruments for disease evaluation or patient oriented measurement of treatment success, in the introduction of treatment recommendations, and in studies evaluating the importance of biomarkers for the disease course.

The data source for our work is the National Database of the Collaborative Arthritis Centres which has continuously been conducted since 1993. Each year, data of about 17,000 patients with inflammatory rheumatic diseases have been collected in a standardized manner. This allows observing trends in routine care for various disease entities. In addition, we analyse practice variation within rheumatology. All participating centres are provided with analyses of their own data in comparison with structurally similar centres and the entire data base. Differences in health care provision which cannot be explained by the clinical presentation of the patients of the respective unit are addressed in the setting of internal quality control.

In the past, the group has investigated the disease burden of several inflammatory rheumatological diseases, age-specific differences in clinical features of RA, has compared criteria for remission and analysed trends in health care provision over the years. Currently, we explore developments in treatment and outcomes of ankylosing spondylitis over the last decade and re-evaluate the formula to transfome FFbH values into HAQ values.

COOPERATION PARTNERS

Dr. R Alten, Rheumatology, Schlossparkklinik Berlin; **Prof. Dr. M Aringer,** Rheumatology, University of Dresden; **Prof. Dr. M Backhaus, Prof. Dr. F Buttgereit, Prof. Dr. G Riemekasten,** Rheumatology, Charité – University Medicine Berlin; **Dr. X Baraliakos,** Ruhr-Universität Bochum; **Prof. Dr. H Burckhardt,** Rheumatology, University of Frankfurt; **Dr. R de la Camp,** Erlangen; **Dr. K Fischer,** Greifswald; **Prof. Dr. E Gromnica-Ihle,** Berlin; **Dr. T Eidner,** Rheumatology, University of Jena; **Dr. U von Hinüber, Dr. W Demary,** Hildesheim; **Dr. G Hoese,** Stadthagen; **Dr. K Karberg,** Berlin; **Prof. Dr. I Kötter,** Robert-Bosch-Krankenhaus, Stuttgart; **Prof. Dr. A Krause,** Immanuel-Krankenhaus, Berlin; **Dr. W Ochs,** Bayreuth; **Prof. Dr. M Rudwaleit,** Berlin; **Prof. Dr. M Schneider,** Rheumatology, University of Düsseldorf; **Dr. A Seifert,** Berlin; **Dr. S Späthling-Mestekemper,** München; **Dr. S Wassenberg, Dr. R Weier,** Ratingen

SELECTED PUBLIKATIONS

Denton CP, Avouac J, Behrens F, Furst DE, Foeldvari I, Humbert M, Huscher D, Kowal-Bielecka O, Matucci-Cerinic M, Nash P, Opitz CF, Pittrow D, Rubin LJ, Seibold JR, Distler O. Systemic sclerosis-associated pulmonary hypertension: why disease-specific composite endpoints are needed. Arthritis Res Ther 2011;13:114.

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Changes in treatment and outcomes of ankylosing spondylitis in routine care in Germany 2000 to 2010

SCIENTISTS
S Bischoff, G Förster, D Huscher, A Pfäfflin, K Thiele, A Zink

COOPERATION PARTNERS
R Alten, Schlossparkklinik Berlin; M Aringer, University hospital Dresden; M Backhaus, Charité – University Medicine Berlin; X Baraliakos, Ruhr-Universität Bochum; H Burckhardt, University hospital Frankfurt; R de la Camp, Erlangen; K Fischer, Greifswald; E Gromnica-Ihle, Berlin; T Eidner, University hospital Jena; U von Hinüber and W Demary, Hildesheim; G Hoese, Stadthagen; K Karberg, Berlin; I Kötter, University hospital Tübingen; A Krause, Immanuel-Krankenhaus Berlin; W Ochs, Bayreuth; M Rudwaleit, Berlin; M Schneider, University hospital Düsseldorf; A Seifert, Berlin; S Späthling-Mestekemper, Munich; S Wassenberg and R Weier, Rheuma Zentrum Ratingen

The National Database of the German Collaborative Arthritis Centres collects clinical and patient-derived data from unselected outpatients with inflammatory rheumatic diseases. Each patient is recorded once a year. Cross-sectional data of patients with ankylosing spondylitis (AS) from nine years were compared with regard to clinical presentation and quality of life indicators. During the past decade, routine care of patients with AS has changed tremendously. Increasingly, these better treatment options are reflected in better clinical outcomes, quality of life and participation in the labour market. The increasing physical activity of patients with AS reflects successful translation of treatment recommendations to patients.

Introduction

Routine care of patients with ankylosing spondylitis (AS) has changed tremendously since the introduction of biological therapies. Tumor necrosis factor inhibitors (TNFi) are the most commonly used biologics in AS patients. We have analysed how treatment changes and their implications affected patients with AS in routine rheumatological care in Germany.

Methods

In the current study, data from the years 2000 to 2010 were used from selected units who reported their patients in each of the years considered. Cross-sectional data from nine years were compared with regard to clinical presentation and quality of life indicators. Due to changes in the documentation routine, the data from 2005 and 2006 are not representative and therefore not shown. Data on BASDAI, BASFI, BASMI and EuroQoL are available from 2007 onwards.

Results

The numbers of patients enrolled per year increased since 2007. The mean age was about 49 years, and the mean disease duration 15 to 16 years (Tab. 1).

From 2007 onwards, nearly half of all patients with AS received biologic agents. The use of synthetic DMARDs decreased significantly, steroids were given to 21% of the patients in 2000 and only 11% in 2010. NSAIDs and Coxibs remain important therapeutic options with 55% of the patients receiving nonselective NSAIDs and 24% Coxibs in 2010 (Fig. 1).

The proportion of patients whose disease was rated severe or very severe by the treating physician decreased from 20% to 12% between 2000 and 2010 (Fig. 2). The disease specific indices BASDAI, BASFI and BASMI indicate a further improvement during the past years. The proportion of patients reporting no limitations in daily activities and mobility in the EuroQoL increased by about 5% since 2007, while almost no patients reported severe physical restrictions (<=2.5%) (Tab. 2).

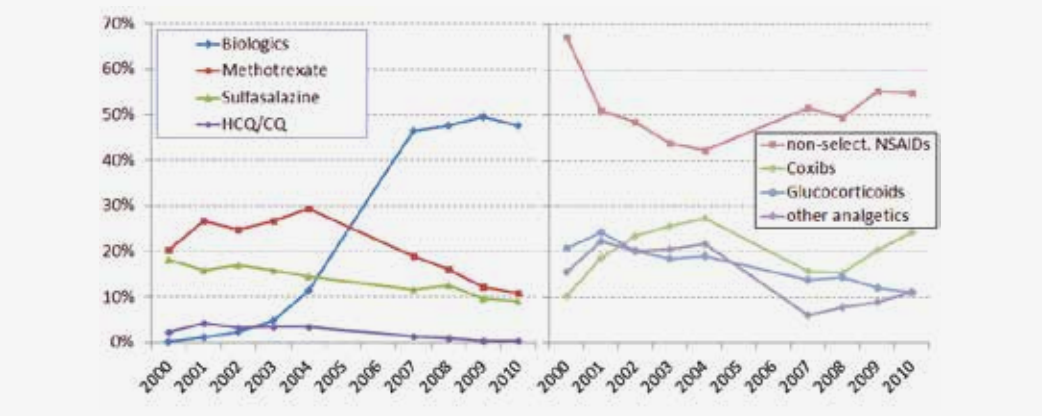

Figure 1:
Use of biologic and synthetic DMARDs and supplementary therapy

Table 1: Patient characteristics in individual years

	2000	2001	2002	2003	2004		2007	2008	2009	2010
N	828	764	926	901	772		1,197	1,170	1,289	1,316
female (%)	38	40	41	42	42		37	35	33	33
age, years (μ)	49	51	50	51	52		48	48	48	49
disease duration, years (μ)	14.8	15.1	14.5	15.0	15.6		14.6	15.3	15.7	16.4

While prescription of physiotherapy has decreased, past year's slogan of the League against Rheumatism was "Rheumatism needs exercise". For the year 2010 we analyzed patient's sporting activities and compared them to age- and sex-matched population data. In general, 75% of the patients with AS reported any sporting activities. For age groups >30 years their sporting acitivities were above the normal population rates. The influence of the disease became apparent when comparing the duration of exercise: while in the normal popoluation 29-46% of women and men exercise at least 2h per week, only 15-29% of the patients achieved such an extent. As expected, active patients reported less limitations in the EQ-5D, better FFbH and BASFI scores, had a better global health and suffered less from fatigue. While the proportion of underweight patients was the same in active and inactive patients, inactive patients had more often a BMI ≥30.

Significant changes occurred in the working situation: 54% of the patients in the working age were employed in 2000 and 65% in 2010, while early retirement declined to 16%. During this time period the percentage of patients with sick leave during the last 12 months fell from 43% to 33% (Fig. 3).

Perspectives

Our study shows that therapeutic success during the past decade is not restricted to rheumatoid arthritis. Important aspects of quality of life have improved considerably. Our next studies will be on other major disease groups such as psoriatric arthritis and systemic lupus erythematodus as well as smaller subgroups of connective tissue diseases. Research into cost trends and identification of predictors of high resource utilization will be continued. Revision of the transcription formula of the German FFbH to HAQ values is in the validation process.

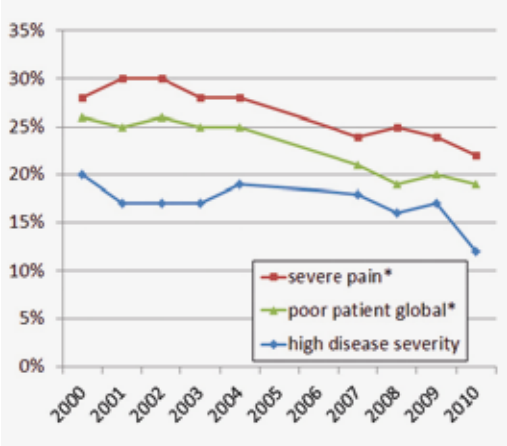

Figure 2: Outcome of disease

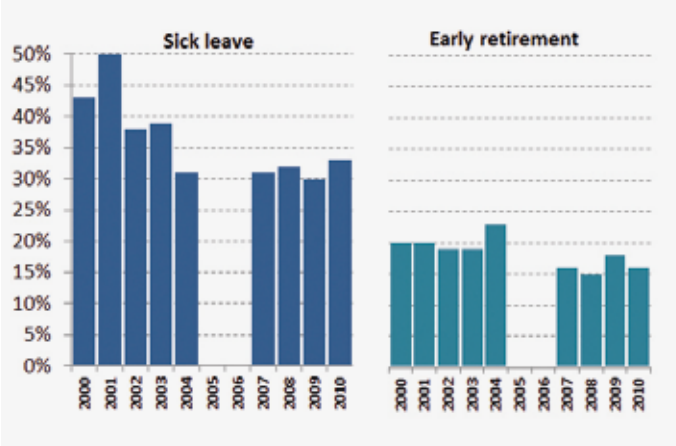

Figure 3: Sick leave of employed patients and early retirement of patients < 65 years of age

Table 2: Disease specific indices and quality of life

	2007	2008	2009	2010
BASMI (µ)	3.6	3.5	3.3	3.2
BASDAI (µ)	3.8	4.0	3.8	3.7
BASFI (µ)	3.8	3.9	3.7	3.6
EQ-5D: Mobility				
I have no problems in walking about	51%	54%	55%	57%
I have have some problems in walking about	49%	46%	45%	43%
I am confined to bed		0.1%	0.1%	0.1%
EQ-5D: Usual Activities				
I have no problems with performing my usual activities	43%	44%	45%	47%
I have some problems with performing my usual activities	55%	54%	53%	50%
I am unable to perform my usual activities	2%	2%	2%	2%

Central Units

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Flow Cytometry & Cell Sorting (FCCF) 166

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Animal Facility 170

Joachim R. Grün

Bioinformatics

KEYWORDS

Bioinformatics, High Performance Chip Data Analysis (HPCDA), HPCDA-Score, Gene List Significance Index (GLSI), Multi-Color FACS Analysis, Database-development

GROUP LEADER

Dr. Joachim R. Grün

COOPERATION PARTNERS

AG Baumgraß, AG Berek, AG Fillatreau, AG Grützkau, AG Hamann, AG Hauser, AG Radbruch, AG Romagnani, AG Tokoyoda, Dr. Henrik Mei, Dr. Zhuo Fang, Heidi Schliemann, DRFZ, Berlin.

Dr. O. Arbach, Dr. R. Biesen, Prof. G.-R. Burmester, AG Dörner, AG Prof. Hiepe, Dr. G. Heine (AG Worms), Dr. T. Häupl, Dr. B. Rosche, Dr. B. Stuhlmüller, T. A. Sörensen, Charité CCM, Berlin.

Dr. C. Dang-Heine, Dr. K. Klugewitz, Prof. J. Sieper, Charité CCBF, Berlin.

PD Dr. K. Pels, PD Dr. Schott, Charité CVK, Berlin.

AG Thiel, U. Stervbo-Kristensen, Dr. A. Sattler (AG Babel), BCRT, Berlin

Dr. A. Hutloff, R. Franke, Robert Koch Institut, Berlin

Prof. F. Melchers, Dr. H. J. Mollenkopf, Dr. M. Tsuneto, Dr. S. Beetz, Dr. A. Mäurer, Max Planck Institut für Infektionsbiologie, Berlin.

Group Bioinformatics is working together with group Immune-Monitoring of Andreas Grützkau. FACS data of that group are analyzed with similar tools as used for chip data. In Ramin laboratory hybridized chips of DRFZ groups or cooperation partners, were analyzed with High Performance Chip Data Analysis (HPCDA) using BioRetis database of Thomas Häupl (Charité CCM). These analyses give us much more possibilities

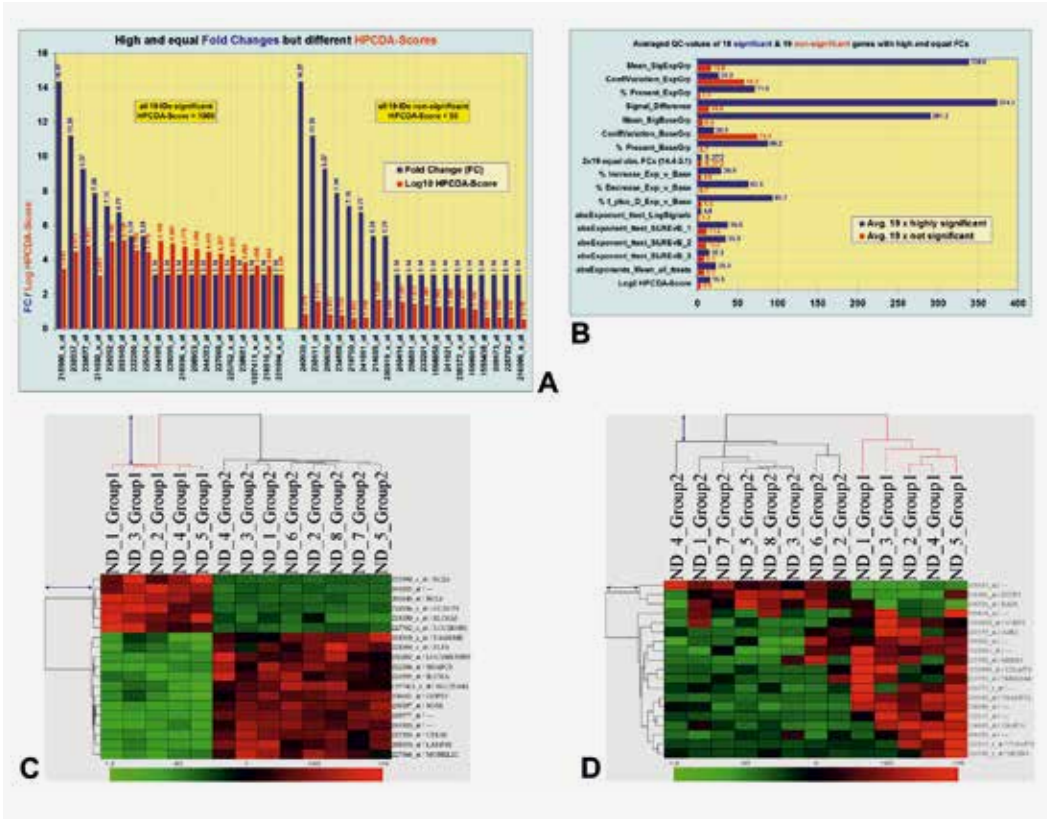

Figure 1: A: 19 significant (left) and 19 non-significant IDs (right) were selected from a 5 vs. 8 chips dataset (AG Radbruch). All significant genes have an exact absolute FC counterpart in the non-significant group. Significant IDs have HPCDA-scores ›1000 and those of non-significant ones are all ‹50. B: Averaged quality values of significant (blue) or non-significant (red) groups of 19 IDs as in A. Mean absolute FCs are 5.372 in both cases, all other mean values are much better for the significant group resulting in much higher HPCDA-scores. C: Clustered 19 significant IDs as in A. D: Clustered 19 non-significant IDs as in A. Due to much higher variability of Signals in D differences of group 1 and 2 and between up- and down-regulated gene groups are lower in D than in C (cf. blue arrows). Quality differences are better visible with HPCDA-score (1A) or quality values (1B) than in cluster pictures, but it is obvious in D that FC is only an averaged value.

than only with calculating fold changes (FCs). In this report usage of FC versus HPCDA possibilities are compared.

In former times of gene expression profiling a FC of 2.0 was criterion to include a gene in a list of significant genes. However, even if FC is one important it is also a limited number to judge relevance of a gene of interest. To visualize this, a list of IDs with significant and one with non-significant genes was chosen, both with identical FC distribution and after that the relevance of genes with HPCDA-score or FC ranking is shown additionally. Figure 1 shows selection of 2 x 19 genes. Left part of Fig. 1A shows significant IDs (BioRetis), with FCs of 3.14 - 14.37 (mean FC = 5.372) and with HPCDA-scores all above 1000. Right part of Fig. 1A shows 19 IDs with exactly same absolute FC distribution, but all judged non-significant, resulting in HPCDA-scores below 50. HPCDA-score shows relevance of a single gene, is calculated including values of Fig. 1B, and was developed empirically with help of Gene List Significance Index (GLSI). GLSI reflects quality of a list of genes. Fig. 1B shows averaged values for both groups of Fig. 1A, relevant in HPCDA. FCs are identical, nevertheless, the difference between significant (blue) and non-significant (red) groups is dramatically and better visible as in cluster pictures of both groups, shown in Fig. 1C (significant) and 1D (non-significant IDs).

In Figure 2, 13037 significant or all 54675 IDs of same dataset as in Fig 1 were ranked by HPCDA-Score or FC. To show quality trends of ranked genes median values for first 13037, 6518, 3258, 1628, 814, and 407 IDs were calculated. Fig. 2A shows median HPCDA-scores of all 4 rankings and 2B the median FCs for same IDs. It is known that ranking the complete dataset by FC brings a lot of really non-significant and even absent genes with high FCs in front. Therefore, it's no wonder that these gene lists have the highest FCs but lowest quality (lowest HPCDA-scores in 2A). Ranking the list of significant genes with FC increases quality only up to a number of 3258, after that, the quality of further reduced lists is decreasing even if FCs are strongly increasing.

In Fig. 1D, it is visible, that FC is an averaged value, sometimes a high FC is realized only by 2 or 3 very high values, and it is not clear if these are only outliers. It should be easily acceptable that a gene with high FC with Signal values of one group all in the same range is much more relevant than a second gene with same FC but with a few very high Signals and a big variation in both groups or with Signals, all called absent or of very low expression height. All these values are included in HPCDA-score calculation, giving a better ranking of gene relevance as that with FC alone.

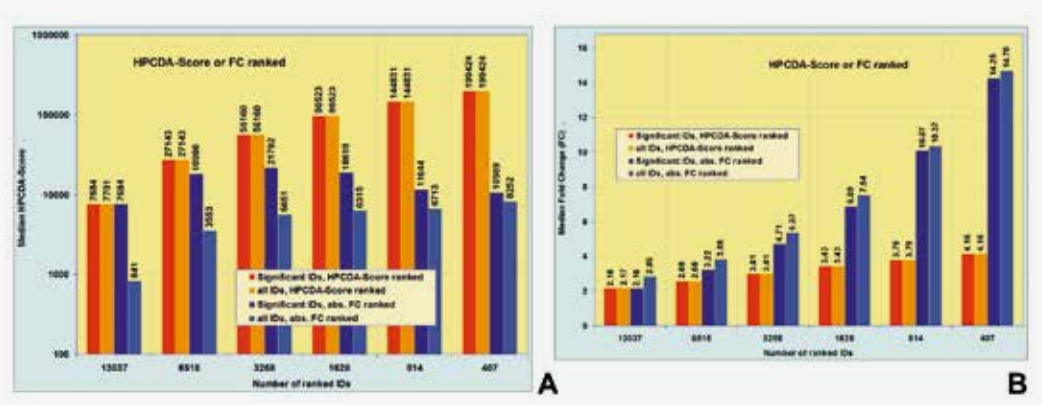

Figure 2: A: Same dataset as in Fig. 1 ranked in two different ways: Red color shows median HPCDA-scores of all 13037 significant IDs, ranked by HPCDA-Score; values for all and first 6518, 3258, 1628, 814, and 407 significant IDs are shown; HPCDA-score ranking of all 54675 IDs with median scores of first 13037 - 407 IDs in orange. Median scores of absolute FC ranked group of HPCDA significant genes in dark blue, and lighter blue shows median scores of the complete dataset, ranked by FC. B: Median FCs for the same four different rankings and the same lists of IDs as in A.

LECTURES/COURSES

Joachim R. Grün, Statistik in der Bioinformatik (DNA-Chipdaten Analyse), Consulting & Qualification Bildungszentrum Haberhauffe GmbH, 16.8.-19.8.2011,

Joachim R. Grün, Statistik in der Bioinformatik (Microarrays), Consulting & Qualification Bildungszentrum Haberhauffe GmbH, 12.12.-18.12.2012,

Dr. J.P. David, Universitätskrankenhaus Eppendorf, Hamburg

Dr. S. Beetz, Prof. D. Kabelitz, Universität Kiel, Kiel.

A. Derer (AG Hüber), Universitätskrankenhaus Erlangen, Erlangen

Prof. A. Tarnok, Klinik für Kinderkardiologie, Leipzig.

Prof. W. Maslinski, Institut of Rheumatology Warsaw, Poland.

Prof. C. L. Verweij, VU University Medical Center Amsterdam, the Netherlands.

Dr. F. Apparailly, INSERM, Montpellier, France.

Prof. D. Boumpas, Dr. P. Verginis, University of Crete, Heraklion, Greece.

Dr. H. Yssel, Institut de Génétique Moléculaire de Montpellier, Montpellier, France.

Ph.D. A. Inamine, Head and Neck Surgery Graduate School of Medicine, Chiba University, Chiba, Japan

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Anja E. Hauser
Raluca Niesner

Central Laboratory for Microscopy
Cinima - Core facility for Innovative Imaging and Microscopy Approaches

GROUP MEMBERS

of CINIMA and JIMI:

Prof. Dr. Anja E. Hauser
Dr. Raluca Niesner
Dr. Zoltan Cseresnyes
Dr. Franziska Mech

Fabian Kriegel,
Master Student / Bioinformatics

Julian Pohlan,
Medicine Student

Due to the successful combination of intrinsic molecular specificity with high spatial resolution, fluorescence microscopy is a versatile technique largely employed in both routine and high-end research applications of biosciences and biomedicine. Currently, these applications reach from the investigation of fixed biopsies, living cell cultures, vital organ models or even living organisms on a molecular basis and down to electron-microscope-like spatial resolution, i.e. supra-resolution techniques (like STED microscopy, PALM, STORM or SSIM/SPEM). The expertise at the **C**ore facility for **in**ovative **im**aging und **a**nalysis (**CINIMA**) at the DRFZ covers standard fluorescence techniques like wide-field and confocal fluorescence microscopy to the newest developments in vital organ explants and intravital multi-photon microscopy.

Confocal laser-scanning microscopy and wide-field fluorescence microscopy

For fast screening of histology samples of different organs (e.g.bone-marrow, gut,lymph node or central nervous system), the DRFZ research groups employ the wide-field fluorescence microscope (Zeiss). In order to achieve three-dimensional images in up to five or six different colors, e.g. spectral resolution of up to six different cellular subsets, the high-resolution Zeiss LSM710 confocal microscope is used (Figure 1). Both whole field images reaching to a few mm² (when tile scans are made) and images depicting cellular interaction/co-localization at sub-cellular level using the 63x oil-immersion lens (NA = 1.3, i.e. resolution at 488 nm excitation wavelength of 229 nm laterally and 577 nm axially) can be acquired.

A central feature of the confocal microscope at the DRFZ is the possibility of live cell visualization and monitoring in a temperature- and CO2-regulated incubation chamber.

Multi-photon laser-scanning microscopy for deep-tissue and intravital microscopy

While the standard wide-field and confocal microscopy based on single-photon excitation intrinsically allows for high resolution due to UV/visible illumination, they do not allow for high imaging depths in intact, living organs. Due to the benefits of ultra-short pulsed near infrared (NIR) and infrared (IR) illumination, multi-photon microscopy counteracts exactly this shortcoming of standard fluorescence microscopy. The intrinsic 3D resolution and the excitation within the tissue optical window, i.e. at wavelengths where neither water nor hemoglobine absorb radiation, makes multiphoton microscopy an ideal tool for the visualization of cellular motility and communication deep in vital organ models or in organs within living organisms at low endogenous fluorescence and sub-cellular resolution.

The DRFZ and its partners at the Charité already have a wide expertise concerning multi-photon imaging within explanted organs, e.g. hippocampal brain slices (Dr. Jan Leo Rinnenthal, Dr. Helena Radbruch, Prof. Frank Heppner, Institute for Neuropathology, Charité) and spleen slices (Prof. A.E. Hauser, Dr. R. Niesner), and even more, within living organisms in intestine (group of Prof. A.E. Hauser), lymph node (group of Prof. A.E. Hauser), bone-marrow (group of Prof. A.E. Hauser) and organs of the central nervous system, i.e. brain stem (Dr. Helena Radbruch, Figure 2) and brain cortex (Dr. Jan Leo Rinnenthal, Prof. Frank Heppner,

Simon Bayerl, Prof. Vajcozy, Dr. Martina Füchtemeier). Currently, we focus our attention in extending this expertise to intravital spleen and kidney imaging and to longitudinal bone marrow endoscopy.

While extending the expertise in the field of deep-tissue and intravital multi-photon imaging with applications especially in immunology plays a central part in the work at the DRFZ imaging core-facility CINIMA, tight cooperations with other imaging facilities in Berlin as well as with systems biology groups ensure the access of DRFZ scientists to a broad palette of fluorescence imaging techniques and data evaluation. In 2011 **JIMI** (**J**oint network for **I**ntravital **MI**croscopy) was founded in order to bundle the expertise of Multi-Photon intravital imaging present at the DRFZ, Berlin (Dr. R. Niesner und Prof. Dr. A. Hauser) with the expert knowledge of microscopy at the MDC (Dr. A. Sporbert and Dr. Zoltan Cseresnyes). The JIMI network is complemented by Prof. Dr. Marc-Thilo Figge (Hans-Knöll-Institut, Jena) in the field of image analysis and systems biology. The goal of **JIMI** is to make intravital microscopy accessible for researchers from different fields in life science. We provide help in planning and performing intravital microscopy experiments as well as in the analysis of the data. This is completed by consulting and assistance through regular seminars of the JIMI members and of invited experts of the field of optical imaging in biosciences and biomedicine. Starting 2012, **JIMI** has been granted funding for 3 years by the DFG. More information can be found on: www.jimi-network.de.

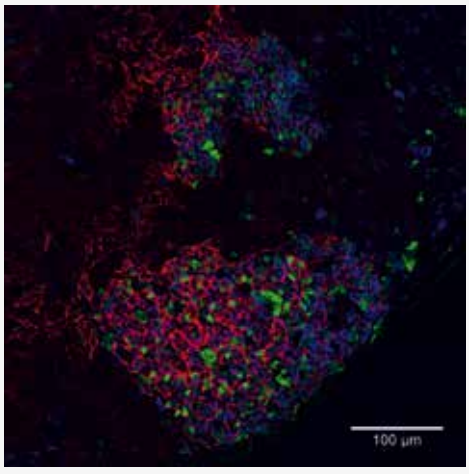

Figure 1: Fluorescence image acquired with our confocal microscope (objective lens 20x) in Peyer's patches from a B1-8 GFP+ mouse immunized with NP-CγG x days before imaging. GL7 – blue, B1-8 GFP+ cells – green, follicular dendritic cells (CD21/35+) – red.

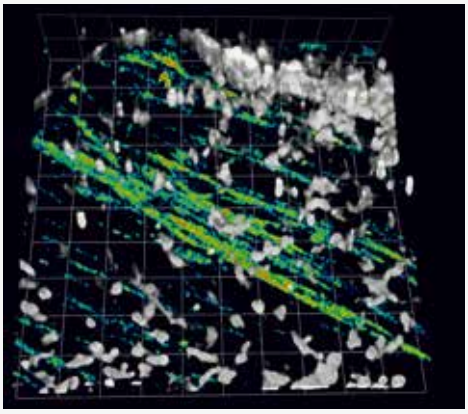

Figure 2: 3D fluorescence image in the brain stem of a CerTN L15 mouse affected by experimental autoimmune encephalomyelitis. The false colors scale (rainbow) indicates the calcium level in neuronal structures (neuronal somata, axons and dendrites are labeled via Thy1 expression cassette). The immune cells (grey) invade the parenchyma and damage the components of the central nervous system (indicated by increased calcium). Mesh unit = 30 µm.

Foto: J. Hirscher

Immune Monitoring

Immune monitoring is a general term referring to several diagnostic procedures for determining the immune status of a patient. Depending on the clinical field, humoral factors such as cytokines, antibody-titers or complement factors, the cellular composition of whole-blood, functional-cellular parameters or a combination of any of these can be determined. Immune monitoring programs are already available for different clinical disciplines, such as those relevant for transplantations, sepsis, immunomodulatory therapies and vaccine studies in which following the specific immune responses is critical.

The idea for establishing a comprehensive immunomonitoring program in the field of inflammatory-rheumatic illnesses is based on the recognition that biomarkers dependent on signaling from single molecules have, with few exceptions, little predictive value with regard to diagnosis or prognosis. Notable exceptions include measures of auto-antibodies directed against citrullinated peptides/proteins (so-called ACPA's) in RA, measures of plasmablasts (Odendahl et al., 2000; Jacobi et al., 2010) or of Siglec-1 expressed on monocytes in cases of SLE (Biesen et al., 2008) - two parameters that correlate strongly with disease progression in Lupus patients. Interestingly, the expression of Siglec-1 in human monocytes has been validated as a highly specific type I interferon surrogate marker in SLE that revealed as the most robust biomarker if compared to the measurement of IFN itself in serum or measuring soluble response proteins, such as IP-10 (Rose et al., 2012). But nevertheless, the identification of biomarker-signatures instead of singular parameters will pave the way for new diagnostic procedures that are urgently needed in therapy-prognosis. Actaully, there are a lot of expensive therapy options available for treating rheumatic patients, but unfortunately at this point there are no companion diagnostics available, which can predict the individual responsiveness already before therapy has been started. This is, however, precisely the goal of personalized medicine, namely to find the best possible treatment for each patient patient. This reduces the risk of potentially harmful side-effects as well as the economic burden on health care systems. The latter is especially relevant in this case as only 50-60% of patients treated with expensive biologics profit from a noticeable improvement in their symptoms.

Because of the technological possibilities opened by the latest-generation of analytical flow-cytometers, as many as 17 different fluorochromes can be measured simultaneously at the single cell level. Based on the possibility of these highly advanced methods, we have introduced a state-of-the-art immunomonitoring program that includes 50 different surface-molecules which, when analyzed in the appropriate combinations, will deliver information on nearly 900 phenotypic parameters. After having demonstrated that 38 immune-phenotypic parameters were sufficient to classify blood samples from patients with Bechterew Disease, it is now being investigated if parameters can be identified that will predict a positive response to anti-TNF-α therapy.

The immunomonitoring staining panel currently includes 10 established staining protocols that primarily contain cell-specific and activation-dependent antigens. These protocols, depending on how they are employed, can be expanded with further stains. Figure 1 contains a summary of parameters that characterizes the cellular composition of whole blood leucocytes („cytometric differential blood count"; Figure 1).

The other protocols allow a fine-analysis of lymphocyte populations (plasmablasts/plasmacells, regulatory T- lymphocytes, central-memory and ventral-effector memory T-lymphocytes), dentritic cells (plasmacytoid and myeloid DC's), monocytes, granulocytes and NK-

GROUP MEMBERS

Gruppenleitung
Dr. rer. nat. Andreas Grützkau

Scientists
Dr. rer. nat. Sabine Baumgart
Chieko Kyogoku, PhD

Technische Assistenz
Heike Hirseland

PhD and MD students
Ursula Schulte-Wrede
Thomas Rose
Anna Smorodchenko

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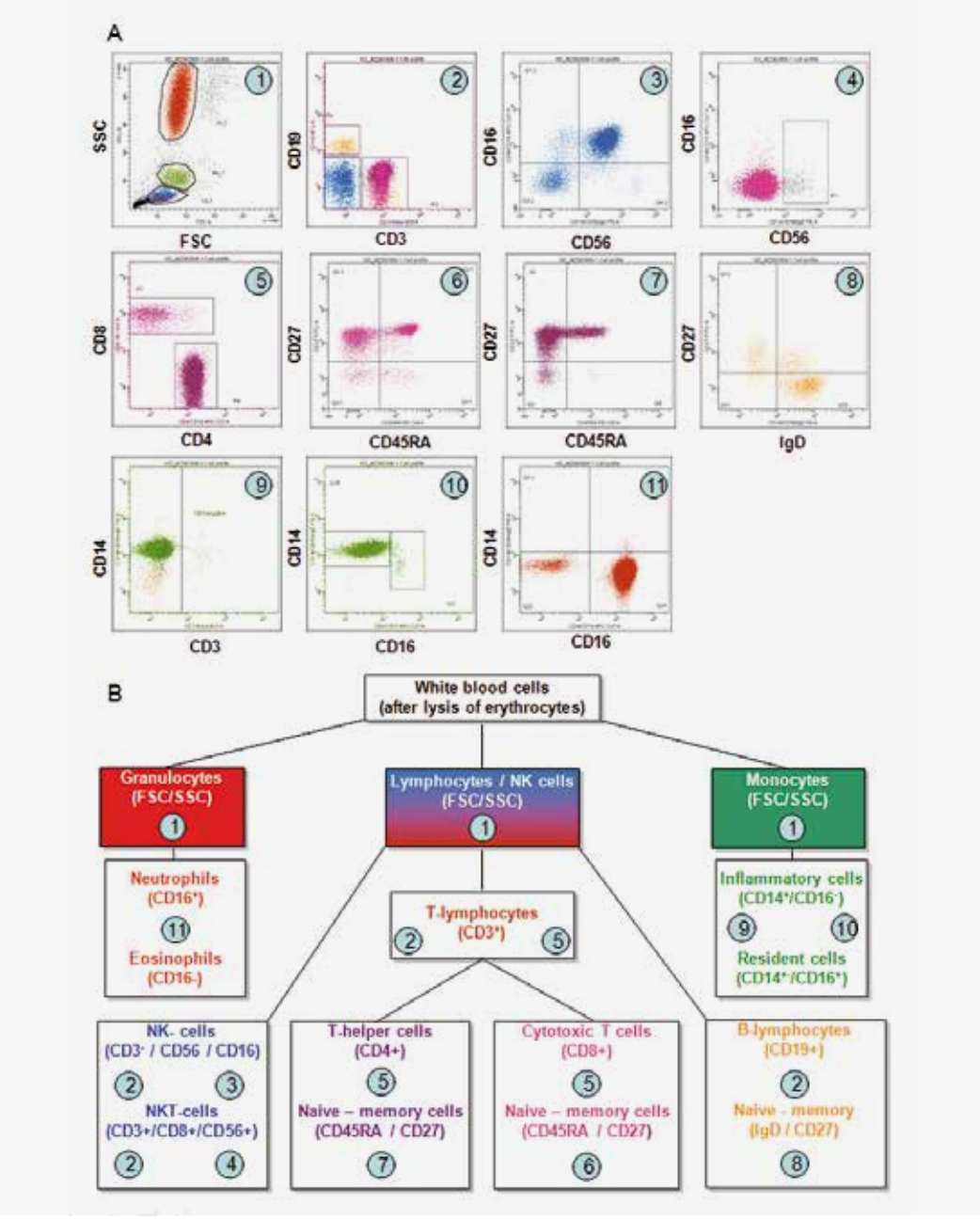

Figure 1: "Cytometric Differential Blood Count" from a healthy control that was generated using a combination of 10 different antibodies directed against surface-proteins. (A) shows the gating-strategy for identifying the different leukocyte populations in peripheral blood. (B) shows the cell populations identified in A and their respective characterizing surface proteins summarized schematically.

COOPERATION PARTNERS

Dipl. Ing.Toralf Kaiser, Dr. rer. nat. Joachim Grün, DRFZ

PD Dr. med. Thomas Häupl, Till Sörensen, Dr. rer. nat. Bruno Stuhlmüller, Dr. med. Robert Biesen, Sylvia Pade, PD Dr. med. Berit Rosche

PD Dr. med. Gabriela Riemekasten, Prof. Dr. med. Thomas Dörner, Dr. rer. nat. Henrik Mei, Prof. Dr. med. Falk Hiepe, Charité – Universitätsmedizin Berlin, Campus Charité Mitte

Prof. Dr. med. Jochen Sieper, Marta Steinbrich-Zöllner, Charité – Universitätsmedizin Berlin, Campus Benjamin Franklin

Prof. Dr. Cor Verweij, VUmc Amsterdam, Niederlande

Prof. Dr. Wlodzimierz Maslinski, Institute of Rheumatology, Warschau, Polen

Dr. rer. nat. Sven Olek, Epiontis GmbH

FUNDING

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cells. The strength of this comprehensive cytometric blood count lies in the fact that other, rarer cell populations are included. These populations, such as regulatory T-lymphocytes, represent a relatively low percentage of cells found, but because of their specialized, immune-modulatory functions, they play a significant role in the inflammatory response and therefore a significant role in influencing therapies.

5-10 mL of whole blood are usually needed for immunemonitoring. Blood is first treated with lysis-buffer to eliminate red blood cells. After staining the cells with the appropriate antibody-cocktails, they are analyzed using cytometers equipped with a blue, violet and red laser.

The enormous amount of phenotypic parameters that can be generated from a single blood-sample made it necessary to develop a specific Access-Databank. This will allow a group-wise comparison of different sample cohorts such as healthy versus diseased or treated versus untreated and comes up with a list of statistical significant immunphenotypic parameters. It is also necessary to develop algorithms that will allow for complete-automatization of the blood-analysis process. To achieve this, cooperation with the Department of Bioinformatics at the Charité lead by Thomas Häupl has been established for the development of automated Clustertools (described in the following paragraph).

Bioinformatics

Aside from the analysis strategies developed by Joachim Grün (Bioinformatic; DRFZ) that will first need to be processed manually, Till Sörensen (from the Thomas Häupl group; Charité) is currently working on algorithms that will enable a fully automated analysis of multi-dimensional data-sets.

A completely automated analysis of flow-cytometry data from the point of measurement to analysis and tests for the purposes of research and diagnostic procedures requires solving problems at several points in the process: appropriate transformation of the fluorescence-parameters, recognition of cell-groups within measurement data-sets, identifying cell-populations between different data-sets, and the extraction of appropriate characteristics for the cell-populations for further examinations and tests.

An algorithm has been developed for the purpose of grouping cell-values into the single measurements, and is applicable to large data-sets (> 1 million cells and up to 10 measurement-parameters). This algorithm is based on an „Expectation Maximization“ – (EM) iteration for a multi-dimensional t-mixture model. Appropriate transformation-parameters are estimated between the iteration-steps to organize the distribution of the cell-groups into a symmetrical form as far as is possible. The number of cell-groups is then determined iteratively. In this way, the algorithm is directly applicable to the measured-data without the necessity of pre-analysis or direct management by an operator. As each step involved in the cell-grouping takes into account all parameters, one can also process uncompensated data directly (Figure 3).

After the cell-groups have been measured in individual experiments, the individual measurements must be allocated to one-another. A meta-cluster-algorithm that takes into account the position and spread of the cell-groups from individual experiments in multi-dimensional space was developed for this purpose (Figure 4). “Generalised Prokustes Analysis” (GPA) is used to even out the individual scaling of parameters between experiments, which enables broad identification of specific cell-populations.

Results & Discussion

Immunomonitoring in cases of Bechterew Disease and Multiple Sclerosis.

In frame of a pilot study conducted in cooperation with the Charité- Campus Benjamin Franklin Dept. of Rheumatology, blood from 12 untreated patients with Bechterew Disease (ankylosing spondylitis) was compared to that of 12 healthy controls. Following primary analysis of the raw data using FacsDIVA-Software (Becton Dickinson), the data were imported into a specifically-adapted Access-database (Joachim Grün). This system was then used to identify the parameters or parameter-combinations obtained from patients with Bechterew Disease that were regulated in a manner significantly different to healthy controls.

Using these methods, it was possible to identify 77 phenotypic parameters that allowed for flawless classification of the patients and healthy controls. Even the

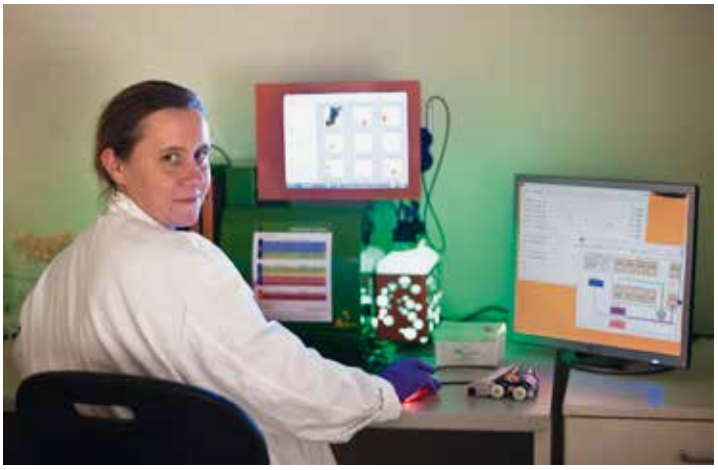

Figure 2: Data acquisition at he MACSQuant cytometer (Miltenyi Biotec.). Up to nine parameters can be detected at the single cell level.

typing of new blood-samples not originally intended for the process of parameter selection could also be predicted with high accuracy.

Anti-TNF-α therapy-induced immune-phenotipic signatures in patients with AS could also be identified in this manner. Currently, signatures that predict positive response to anti-TNF-α before beginning therapy are being validated.

In collaboration with Berit Rosche (Neuroimmunology; Charité), it was also possible to identify disease-specific signatures in patients with Multiple Sclerosis.

Perspectives

The complex immune monitoring described here is currently being used to accompany therapy-studies and to identify immune-phenotype based predictors for positive response to specific biologics. Development of algorithms that enable automation of primary data-analysis is also being pursued. Lastly, methods for high-throughput immunomonitoring of large volumes of samples must be developed.

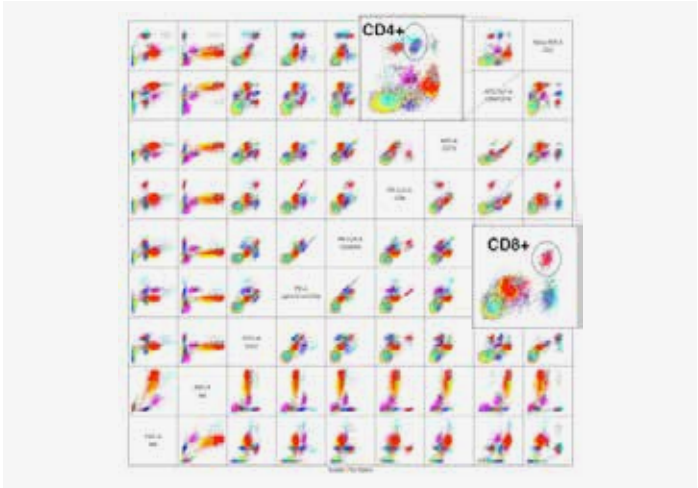

Figure 3: Result of an automatic detection of cell populations defined by nine different cellular parameters (seven fluorescence and two light scatter parameters).

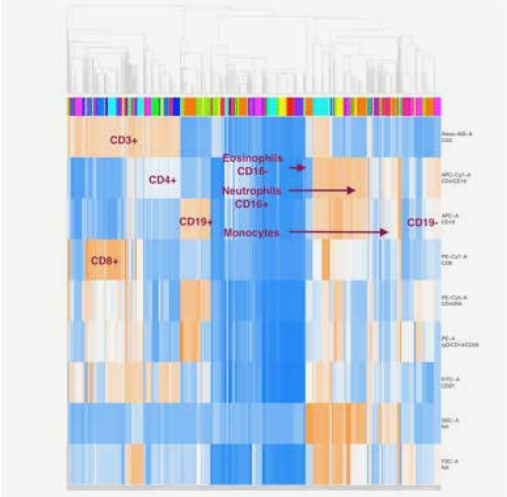

Figure 4: Automated clustering of 691 immunophenotypic populations that discriminate peripheral blood samples from six healthy donors and 12 ankylosing spondylitis patients

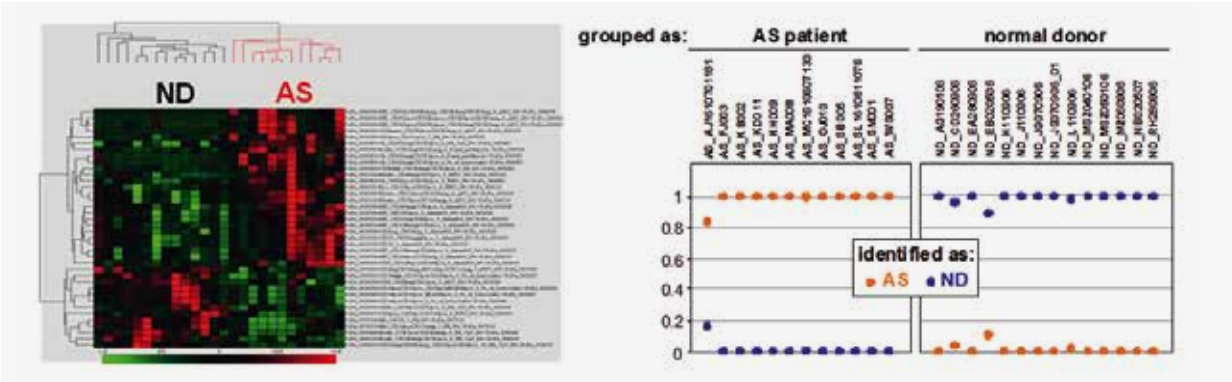

Figure 5: Classification of 12 ankylosing spondylitis patients in comparison to 12 healthy donors by 77 immunophenotypic parameters. These parameters were also used for a successful prediction analysis by the software tool PAM (Prediction Analysis of Microarray data). All samples were correctly predicted with a propability of ›80%.

Andreas Grützkau
Claudia Berek
Thomas Häupl

Regine von Ramin-Laboratory for Molecular Rheumatology

GROUP MEMBERS

Gruppenleitung
Dr. rer. nat. Claudia Berek
Dr. rer. nat. Andreas Grützkau
PD Dr. med. Thomas Häupl

Technische Assistenz
Heidi Schliemann
Heike Hirseland
Gudrun Steinhauser

REFERENCES

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Through the generous bequest from Mrs. Regine von Ramin and further financing by the Berlin senate-administration WiFoku, the Regine-von-Ramin Laboratory for Molecular Rheumatology (RvR-Laboratory) was founded in December of 2004. This facility will be used jointly by research groups of the DRFZ and the affiliated groups of the Charité for the purposes of genome-wide gene expression analysis using Affymetrix-Technology. In addition to global transcription analysis, it will also be possible to carry out microarray-based miRNA expression analysis.

Technical equipment belonging to the Regine von Ramin Laboratory

The RvR-Laboratory contains an Affymetrix-station, which is equipped with the the Scanner 3000 7G and the Fluidics FS450. For the purposes of RNA-sample quality control, a Bioanalyzer (Agilent) and Nanodrop ND-1000 spectral photometer are available in addition to standard instruments. Additionally, the aHybTM-Hybridisation station (Milenyi Biotec) allows for processing of array formats that are spotted on slides, and thereby processes like a miRNA expression profiling using the miRXplore Array (Miltenyi Biotec).

An ideal supplement to this facility is a Laser Capture Microdissection machine (LCM) from Fa. Arcturus, which allows a cutting out of morphologically and immune-histologically characterized tissue samples for molecular analysis without enzymatically digesting them. Both systems are primarily used for molecular analysis of cells at the level of their DNA, RNA and proteins. Through the integration of LCM-technology in the RvR-Laboratory, it is possible to perform global gene expression analysis on tissue samples that are morphologically and immuno-histologically identifiable (Figure 1).

Bioinformatic Analysis

A close cooperation with the Department of Bioinformatics (Joachim Grün, DRFZ; Thomas Häupl, Charité) and the Charité spin-off company "BioRetis", , allowed the establishment of a comprehensive mouse- and disease-relevant human transcriptome database. The data-warehousing of these data by the BioRetis-analysis platform allows for comfortable and precise group comparative analysis that can be implemented worldwide by existing national and international research organizations within the framework of a defined rights allocation system. An overview of the basic analysis strategies can be found in the following chapter on "Bioinformatics" by Joachin Grün.

Generating disease-, cell- and cytokine-specific gene expression profiles.

One research focus at the DRFZ is the analysis of disease- and cell-specific global gene expression profiles. It has been shown that peripheral monocytes isolated from RA, SLE and Bechterew's disease-patients show disease-specific gene signatures that couldbe successfully used for disease-classification (Figure 2). The large number of differentially expressed genes reflects the complexity of chronic-inflammatory rheumatic diseases. The central pathophysiological role of pro-inflammatory cytokines, such as TNF-α or INF-α/γ, in the inflammatory-processes could be shown clearly with the clinical success of cytokine-specific biologicals. However, there is currently no reliable molecular biomarker that would allow for a targeted and thereby individualized therapy. Therefore, cytokine-specific gene signatures have been generated and analyzed ex-vivo in two EU-supported research-projects (Autocure and IMI JU BTCure) (Figure 3).

Through a comparison of these expression profiles with the disease-specific profile, Biljana Smiljanovic was able to show, in the course of the research conducted for her dissertation, that peripheral monocytes are highly-sensitive biomarkers that express disease-dependent, qualitative and quantitative variability in their cytokine signature. While monocytes from Lupus-patients are characterized primarily by an IFN-α/γ-induced immune-response, RA-patients were characterized by a dominance of TNF-α in this process. This knowledge will be used in further investigations to clarify if these molecular cytokine-response signatures can be used to predict a positive response to therapy.

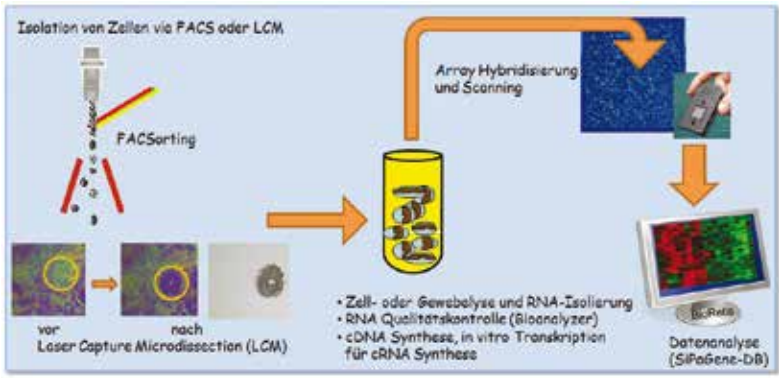

Figure 1: process of global gene expression analysis beginning with cell isolation using FACS or Laser-Microdissection to the generation and analysis of the transcriptome using microarray-technology. These methods allow for the identification of so-called gene-signatures that shed light on the cell-specific pathophysiology and can also be used for diagnostic purposes.

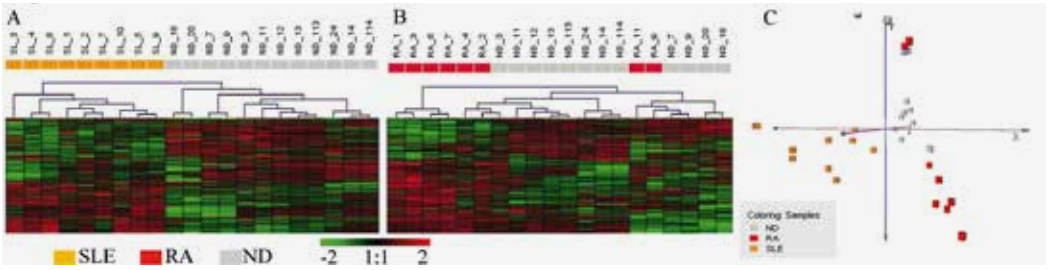

Figure 2: gene expression profile of human monocytes isolated from the peripheral blood of Lupus (A) and RA-patients (B) compared to the monocytes of healthy donors. The red-green pattern summarizes regulated genes (red: enhanced expression; green: reduced expression) that are, however, qualitatively different between SLE and RA and can therefore be used in disease-classification (C; Principal Component Analysis).

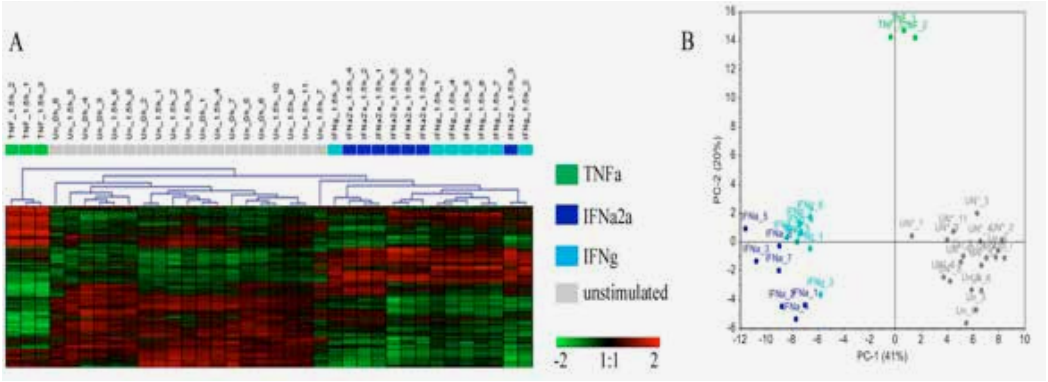

Figure 3: (A) hierarchical gene cluster analysis from cytokine-specific expression profiles that were established from peripheral blood monocytes. (B) Principal Component Analysis (PCA) showing that the cytokine-activated cells are clearly differentiatable from unstimulated samples (Gray), and that the IFN-a and IFN-g gene profiles (in blue and turquoise) are more similar to one-another than those from TNF-a (in green).

COOPERATION PARTNERS

Dr. rer. nat. Joachim Grün, DRFZ

Dr. rer. nat. S Fillatreau, Dr. rer. nat. Chiara Romagnani, Dr. rer. nat. A Hutloff, Dr. rer. nat. K Tokoyoda, DRFZ

PD Dr. med. K Pels, PD. Dr. med. E. Schott, Dr. rer. nat. C Daridon, Prof. Dr. med. T Dörner, Dr. med. S Mercan,Dr. rer. nat. K Kappert, Charité - Universitätsmedizin Berlin

Prof. Dr. rer. nat. A Thiel, BCRT - Charité

Prof. Dr. rer. nat. F Melchers, Max-Planck-Institut für Infektionsbiologie, Berlin

PD Dr. rer. nat. U Höpken, Max Delbrück Zentrum, Berlin-Buch

PD Dr. rer. med. H Eibel, Universitätsklinikum, Freiburg

Prof. Dr. med. B Bröker, Immunologie, Greifswald

Dr. F Apparailly, Dr. H Yssel, INSERM, Montpellier, Frankreich

Prof. Dr. W Maslinski, Institute of Rheumatology, Warschau, Polen

Dr. rer. nat S. Wild, Miltenyi Biotech, Bergisch-Gladbach

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Senatsverwaltung für WiKuFo (Zuwendung aus dem Bund-Länder-Programm "Förderung innovativer Forschungsstrukturen in den neuen Ländern und Berlin)

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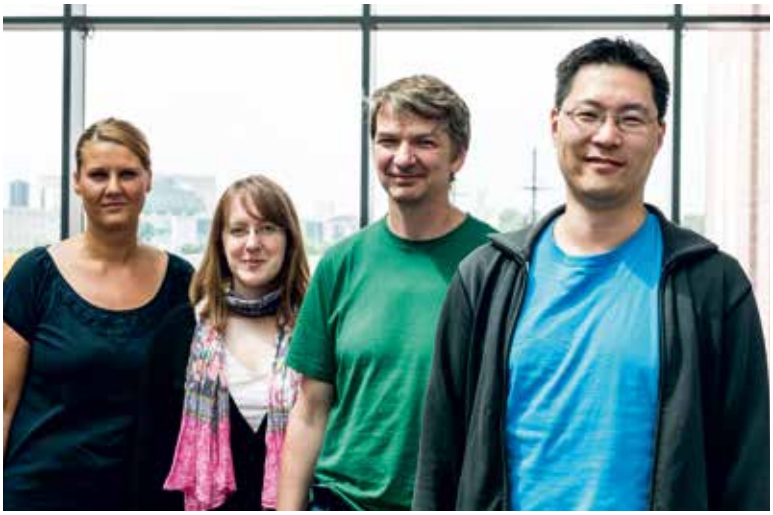

Jenny Kirsch
Alexandra Heeseler
Toralf Kaiser
Hyun-Dong Chang

Flow Cytometry & Cell Sorting (FCCF)

GROUP MEMBERS

Toralf Kaiser,
Technical Head of the FCCF

Jenny Kirsch,
Service operator and advisor for biological issues

Alexandra Heeseler,
Student assistance

Hyun-Dong Chang,
Scientific Head of the FCCF

COOPERATION PARTNERS

Max Planck Institute for Infection Biology

Max Planck Institute for Molecular Genetics

Charité - Universitätsmedizin Berlin (Campus Mitte, Campus Steglitz, Campus Virchow)

Universität Potsdam

Ferdinand-Braun-Institut Leibniz-Institut für Höchstfrequenztechnik

Frank Opitc Products GmbH

PUBLICATIONS

UV-C LED Disinfection Module Integrated in a BD FACS Diva Cell Sorter for Sterile Cell Sorting. Jenny Kirsch, Johannes Glaab, Michael Kneissl and Toralf Kaiser. Poster at Cyto conference, Leipzig, Germany, 2012.

Extensive service features and technological innovations allow state-of-the art cell analysis and cell sorting

The Flow Cytometry Core Facility (FCCF) is located at the DRFZ since the year 2000. It originated from the cooperation between the DRFZ, the Charité-Universitätsmedizin Berlin and the Max Planck Institute for Infection Biology. The FCCF has state-of-the-art technical equipment. Scientists are provided the possibilityto conduct flow cytometric cell sorting and cell analyses. The facility operates five cell sorters and ten cell analyzers. The facility staff offers expertise based on 12 years of experience in the area of flow cytometry. It assists users offering competent consultation in the planning and realization of their experiments.

Technical equipment

2 Analyser FACSCalibur (2-lasers)
4 Analyser MACSQuant (3-lasers)
1 FACS Canto (3-lasers)
1 Analyser LSR II (4-lasers)
1 Analyser LSR Fortessa (4-lasers)
1 Cell Sorter FACSVantage SE with Turbosort and DIVA upgrade (3-lasers)
2 Cell Sorter FACS Aria (3-lasers)
1 Cell Sorter FACS Aria II (4-lasers)
1 Cell Sorter Influx
1 Cytometer CyTOF
1 Dataserver 1TB to save cytometry data files

Methods and range of application of Flow Cytometry

Flow cytometry is an analytical method used for the quantitative analysis of physical, biochemical/cell biological and immunogenetic parameters of cells. On the basis of these parameters various cell features, such as viability, quantification of antigens, phenotype, and cell cycle can be determined. The flow cytometers available at the FCCF allow the simultaneous measurement of up to 15 parameters at the single cell level. Cells can be measured and analyzed at an extremely rapid rate of up to 20,000 events per second. By this means, cell subpopulations cannot only be analyzed; they can also be isolated and grown, or used in downstream experiments. This is achieved by sorting a defined number of cells into tubes or on special cell culture plates. Even single cells can be sorted on slides or in multi-well tissue culture plates.

Fields of activity at the FCCF: Cell analysis and cell sorting

Cell analysis

Using a cell analyzer, cells can be analyzed electronically due to their biological and physical features. These features can be measured using scattered light and an antibody-fluorescent dye complex. The antibody is coupled to fluorescent dye that fluoresces when excited with monochromatic laser light. The high sensitivity of the cell analyzer allows the detection of as few as 50 molecules per cell. Various cell characteristics are measurable due to the attachment of the antibody to the cell. This type of procedure is also possible for fluorescing proteins (e.g. GFP, RFP) located inside a cell. Analytical cytometers, such as the LSR II and the LSR Fortessa, allow multichromatic detection of up to 15 parameters, and which have been used to establish a complex multiparametric, longitudinal immunophenotyping of blood samples of rheumatic patients (Steinbrich-Zoellner et al., 2008). The LSR Fortessa and one of the MACSQuant analyzers are equipped with a yellow-green laser (561 nm) that allows a very sensitive detection of phycoerythrin (PE) and its tandem conjugates. The latest development in analytical flow cytometry is the use of non-radioactive isotopes of so-called rare earths for labeling and detection. The cytometer CyTOF combines flow cytometry with ato-

mic mass spectrometry. This new technology circumvents the inherent problems of fluorescence, e.g. spectral overlap and bleaching of dyes, by detecting and quantifying isotopes of defined atomic mass coupled to antibodies. In this way, 40 parameters, and theoretically up to 100 parameters, can be measured on a single cell level.

Cell Sorting

Cell sorting is another technique used in flow cytometry, whereby cells of interest can be sorted out and collected for further biochemical purposes or functional analyses. Due to their features, single cells can be sorted out selectively from a heterogeneous mixture. At first, these cells are analyzed (as in the cell analyzer) and packed into individual droplets. The sorting of the cells is then accomplished by giving the droplets an electrical charge. The charged droplet is then deflected by charged electrodes into waiting sample tubes.

In 2010 a new BD Influx has been acquired. The BD Influx offers high sensitivity combined with enhanced flexibility, enabling the sorting of cells of different sizes. This enables the isolation of larger and fragile cells, such as cells from different tissues, such as bone marrow, lymph nodes or spleen (Tokoyoda et al., 2009).

In combination with appropriate pre-enrichment strategies, such as magnetic cell sorting, very rare cell subsets, like antigen-specific CD4 and CD8 or Th17 lymphocytes, can be analyzed or even separated to high purities for molecular down-stream analyses (Kirchhoff et al., 2007; Lexberg et al., 2010).

Technological developments

Immune monitoring

The number of parameters used in flow cytometry for a further subdividing of leukocyte subsets increases steadily. Especially, multichromatic immune monitoring for diagnostic and predictive purposes will profit from these developments. Presently, we are establishing the CyTOF technology to increase the multiplicity of parameters that can be detected by a conventional high-end cytometer.

Established technologies applied in practice

Training activities

Every month we offer a two hour basic course on flow cytometry, which provides training and education for the proper use of the techniques used in flow cytometry. The course is open for all who are interested. Because of the high demand, persons interested should register in the discussion platform. The Sorterclub takes place every two weeks and serves as a forum for discussing and planning experimental research with reference to flow cytometry or cell sorting. Together with various groups working at the DRFZ the FCCF offered workshops for users from around the world. In 2013, an EMBO-Practical Course focusing on Cytometry, Cell Sorting, and advanced microscopy will take place once again, as already in the years 2008, 2006 and 2003. Every year the FCCF opens its doors for the "Long Night of Sciences" for making cell sorting and cellular immunology more perceptible for public visitors, such as rheumatic patients, students or pupils.

Service offers accessible via internet

Our service offers for scientists are accessible via internet. After the registration, the users get access to the online-scheduler located on:

http://fccf.drfz.de

There, the use of equipment, the documentation of experiments, and billing can be managed. Users also have access to the central data server in order to view FACS data and for using the discussion forum.

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Kaiser T, Raba K, Sickert M, Radbruch A, Scheffold A. Integration of an ultrasonic wave device in a FACS-Aria cell sorter for continuous, non-invasive mixing of cell suspensions.

PATENTS

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Kaiser T, Kolbe T, Kneissl M: WO 2012/123412, "FLOW CYTOMETER DISINFECTION MODULE".

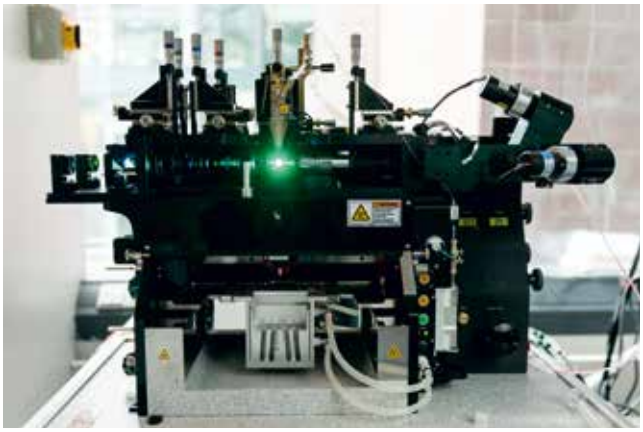


Figure 1: The high-end cell sorter (BD Influx), which allows the isolation of large and fragile cells, such as stroma cells or stem cells, the isolation of extremely small cell components, and for polarisation measurements.

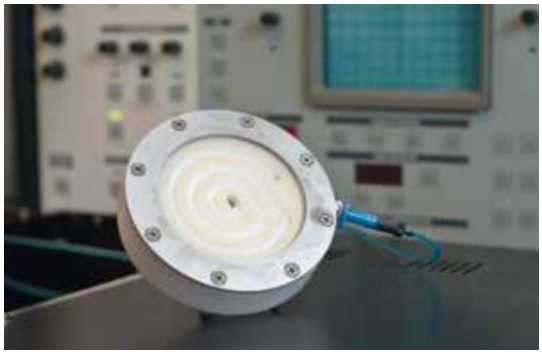


Figure 2: A newly developed module equipped with UV-C LEDs being currently tested for the continuous decontamination of the sheath fluid of cell sorters. The prototype has been developed in collaboration with the Ferdinand Braun Institute and the TU Berlin and is suitable for all currently commercially available sorters and thus could reduce the danger of contamination during the sorting process.

Heidi Schliemann
Gudrun SteinhauserHeidi
Hecker-Kia
Tuula Geske
Anette Peddinghaus

Central Laboratory

Service facility for experimental research

GROUP MEMBERS

Heidi Hecker-Kia
Tuula Geske
Heidi Schliemann
Gudrun Steinhauser
Anette Peddinghaus

COOPERATION PARTNERS

Max Planck Institut für Infektionsbiologie

Charité – Universitätsmedizin Berlin,
Campus Mitte und
Campus Benjamin Franklinww

The central laboratory facility of the DRFZ was founded in the year 2000. The lab managers provide services for all research groups, the main focus being on the supply with antibodies and their conjugates. Apart from this central task, the lab managers are also responsible for infrastructural aspects concerning the DRFZ, such as: the estimation and acquisition of financial contributions of the liaison groups to the DRFZ infrastructure, the ordering system regarding the supply with cell culture and general materials, management of general scientific equipment, including acquisition, calculation and service, in collaboration with the administration of the DRFZ and the supply of groups with basic materials, chemicals and chemical solutions for working with cell cultures.

Antibodies

Specific antibodies and their fluorochrome derivatives are of central importance for doing experimental cell-biological research. Therefore, the team of lab managers working in the central laboratory particularly focuses on the production and supply of more than 1.000 substances. More than 250 antibody-producing hybridomas are cultivated. Specific antibodies are isolated from cell culture supernatants, purified and, if necessary, conjugated with various fluorochromes. Thus, the lab managers provide the basic supply with all relevant biological tools necessary for doing FACS ananlysis, cell sorting, histology, ELISA, and other immunological techniques. (Fig.1)

The huge variety of antibodies and conjugates is accessible to scientists working at the DRFZ, the Max Planck Institute for Infection Biology, the Charite- Universitätsmedizin Berlin, and in other collaborating groups. The intranet folder “OnlineAntibodiesManagement” offers information on the current quantity of samples available in the stock.Users are also provided with background information such as, for instance, the name of clone, concentration, and titer, or with other data regarding the samples.(Fig.4)

Service

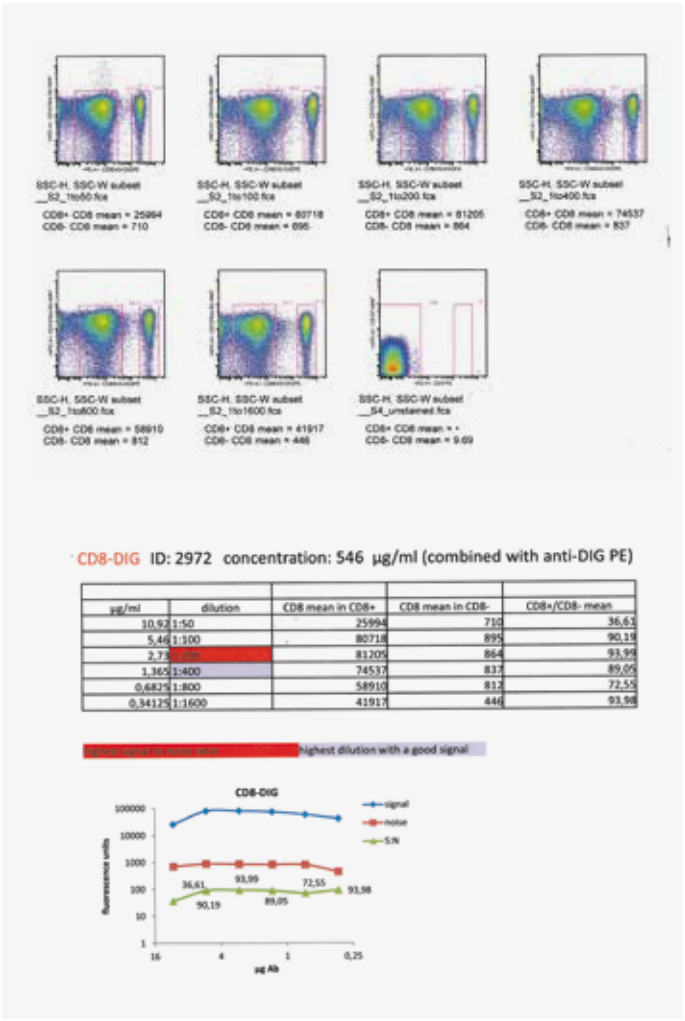
On request, the lab managers also offer their advice and assistance in case of technical problems, for instance concerning the isolation and purification of proteins, derivatisation, fusion or fragmentation of antibodies and other proteins, production of specific affinity matrices for chromatographic purposes and for the establishment of ELISAs. All preparations go hand in hand with specific quality controls. (Fig.2+3)

The field of activity of the central laboratory also includes the introduction and establishment of new methods and techniques, in particular, regarding new fluorochromatic markers.

Perspectives

The acquisition of further hybridomas for new scientific questions is essential.The fight against mycoplasma or endotoxin contamination has to be adapted continuously to state-of-the- art technology. The increasing use of multicolor techniques in FACS requires a continuous increase in the number of available fluorochromes, to provide enough substances with sufficient brightness, photo stability and narrow emission spectra in differnt combinations. Beyond that the introduction of the CyTOF technology in the DRFZ implies new fields of responsibility, e.g. the establishment of antibody conjugation with heavy metals.

The growing number of groups and scientists working at the DRFZ makes the adaption of infrastructural aspects of the central laboratory necessary and represents another great challenge for the lab management. The main aim is to guarantee the availability of necessary equipment and facilities at all times.



µg/ml	dilution	CD8 mean in CD8+	CD8 mean in CD8-	CD8+/CD8- mean
10,92	1:50	25994	710	36,61
5,46	1:100	80718	895	90,19
2,73	1:200	81205	864	93,99
1,365	1:400	74537	837	89,05
0,6825	1:800	58910	812	72,55
0,34125	1:1600	41917	446	93,98

Figure 2: Titrations protocol: amCD8 (53-6.72) - DIG

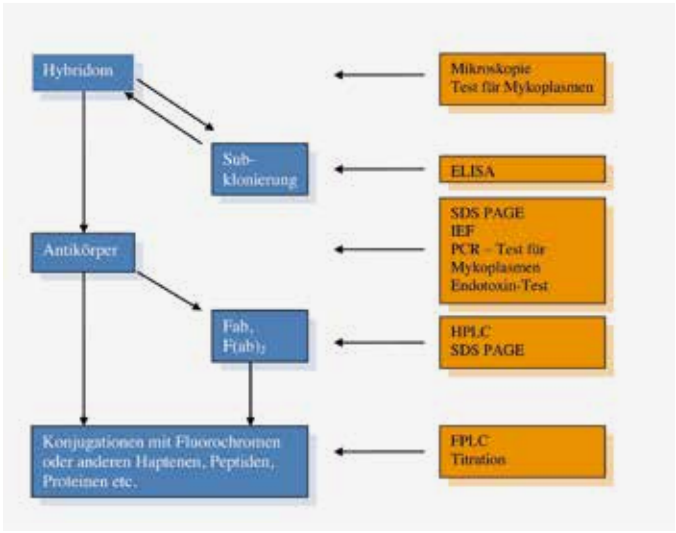


Figure 1: General overwiew of antibody production and quality control

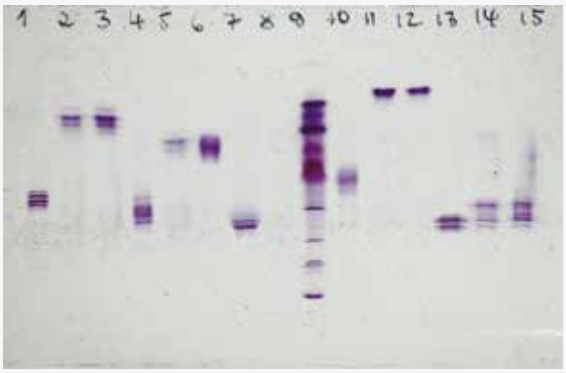
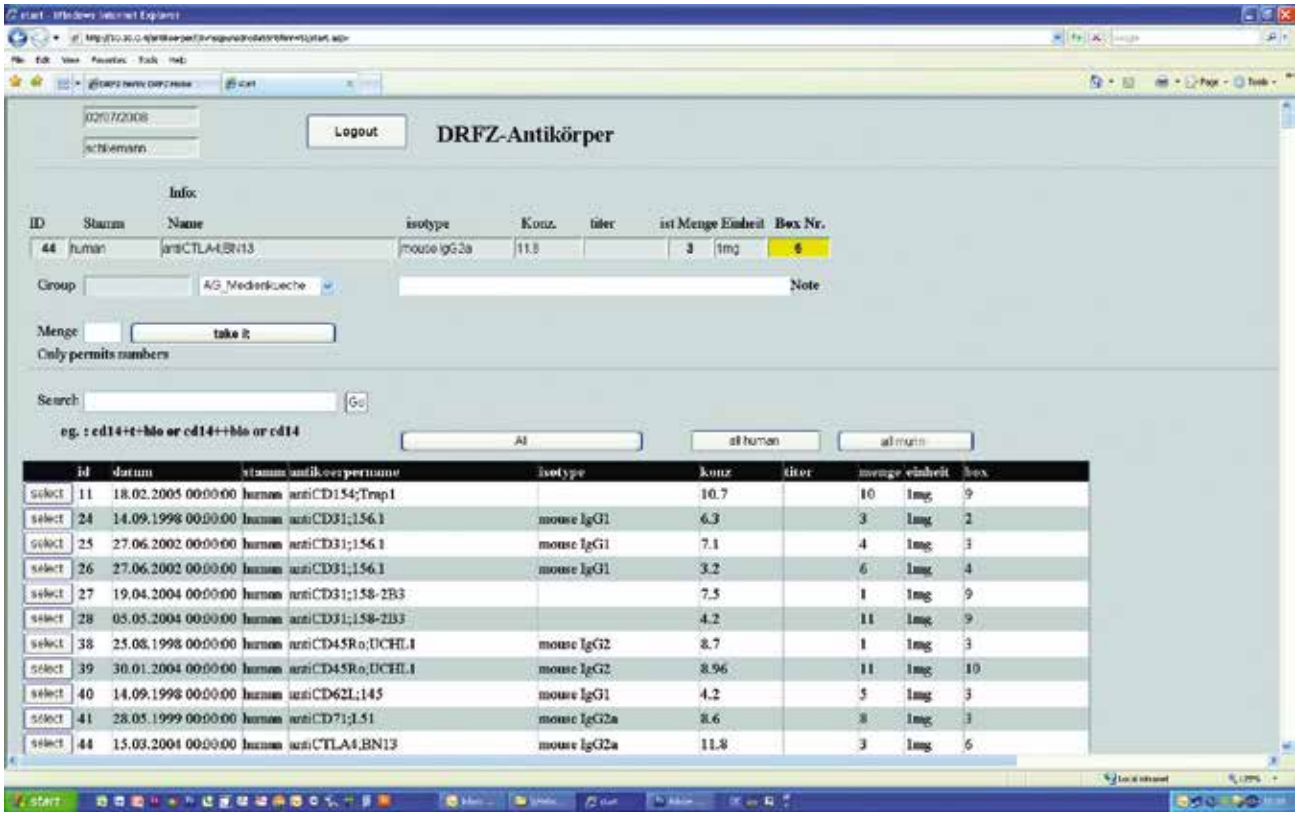


Figure 3 : Quality control of antibodies: isolectric focusing



	id	datum	stamm	antikoerpername	isotype	konz	titer	menge	einheit	box
select	11	18.02.2005 00:00:00	human	antiCD154;Trap1		10.7		10	1mg	9
select	24	14.09.1998 00:00:00	human	antiCD31;156.1	mouse IgG1	6.3		3	1mg	2
select	25	27.06.2002 00:00:00	human	antiCD31;156.1	mouse IgG1	7.1		4	1mg	3
select	26	27.06.2002 00:00:00	human	antiCD31;156.1	mouse IgG1	3.2		6	1mg	4
select	27	19.04.2004 00:00:00	human	antiCD31;158-2B3		7.5		1	1mg	9
select	28	05.05.2004 00:00:00	human	antiCD31;158-2B3		4.2		11	1mg	9
select	38	25.08.1998 00:00:00	human	antiCD45Ro;UCHL1	mouse IgG2	8.7		1	1mg	3
select	39	30.01.2004 00:00:00	human	antiCD45Ro;UCHL1	mouse IgG2	8.96		11	1mg	10
select	40	14.09.1998 00:00:00	human	antiCD62L;145	mouse IgG1	4.2		5	1mg	3
select	41	28.05.1999 00:00:00	human	antiCD71;L51	mouse IgG2a	8.6		8	1mg	3
select	44	15.03.2004 00:00:00	human	antiCTLA4;BN13	mouse IgG2a	11.8		3	1mg	6

Figure 4: Online data bank

Anja Schulz
Astrid Puppe

Animal Facility

GROUP MEMBERS

Facility management:
Dr. med. vet. Anja Schulz

Animal welfare officer:

Dr. med. vet. Kristina Ullmann
(till 10/2012)
Astrid Puppe
(since 11/2012)

COOPERATION PARTNERS

Max Planck Institut für Infektionsbiologie

Charité – Universitätsmedizin Berlin

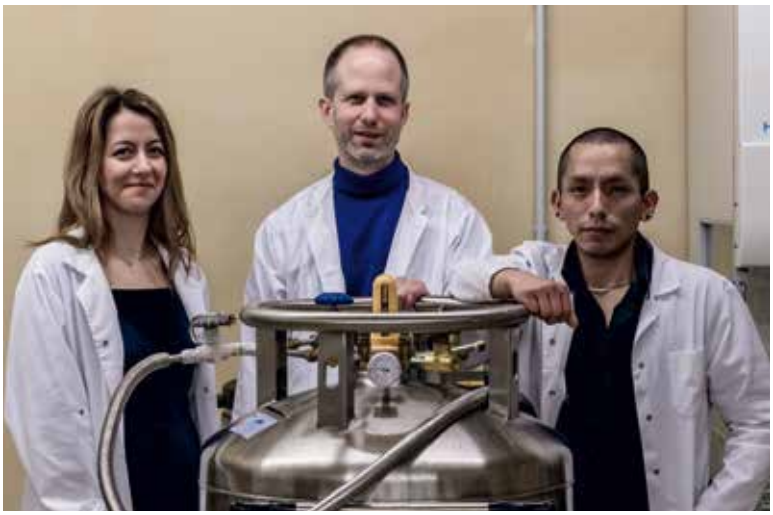
Genetically modified mice are valuable tools for modern biomedical research. Although the list of methods replacing animals in experimental research is constantly growing, some scientific questions still require the use of animal models. Especially to understand multifactorial diseases like autoimmunity, in which different organs and cell types are involved, the complexity of a living organism is needed.

Therefore, the DRFZ operates a state of the art animal facility. This facility is divided into an experimental area in Berlin-Mitte and a separate breeding area in Berlin-Marienfelde, where numerous mouse strains are kept. Specially trained animal care takers and veterinarians ensure best husbandry conditions and a seamless monitoring of the animals in the favor of animal welfare.

All mice are bred and held under so called SPF (specific pathogen free) conditions to ensure that animals are free from pathogens that could interfere with the later planned studies. To guarantee this, the facility is equipped with personnel airlocks, and all material that enters is thoroughly autoclaved. All mice undergo a microbiological check-up on a regular basis.

The DRFZ also provides (along with the MPI) a training program for scientists working with mice, including a theoretical and practical part which is confirmed with a certificate after participating.

Regular veterinary check up in the mouse facility.



SCIENTIST

Scientific facility management:
Dr. Andreas Hutloff

Technical staff:
Paolo Rosellini Tognetti,
Sevim Yanc

Additional assistance:
Janni Breinl

COOPERATION PARTNERS

Dr. Uwe Klemm,
Experimental Animals,
Max Planck Institute for
Infection Biology, Berlin

Dr. Geert Michel,
Transgene Facility, FEM,
Charité University
Medicine Berlin, Germany

Ronald Naumann,
Transgenic Core Facility,
Max Planck Institute of Molecular
Cell Biology and Genetics, Dresden

Frank Zimmermann,
Biotechnology Laboratory,
University Heidelberg

Embryo Technology Laboratory

The Embryo Technology Laboratory was founded in 2010 as a joint core facility together with the Robert Koch Institute. It is located within the animal facility in Berlin-Marienfelde and provides state of the art technologies for modern mouse colony management like in vitro fertilization (IVF) and cryopreservation.

The following services are offered (Fig. 1):

1) Embryotransfer. To guarantee a defined health status in the SPF breeding area, new transgenic mouse lines have to be imported via embryotransfer. Two cell embryos (generated by IVF or classical mating) are washed extensively to remove any pathogens (Fig. 2) and implanted into pseudopregnant foster mice.

2) Cryopreservation of mouse sperm and embryos. Mouse strains which are temporarily not needed can be stored as 2-cell embryos or sperm in liquid nitrogen. Frozen material is also an option for world-wide shipping of transgenic mouse lines.

3) In vitro fertilization. IVF is used to recover mouse lines from frozen sperm and is generally a very efficient method to generate mouse embryos.

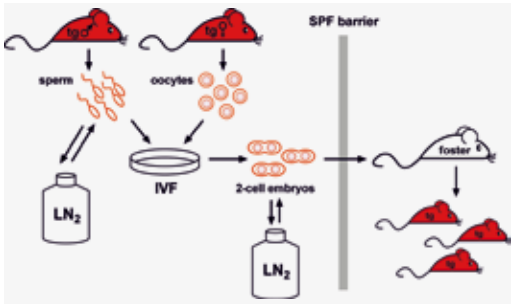


Figure 1: Workflow for services offered by the ET-Lab.

Figure 2: Washing of embryos. The fertilized oocyte is protected by the zona pellucida which is impermeable for pathogens. Residual sperm is still sticking to the zona.

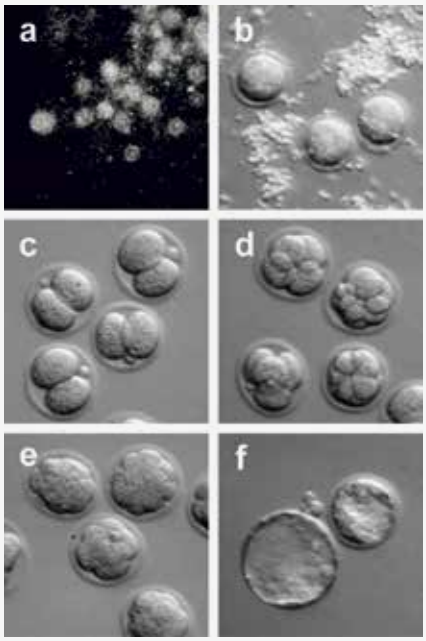


Figure 3: Developmental stages of mouse embryos. a) Sperm and oocytes (hidden in the cumulus cells) 5 min after IVF. b) 45 min after IVF. The sperm almost completely dissolved the cumulus cells. c) 2-cell embryos. d) 4- and 8-cell embryos. e) Morula stage. f) Blastocysts.

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Worm M, Babina M, Hompes S. Causes and risk factors for anaphylaxis. J Dtsch Dermatol Ges. 2013;11(1):44-50.

Zink A. Risiko für nichtmelanotischen Hautkrebs unter TNF-Inhibition [Risk of non-melanoma skin cancer under TNF inhibition : New aspects from the British Biologics Register.]. Z Rheumatol. 2012 Dec 9. [Epub ahead of print] German.

Invited Talks 2011

Jan. 8, 2011 Minden, K., Neues zu alten Erkrankungen: Epidemiologie, Pathologie und Therapie chronisch-entzündlicher rheumatischer Erkrankungen im Kindes- und Jugendalter, Wissenschaftsretreat Charité, Berlin, GER

Jan. 8, 2011 Strangfeld, A., Infektionen unter Biologika - Daten aus RABBIT und anderen Registern, Jahresauftakt-Tagung 2011, Dresden, GER

Jan. 10, 2011 Minden, K., Biologika in der Kinderrheumatologie anti-TNF & Co., Quo vadis, Kinder- und Jugendmedizin?, Berlin, GER

Jan. 13, 2011 Strangfeld, A., Update from the German Biologics Register RABBIT, 7th European Register Meeting, Uppsala, SWE

Jan. 17, 2011 Zink, A., European Biologics Registers - Collaborative Analyses across Registers, EULAR Task Force on Biologics Registers Implementation Workshop, Zürich, CHE"

Jan. 17, 2011 Listing, J., Time-varying risks: Consequences for the analysis and interpretation of the results, EULAR Task Force on Biologics Registers Implementation Workshop, Zürich, CHE"

Jan. 17, 2011 Eveslage, M., Preparing data for analysis, data quality, misclassification: start & stop dates, outcome, EULAR Task Force on Biologics Registers Implementation Workshop, Zürich, CHE

Jan. 21, 2011 Minden, K., Outcome of juvenile idiopathic arthritis, Kinderrheumatologisches Symposium, St. Aug.in, GER

Jan. 26, 2011 Worm, M., Anaphylaxie und Notfallbehandlung, Fortbildung Landesverband der HNO-Ärzte, Halle, GER

Jan. 27, 2011 Worm, M., B cells in allergy, Kolloquium Molekulare Zellbiologie, Lübeck, GER

Jan. 27, 2011 Radbruch, A., Wie uns das Immunsystem schützt, Vortrag im Einstein-Gymnasium (Vortragsreihe organisiert von der Berlin-Brandenburgischen Akademie der Wissenschaften, BBAW), Potsdam, GER

Feb. 8, 2011 Hamann, A., Topografical memory, T cell lineages and the impact of epigenetic regulation, Seminar, Erlangen, GER

Feb. 9, 2011 Berek, C., The plasma cell survival niche, Universitätsklinikum Jena-Friedrich-Schiller-Universität, Jena, GER

Feb. 16, 2011 Baumgrass, R., Mathematical modeling of transcriptional T-cell activation and differentiation, Meeting: Forsys Status Seminar, Heidelberg, GER

Feb. 17, 2011 Worm, M., Atopic Dermatitis and Food Allergy: When and how to test, FAAM - EAACI, Venedig, ITA

Feb. 24, 2011 Radbruch, A., immunological memory as driver of chronic rheumatic inflammation, International Immunology Summit, Berlin, GER

Feb. 24, 2011 "Zink, A., What can the information from patient registries in RA tell us? Interpretation of the data, International Immunology Summit, Berlin, GER"

Mar. 2, 2011 Radbruch, A., The organisation of immunological memory, ESF-JSPS Frontier Science Conference for Young Researchers, Hulshorst, NLD

Mar. 4, 2011 Radbruch, A., Memory Plasma Cells, European Workshop for Rheumatology Research, Amsterdam, NLD

Mar. 11, 2011 Hiepe, F., Systemischer Lupus erythematodes – neue Therapieansätze und "unmet medical need", UCB-Kamingespräch “Aktuelle Herausforderungen der Immunologie”, Köln, GER

Mar. 11, 2011 Strangfeld, A., Optimizing Treatment Now and for the Long Term, The REAL-Meeting, München, GER

Mar. 13, 2011 Radbruch, A., Immunological memory, 7th Spring School on Immunology of the DGfI, Ettal, GER

Mar. 17, 2011 Minden, K., Course and prognosis of JIA - Data from observational studies, 1st Transalpe Meeting in Pediatric Rheumatology, Innsbruck, AUT

Mar. 17, 2011 Worm, M., Are there different immunological “finger-prints” of vitamine-A-derivates?, Symposium Management of type I and type IV allergies - new persectives and treatment options (Annual Congress SSAI-SGAI), Lugano, CHE

Mar. 18, 2011 Hamann, A., Functional memory, T cell lineages and the impact of epigenetic regulation, 7th Spring School on Immunology of the DGfI, Ettal, GER

Mar. 22, 2011 Radbruch, A., Immunablation und autologe Stammzelltransplantation, Gastdozentur beim Lebenswissenschaftlichen Kolleg der Studienstiftung, Köln, GER

Mar. 23, 2011 Niewerth, M., Transition in der pädiatrischen Rheumatologie - Status quo, Symposium der Ständigen Koordinationsgruppe Versorgungsforschung, Berlin, GER

Mar. 23, 2011 Strangfeld, A., Versorgungsrealität und ACR-Update Epidemiologie, RheumaWissen, Bremen, GER

Mar. 25, 2011 Radbruch, A., Immuntherapie der Zukunft, Kurs „Spondylarthritiden“, Berlin, GER

Mar. 26, 2011 Worm, M., Gentechnik in der Diagnostik von Nahrungsmittelallergien, 6. Tiroler Allergie-Tagung Gentechnik und Allergie, Innsbruck, AUT

Mar. 28, 2011 Westhoff, G., Beobachtungsstudie Sjögren-Syndrom, Sjögren-Selbsthilfe Berlin, Berlin, GER

Mar. 30, 2011 Worm, M., Auslöser und Epidemiologie der Anaphylaxie, 46. DDG-Tagung, Dresden, GER

Mar. 31, 2011 Chu, VT., Eosinophils are required for the longevity of plasma cells, 9th B cell forum (DGfI), Bad Sooden-Allendorf, GER

April 4, 2011 Radbruch, A., Modelling and experimentation: approaching the complexity of the immune system, Max Planck Institute für Physik Komplexer Systeme Workshop: “Physics of immunity: complexity approach (PICA)”, Dresden, GER

April 4, 2011 Löhning, M., Plasticity Of T Helper Cell Differentiation In Antiviral Immune Responses, SFB 841 Symposium, Hamburg, GER

April 6, 2011 Baumgrass, R., Activation-induced cell fate decisions in Th cells, Meeting: Physics of Immunity, Dresden, GER

April 7, 2011 Radbruch, A., haben Morbus Crohn, Rheuma, Allergie und Transplantation gemeinsam?, 3. Nationales Innovationsforum Medizin. Fokus: Immunologie, Berlin, GER

April 7, 2011 Chang, H-D., Das immunologische Gedächtnis (als Treiber chronischer Entzündung), 3. Nationales Innovationsforum Medizin. Fokus: Immunologie, Berlin, GER

April 7, 2011 Minden, K., Outcome of juvenile idiopathic arthritis - Data from observational studies, Kinderrheumatologisches Symposium, St. Aug.in, GER

April 7, 2011 Minden, K., Observational studies - Paediatric rheumatology group, Kinderrheumatologisches Symposium, St. Aug.in, GER

April 8, 2011 Hamann, A., ImmunoReset, 3. Nationales Innovationsforum Medizin. Fokus: Immunologie, Berlin, GER

April 12, 2011 Zink, A., Establishing and running a biologics register: Run, run RABBIT!, British Society for Rheumatology Annual Conference, Brighton, GBR

April 12, 2011 Berek, C., Eosinophils are required for the long term survival of plasma cells in the bone marrow, Keystone Meeting B Cells: New Insights into Normal versus Dysregulated Function, Whistler, CAN

April 19, 2011 Romagnani, C., NK2011, Mainz, GER

April 28, 2011 Minden, K., Transition of patients included in biologic registers, Pharmachild Meeting, Utrecht, NLD

May 1, 2011 Mei, HE., Heterogeneity of human bone marrow plasma cells, 8. B-Zell-Forum der DGfI, Dresden, GER

May 2, 2011 Hiepe, F., Die Rolle langlebiger Plasmazellen in der Pathogenese des SLE, 117. Kongress der Deutschen Gesellschaft für Innere Medizin (DGIM), Wiesbaden, GER

May 5, 2011 Minden, K., Wie viele und welche Rheumapatienten werden Sie in der Praxis sehen? Epidemiologie wichtiger kinder- und jugendrheumatologischer

Erkrankungen, Trainingskurs in Kinder- und Jugendrheumatologie für Kinder- und Jugendärzte, Würzburg, GER"

May 5, 2011 Minden, K., Nichtapperative Basisdiagnostik mit Fallbeispielen, Trainingskurs in Kinder- und Jugendrheumatologie für Kinder- und Jugendärzte, Würzburg, GER

May 5, 2011 Minden, K., Differentialdiagnose der JIA anhand von Fallbeispielen (II), Trainingskurs in Kinder- und Jugendrheumatologie für Kinder- und Jugendärzte, Würzburg, GER

May 5, 2011 Minden, K., Therapie der JIA mit Fallbeispielen, Trainingskurs in Kinder- und Jugendrheumatologie für Kinder- und Jugendärzte, Würzburg, GER

May 7, 2011 Zink, A., Safety Aspects of Biologics Data from the German and European Registers, Experiencia Meeting, Berlin, GER

May 15, 2011 Kruglov, A., Critical Role Of Soluble Ltα Produced By Lti Cells In Intestinal Iga Production, 13th International Conference on Tumor Necrosis Factor, Awaji, JPN

May 15, 2011 Nedospasov, S., Distinct functions of TNF and lymphotoxin as defined by mouse studies,

13th International conference on Tumor Necrosis Factor, Awaji, JPN

May 15, 2011 Radbruch, A., The organisation of immunological memory, 6th ENII EFIS/EJI Immunology Summer School 2011, Capo Caccia, ITA

May 15, 2011 Mei, HE., Chronic differentiation of human mucosal IgA-secreting cells during B cell depletion therapy with rituximab, 98th Annual Meeting of the AAI, San Francisco, USA

May 15, 2011 Mei, HE., Chronic Differentiation Of Human Mucosal Iga-Secreting Cells During B Cell Depletion Therapy With Rituximab, 98th Annual Meeting of the AAI, San Francisco, USA

May 15, 2011 Hiepe, F., Memory Plasma Cells As Therapeutic Target in Autoimmune Disease, 11th International Workshop on Autoantibodies and Autoimmunity, Shanghai, CHN

May 20, 2011 Niesner, R., Enhancing the optical performance in dynamic intravital two-photon microscopy, CYTO 2011, Baltimore, USA

May 20, 2011 Chang Hyun-Dong, Maria Lexberg, Andreas Radbruch, Stability And Plasticity Of Th17 Cells Revealed By The Cytokine Secretion Assay, CYTO 2011, Baltimore, USA

May 20, 2011 Niesner, R., "Intravital striped-illumination multi-beam multi-photon microscopy", CYTO 2011, Baltimore, USA

May 21, 2011 Radbruch, A., Cytometric tracking of immunological memory – it´s not in the blood, XXVI Congress of the International Society for The Advancement of Cytometry (ISAC), Baltimore, USA

May 21, 2011 Minden, K., Der Einsatz von Biologika - ständig neue Aspekte, Kinderrheumatologisches Symposium, Berlin, GER

May 21, 2011 Minden, K., Pädiatrische Rheumatologie, PräEULAR - Neue Standards in der Rheumatologie - Kurs auf London, Berlin, GER

May 25, 2011 Baumgrass, R., Cytometry – challenges and opportunities to study transcriptional regulation (and dysregulation), XXVI Congress of the International Society for The Advancement of Cytometry (ISAC), Baltimore, USA

May 25, 2011 Buttgereit, F., Integrated Summary of Safety for Modified-Release Prednisone Compared to Immediate-Release Prednisone: Results from the "Circadian Administration of Prednisone in Rheumatoid Arthritis" (CAPRA) Studies, EULAR, London, GBR

May 25, 2011 Strangfeld, A., Interpreting safety data from registries, EULAR, London, GBR

May 25, 2011 Mei, HE., Incomplete targeting of mucosal B cells by anti-CD20 therapy with rituximab in patients with rheumatoid arthritis, EULAR, London, GBR

May 25, 2011 Hiepe, F., The mechanism of action of Belimumab, a BLYS-specific inhibitor, is consistent with biomarker and vaccine results from the phase 3 BLISS studies, EULAR, London, GBR

May 25, 2011 Hiepe, F., Case 1 presentation: Development of factor VIII inhibitor haemophilia in a SLE patient after immunoablation with ATG followed by autologous stem cell transplantation, EULAR, London, GBR

May 25, 2011 Hiepe, F., Case 1 discussion: Autologous stemm cell transplantation in lupus, EULAR, London, GBR

May 25, 2011 Sieper, J., Strengths and limitations of inflammatory back pain (IBP) in the diagnosis of SpA, EULAR, London, GBR

June 9, 2011 Berek, C., Eosinophils are required for the long term survival of plasma cells in the bone marrow, Basel Institut für Immunology Meeting, Basel, CHE

June 11, 2011 Worm, M., Epidemiology of food allergy, EAACI Congress, Istanbul, TUR

June 12, 2011 Hiepe, F., Long lived plasma cells and autoimmunity, B Cells an Protection: Back to Basics, San Feliu de Guixols, ESP

June 16, 2011 Baumgrass, R., Binary Decision Making in Gene Expression Control of T Lymphocytes, TR 52 Meeting, Berlin, GER

June 16, 2011 Fillatreau, S., Activated B cells: their role in immune regulation, NAT meeting, Nantes, FRA

June 18, 2011 Worm, M., Aktualisierte Leitlinie zur Diagnostik und Therapie der Insektengiftallergie, Insektenworkshop, Motzen, GER

June 20, 2011 Shebzukhov, Yu V., Epigenetic Regulation Of Tnfα Transcription, 14th German Meeting on T-cells: Subsets & Functions, Marburg, GER

June 20, 2011 Neumann, C., T cell meeting Marburg, Marburg, GER

June 21, 2011 Chu, VT., Eosinophils Are Essential For The Long-Term Survival Of Plasma Cells In The Bone Marrow, Eosinophils for ever young International Eosinophil Society, Biennale Symposium, Quebec, CAN

June 21, 2011 Berek, C., Eosinophils support the long term survival of plasma cells in the murine bone marrow, Eosinophils for ever young International Eosinophil Society, Biennale Symposium, Quebec, CAN

June 22, 2011 Radbruch, A., Resting memory: memory plasma cells and memory T helper cells, Joint HKS and IMPAM Meeting, Washington, D.C., USA

June 24, 2011 Hiepe, F., Ursache, Diagnostik und Differenztialdiagnostik rekativer Arthritiden, Vortragsveranstaltung Seramun Diagnostica GmbH, Heidesee, GER

June 24, 2011 Strangfeld, A., What can we learn from registries?, CEE Inflammation Forum, St. Petersburg, RUS

July 1, 2011 Worm, M., Anaphylaxie-Register-Vorteile für den Arzt, Galen SummerSchool, Charité Berlin, Berlin, GER

July 2, 2011 Zink, A., Kardiovaskuläre Morbidität und Mortalität bei Patienten mit rheumatoider Arthritis, Symposium Rheuma und Herz, Göttingen, GER"

July 7, 2011 Zink, A., Versorgung Rheumakranker in Deutschland, 3. Nationales Innovationsforum Medizin. Fokus: Immunologie, Berlin, GER

July 10, 2011 Berek, C., Making and using monoclonal antibodies in research and medicine, People's University of Beijing, Peking, CHN

July 14, 2011 Giesecke, C., Secondary Tetanus Immunization Generates Clonally Related Antigen-Specific Plasma Cells and Memory B cells, AR:O.S.A. Expert Workshop Current Rheumatology, Berlin, GER

July 14, 2011 Mei, HE., Incomplete targeting of mucosal B cells by anti-CD20 therapy with rituximab in patients with rheumatoid arthritis, AR:O.S.A. Expert Workshop Current Rheumatology, Berlin, GER

July 14, 2011 Taddeo, A., Effect of anti-CD20 therapy on plasma cell populations and on the dynamics between plasma cells and B cells in murine SLE, The VI. Expert Workshop AR:O.S.A. VI: Aktuelle Rheumatologie - Outcome, Science, Advances, Berlin, GER

July 14, 2011 Schmidt, S., Enhanced levels of circulating IgA plasmablasts suggests over-activation of mucosal immunity in patients with active SLE., AR:O.S.A. Expert Workshop Current Rheumatology, Berlin, GER

July 14, 2011 Wirries, I, Heterogeneity of plasma cells in human bone marrow, AR:O.S.A. Expert Workshop Current Rheumatology, Berlin, GER

July 15, 2011 Hiepe, F., Labordignostik bei Autoimmunerkrankungen, Rheumatologische Sommerakademie, Potsdam, GER

July 24, 2011 Zink, A., Studientypen: RCT, Register und Kohorten, DGRh Summer School Rheumatologie, Düsseldorf, GER

July 27, 2011 Radbruch, A., Neue Targets in der Therapie, Rheumatologische Summer School, Düsseldorf, GER

Aug. 15, 2011 Minden, K., Kinderrheumatologie - Schwierigkeiten in Diagnose und Therapie, Qualitätszirkel Orthopädie, Berlin, GER

Aug. 23, 2011 Hiepe, F., BLyS als Ziel einer selektiven SLE-Therapie, Launch-Tagung Benlysta, Berlin

Aug. 25, 2011 Mei, HE., Blood-borne human plasma cell in steady state are derived from mucosal immune responses, 14th International Congress of Immunology, ICI, Kobe, JPN

Aug. 27, 2011 Worm, M., Vortrag, Studien, Leitlinien, eigene Erfahrungen? Fragen und Antworten, Allergie Akademie, Berlin, GER

Aug. 31, 2011 Gaber, T., Jakstadt, M., Hahne, M., Fangradt, M., Strehl, C., Hoff, P., Burmester, G.-R., Buttgereit, F., Hypoxia Affects The Impact Of Tocilizumab Treatment On The Cytokine Secretion From Chronically Activated Human Cd4+ T Cells, DGRh, München, GER

Aug. 31, 2011 Minden, K., Gesundheitsbezogene Lebensqualität von erwachsenen Patienten mit juveniler idiopathischer Arthritis (JIA), die im Kindesalter mit Etancercept behandelt wurden, DGRh, München, GER

Aug. 31, 2011 Minden, K., Transition in Deutschland: Status quo aus pädiatrischer und internistischer Sicht, DGRh, München, GER

Aug. 31, 2011 "Niewerth, M., Erfahrungen junger Rheumatiker beim Wechsel in die Erwachsenenmedizin zwei Jahre nach Verlassen der pädiatrischen Versorgung, DGRh, München, GER"

Aug. 31, 2011 Chang, H-D., The pathogenic T cell memory in chronic inflammation, DGRh, München

Aug. 31, 2011 Chang, H-D., IMPAM Netzwerk, DGRh, München, GER

Aug. 31, 2011 Strangfeld, A., Abatacept im deutschen Biologika-Register, Abatacept-Experten-Forum, München, GER

EULAR, Annual European Congress of Rheumatology
DGRh: 39. Annual Meeting of the Deutschen Gesellschaft fur Rheumatologie

Aug. 31, 2011 Strangfeld, A., Unterschiede zwischen Mann und Frau bei rheumatoider Arthritis, DGRh, München, GER

Aug. 31, 2011 Minden, K., RA-Patienten, die eine Remission nach den neuen EULAR/ACR-Kriterien erreichen, haben eine der Normalbevölkerung vergleichbare Funktionsfähigkeit, DGRh, München, GER

Aug. 31, 2011 Westhoff, G., Fatigue - eine unterbewertete Manifestation rheumatischer Krankheiten, DGRh, München, GER

Aug. 31, 2011 Westhoff, G., Sensitivität und Spezifität der neuen ACR-EULAR RA Klassifikationskriterien in der Praxisroutine (CAPEA), DGRh, München

Aug. 31, 2011 Westhoff, G., Verlauf und Prognose der frühen Arthritis - CAPEA, DGRh, München, GER

Aug. 31, 2011 Huscher, D., Vergleich der neuen ACR/EULAR Remissionskriterien mit der DAS28-Remission bei Patienten mit rheumatoider Arthritis – Daten der Kerndokumentation, DGRh, München

Sep. 1, 2011 Mei, HE, Regulation of protective and autoreactive humoral memory, DGRh, München

Sep. 1, 2011 Tokoyoda, K., Bone marrow and Immunological memory, 20th Annual Meeting of Bioimaging Society, Chitose, JPN

Sep. 1, 2011 Daridon, C., The humanized anti-CD22 antibody epratuzumab affects adhesion molecule expression and migration of B-cells in systemic lupus erythematosus, DGRh, München, GER

Sep. 1, 2011 Riemekasten, G., Vorsitz Symposium Systemsklerose, Sjögren, Myositiden. Und "Systemsklerose", DGRh, München, GER

Sep. 1, 2011 Riemekasten, G., Management digitaler Ulzerationen bei Patienten mit SSc, DGRh, München, GER

Sep. 1, 2011 Riemekasten, G., Undeutsch, R., Symposium Start-Up Projekte - Evaluation der Behandlung eines Lupus mit wiederholter Gabe einer Autoantigen-Aminosäuresequenz, DGRh, München, GER

Sep. 1, 2011 Daridon, C., The humanized anti-CD22 antibody epratuzumab affects adhesion molecule expression and migration of B-cells in systemic lupus erythematosus., DGRh, München, GER

Sep. 1, 2011 Mei, HE., Success and limits of B cell depletion therapy., DGRh, München, GER

Sep. 1, 2011 Hiepe, F., Vom Schrotschuss zur zielgerichteten Therapie., DGRh, München, GER

Sep. 1, 2011 Radbruch, A., Regulation of memory Th cell differentiation and survival by microRNA, 2nd Chinese-German Immunology Meeting of the DGfI: Immunotherapy: From Basic to Clinics, Peking, CHN

Sep. 2, 2011 Riemekasten, G., Rheuma und Lunge, DGRh, München, GER

Sep. 2, 2011 Mei, HE., The human bone marrow is a major site of CD19-negative plasma cells characterized by advanced maturity and IgG production., DGRh, München, GER

Sep. 3, 2011 Riemekasten, G., Symposium Gender Aspekte rheumatischer Erkrankungen - Einfluss des Geschlechts auf Epidemiologie und Verlauf der Kollagenosen, DGRh, München, GER

Sep. 4, 2011 Hutloff, A., Costimulatory Molecules in the Regulation of the Immune Response, 2nd Polish Congress of Biochemistry and Cell Biology (Joint Meeting of the Polish Biochemical Society and Polish Cell Biology Society), Krakau, POL

Sep. 5, 2011 Chang, H-D., The adaptive immune system and bone biology, International Society for Fracture Repair, Würzburg, GER

Sep. 7, 2011 Sieper, J., Seronegative spondyloarthropathies (d), Schweizerische Ges. für Rheumatologie, Bern, CHE

Sep. 8, 2011 Worm, M., Sektions- und AG-Sitzungen Dermatologie; Praxisnahes Forbildungssymposium: Anaphylaxie, "Anaphylaxie Register Update 2011, 6. Deutscher Allergiekongress Wiesbaden "100 Jahre spezifische Immuntherapie", Wiesbaden, GER

Sep. 9, 2011 Strangfeld, A., Risk of herpes zoster in patients treated with biologicals, DANBIO 10th anniversary, Kopenhagen, DNK

Sep. 13, 2011 Sieper, J., Psoriasis-Arthritis, Spondyloarthritiden: Update, DGRh, München, GER

Sep. 13, 2011 Song, IH., Patientenmit aktiver ankylosierender Spondylitis zeigen ein klinisches Ansprechen auf einen zweiten Zyklus von Rituximab - Ergebnisse einer offenen Follow-up-Studie, DGRh, München, GER

Sep. 13, 2011 Song, IH., Klinisches Ansprechen und Ansprechen in der Magnetresonanztmographie auf eine Behandlung mit Etanercept vs Sulfasalazin bei früher axialer Spondyloarthritis - 2-Jahresdaten der ESTHER-Studie, DGRh, München, GER

Sep. 13, 2011 Poddubnyy, D ., NSAIDs retard radiographic spinal progression over two years in ankylosing spondylitis but not in non-graphic axial spondyloarthritis, DGRh, München, GER

Sep. 13, 2011 Sieper, J., Der lange Weg zur Diagnose - wie relevant und effektiv sind Screening-Strategien bei axialer SpA?, DGRh, München, GER

Sep. 14, 2011 Zink, A., Safety Data from RABBIT, Registries Convention, Rome, ITA

Sep. 15, 2011 Radbruch, A., The resting and the pathogenic immunological memory, Jahrestagung der Österreichischen Gesellschaft für Immunologie, Graz, AUT

Sep. 17, 2011 Hiepe, F., Laborparmeter zur Prädiktion von systemischer Sklerose und digitaler Ulzerationen, 3. Deutsches SSc-Forum, Berlin, GER

Sep. 18, 2011 Hiepe, F., Vom Schrotschuss zur zielgerichteten Therapie: Benlysta und andere neue Medikamente für Lupus., Fortbildungsveranstaltung der Lupus-Selbsthilfegemeinschaft, Kassel, GER

Sep. 20, 2011 Minden, K., Juvenile Idiopathische Arthritis (JIA) von Fall zu Fall, Pressekonferenz Pfizer, Berlin, GER

Sep. 21, 2011 Hamann, A., Peptides reloaded - new strategies for tolerogenic vaccination, Kitasato Symposium, Potsdam, GER

Sep. 22, 2011 Minden, K., Rheumatologische Kerndokumentation, Arbeitstreffen "Curriculare Modernisierung und bessere Versorgung der Rheumapatienten in der Republik Moldova (CuMoRheM)", Leipzig, GER

Sep. 22, 2011 Fillatreau, S., Activated B cells: Pathogenic and Protective Cytokine Producers, Kitasato Symposium, Potsdam, GER

Sep. 22, 2011 Radbruch, A., Cytokine imprinting – mechanisms for memory, Kitasato Symposium, Potsdam, GER

Sep. 23, 2011 Hiepe, F., Concepts of plasma cell targeting, 10th Dresden Symposium on Autoantibodies, Dresden, GER

Sep. 28, 2011 Worm, M., Retinoide und Hautbarriere, 27.Fortbildungskongress „Fortschritte der Allergologie, Dermatologie, Pneumologie“, Davos, CHE

Sep. 28, 2011 Hiepe, F., Die Bedeutung der B-Zellen in der Pathogenese entzündlich-rheumatischer Erkrankungen., Immunologische Grundlagen der Rheumatologie 2011, Herne, GER

Sep. 28, 2011 Krüger, M., Regulated Heterogeneity In Endogenous C-Fos Protein Levels Ensures Variability And Robustness In Il-2 Decision Making Within The Memory Th Cell Population, DGfI / SIICA Joint Annual Meeting, Riccione, ITA

Sep. 28, 2011 Chu, VT., Plasma cell survival niche: Eosinophil and plasma cell communication, DGfI / SIICA Joint Annual Meeting, Riccione, ITA

Sep. 28, 2011 Smiljanovic, B., The Multifaceted Balance Of Tnf-Alpha And Type I/Ii Ifn Responses In Sle And Ra: How Monocytes Manage The Impact Of Cytokines, DGfI / SIICA Joint Annual Meeting, Riccione, ITA

Sep. 28, 2011 Mir-Farzin, M., Regulation of memory Th cell differentiation and survival by microRNA, DGfI / SIICA Joint Annual Meeting, Riccione, ITA

Sep. 28, 2011 Strangfeld, A., Neue Immuntherapeutika vor und nach der Zulassung: was wir von Rheumatologen lernen können, 84. Kongress der GERtschen Gesellschaft für Neurologie, Wiesbaden

Sep. 29, 2011 Minden, K., Therapie mit Biologika sowie deren Einfluss auf den Krankheitsverlauf der juvenilen idiopathischen Arthritis, 109. Kongress der DOG, Berlin, GER

Sep. 29, 2011 Hauser, AE., Lifetime Of Antibody Secreting Cells In Mucosal Immune Responses, DGfI / SIICA Joint Annual Meeting, Riccione, ITA

Sep. 30, 2011 Hiepe, F., The Role of Plasma Cells in Autoimmunity, 11 th International Symposium on Sjögren´s Syndrome, Athen, GRC

Sep. 30, 2011 Löhning, M., Virus Infection Reprograms Th2 Cells Into A Stable Gata-3+ T-Bet+ “Th2+1” Hybrid Cell Subset, DGfI / SIICA Joint Annual Meeting, Riccione, ITA

Oct. 5, 2011 Scheel, T., Quantitative Single Cell Analysis Of Endogenous Transcription Factor Expression Levels Reveals Nfatc2 And C-Fos As Limiting Factors For Il-2 Production, 7th Workshop Molecular Interactions, Berlin, GER

Oct. 5, 2011 Scheel, T., Quantitative Single Cell Analysis of Endogenous Transcription Factor Expression Levels Reveals NFATc2 and c-fos as Limiting Factors for IL-2 Production, 7th Workshop Molecular Interactions, Berlin, GER

Oct. 7, 2011 Radbruch, A., The organization of the resting immunological memory, Annual Meeting of the Croatian Immunological Society, Rabac, HRV

Oct. 7, 2011 Niewerth, M., Sozioökonomische Relevanz entzündlich-rheumatischer Erkrankungen im Kindesalter, 49. Jahrestagung der Österreichischen Gesellschaft für Kinder- und Jugendheilkunde, Villach, AUT

Oct. 8, 2011 Zink, A., Aktiv im Leben trotz Rheuma, Jahrestagung der Gesellschaft Deutscher Naturforscher und Ärzte, Berlin, GER

Oct. 8, 2011 Minden, K., Transition - Übergang vom Kind zum Erwachsenen, JIA Symposium, Leipzig, GER

Oct. 9, 2011 Gabriel, C., Identification And Functional Characterization Of Interaction (Partners) In Tcr Induced Signalling Pathways, 3rd Autumn School, DGFI, Bad Schandau, GER

Oct. 9, 2011 Minden, K., Identification And Functional Characterization Of Critical Transcription Factors During T Helper Cell Fate Decisions, 3rd Autumn School, DGFI, Bad Schandau, GER

Oct. 9, 2011 Berek, C., Plasma cells, 3rd Autumn School, DGFI, Bad Schandau, GER

Oct. 12, 2011 Scheel, T., Quantitative Single Cell Analysis Of Endogenous Transcription Factor Expression Levels Reveals Nfatc2 And C-Fos As Limiting Factors For Il-2 Production, 21. Jahrestagung der Deutschen Zytometrie Gesellschaft, Bonn, GER

Oct. 12, 2011 Niesner, R., Advances in dynamic intravital two-photon microscopy - focus on optical performance and molecular specificity, 21. Jahrestagung der Deutschen Zytometrie Gesellschaft, Bonn, GER

Oct. 13, 2011 Sieper, J., Mechanisms of bone formation in Ankylosing Spondylitis, Internat.Congress on Bone Involvement in Arthritis, Santa Margherita Ligure, ITA

Oct. 14, 2011 Scheel, T., Quantitative Single Cell Analysis of Endogenous Transcription Factor Expression Levels Reveals NFATc2 and c-fos as Limiting Factors for IL-2 Production, 21. Jahrestagung der Deutschen Zytometrie Gesellschaft, Bonn, GER

Oct. 14, 2011 Zink, A., Safety of TNF Antagonists, International Immunology Masterclass: Mini-Symposium 2: Efficacy and safety of TNF antagonists – Data obtained from registries, Bratislava, SVK

Oct. 19, 2011 Niesner, R., Technological advancement in intravital multi-photon microscopy, Jahrestagung DGfZ, Bonn, GER

Oct. 21, 2011 Hiepe, F., Der SLE - eine Geschichte mit offenem Ende, Benlysta- Launsch-Symposium, München, GER

Oct. 26, 2011 Hiepe, F., Diagnose des Systemischen Lupus Erythematodes (SLE), Systemischer Lupus Erythematodes - ein Update zu Diagnostik und Therapie, EuMeCom-Fortbildung, Berlin, GER

Oct. 26, 2011 Worm, M., Anaphylaxie, Investigator Meeting, Berlin, GER

Oct. 26, 2011 Hiepe, F., Therapie des Systemischen Lupus Erythematodes (SLE), Systemischer Lupus Erythematodes - ein Update zu Diagnostik und Therapie, EuMeCom-Fortbildung, Berlin, GER

Oct. 27, 2011 Worm, M., Systemtherapie des chronischen Handekzems - warum eine frühe Behandlung sinnvoll ist, ABD Tagung, Dresden, GER

Nov. 4, 2011 Chang, H-D., Organisation of immunological memory by bone marrow stroma, 75th Annual Scientific Meeting of the American College of Rheumatology, ACR, Chicago, USA

Nov. 4, 2011 Huscher, D., Performance of the new ACR/EULAR remission criteria compared to DAS28 remission in unselected real-life patients with rheumatoid arthritis, 75th Annual Scientific Meeting of the American College of Rheumatology, ACR, Chicago, USA

Nov. 4, 2011 Strangfeld, A., Impact of Different Biologic Agents on the improvement of fatigue, 75th Annual Scientific Meeting of the American College of Rheumatology, ACR, Chicago, USA

Nov. 5, 2011 Worm, M., Anaphylaxie bei Neurodermitis Patienten, Derma Kompakt, Berlin, GER

Nov. 7, 2011 Shebzukhov, Yu V., Decreased Activity Of Mapk/Ap1 Signaling Pathway Limits The Expression Levels Of Inflammatory Cytokines In Th0 And Th2 Cells, The 15th STS Meeting 2011: Signal Transduction - Receptors, Mediators and Genes, Weimar, GER

Nov. 12, 2011 Berek, C., Eosinophils are required for the long–term survival of plasma cells, Universität Würzburg, Würzburg, GER

Nov. 14, 2011 Grützkau, A., Multiparameter flow cytometry analysis of peirpheral blood mononoclear cells in ankylosing spondylitis, RCIS Berlin Immunology Day, Berlin, GER

Nov. 14, 2011 Löhning, M., Generation, Maintenance And Reprogramming Of Immunological Memory, RCIS Berlin Immunology Day, Berlin, GER

Nov. 14, 2011 Hutloff, A., Regulation of T Follicular Helper Cell Development, RCIS Berlin Immunology Day, Berlin, GER

Nov. 15, 2011 Radbruch, A., Erfahrungen eines erfolgreichen Antragstellers, European Research Council – Advanced Grants: Workshop für Antragsteller/Innen der Leibniz-Gemeinschaft, Berlin, GER

Nov. 16, 2011 Hiepe, F., Plasmazellen als Zielzellen in der Therapie des SLE., Fortbildung EUMECOM, Heidelberg, GER

Nov. 17, 2011 Hiepe, F., Plasma cells and autoimmunity, Seminar MerckSerono, Genf, CHE

Nov. 19, 2011 Niewerth, M., Aktuelle Daten zur Versorgung junger Rheumatiker, Veranstaltung des Rheumazentrums München zum Thema "Transition in der Rheuamtologie, München, GER

Nov. 24, 2011 Hiepe, F., Bedeutung der Interferon-Gensignatur bei Konnektivitiden, 13. Berner Immunologie - Tag Systemischer Lupus Erythematodes, Bern, CHE

Nov. 25, 2011 Strangfeld, A., Tumorrisiko bei Patienten unter konventioneller und Biologika-Therapie, B Cell Forum, Nürnberg, GER

Nov. 26, 2011 Tokoyoda, K., Establishment of T helper memory in bone marrow, 40th Annual meeting of the Japanese Society for Immunology, Chiba, JPN

Nov. 26, 2011 Worm, M., Anaphylaxie - Was können wir aus Registerdaten lernen und wie können wir die Versorgung verbessern, Anaphylaxie-Konferenz, Szeczin, POL

Nov. 26, 2011 Minden, K., Neue Therapien in der Kinderrheumatologie und ihre Konsequenzen, Rheumatologisches Harzsymposium, Werningerode, GER

Nov. 30, 2011 Zink, A., Epidemiologische Studien am DRFZ: Aktuelle Ergebnisse, Jahrestagung des Regionalen Rheumazentrums Berlin, Berlin, GER

Dec. 1, 2011 Berek, C., B lymphocytes and sustained autoantibody production, Expanding Autoimmunity Menarini Diagnostics, Lissabon, PRT

Dec. 2, 2011 Löhning, M., Programmierung Von Gedächtnis-Zellen Des Immunsystems Als Beispiel Für Zelluläre Lernprozesse, BBAW-Minisymposium Biochemie – Molekularbiologie, Berlin, GER

Dec. 3, 2011 Worm, M., Das chronische Handekzem, 21. Jahrestagung der Dermatologen Brandenburgs, Potsdam, GER

Dec. 6, 2011 Strangfeld, A., Transformation von Registerdaten in die Praxis, 18. Charité-Trainingskurs Rheumatologie, Berlin, GER

Dec. 7, 2011 Löhning, M., Virus Infection Reprograms Th2 Cells Into A Stable Gata-3+ T-Bet+ "Th2+1" Hybrid Cell Subset, Annual Congress of the British Society of Immunology, BSI, Liverpool, GBR

Dec. 9, 2011 Hiepe, F., Der komplizierte Fall: Falldiskussionen zu autoimmunen Gerinnungsstörungen und Kollagenosen, 18. Charité-Trainingskurs Rheumatologie, Berlin, GER

Dec. 12, 2011 Worm, M., Training on anaphylaxis, Investigator Meeting, Washington, D.C., USA

Dec. 14, 2011 Romagnani, C., Signatures of NK cell differentiation, CIM Karolinska, Stockholm, SWE

Dec. 16, 2011 Baumgrass, R., Impaired TcR-signaling promotes Treg cell differentiation, SFB 650 Meeting, Berlin, GER

Dec. 16, 2011 Mei, HE., B and plasma cell disturbances in SLE, Biomarkers in Rheumatology: 2nd GISEA International Meeting, Rome, ITA

Dec. 17, 2011 Hiepe, F., Innovative Therapieoptionen bei SLE, Kollagenose-Club, Hamburg, GER

Dec. 17, 2011 Radbruch, A., Good memory – bad memory, Wissenschaftliches Symposium „Immunität und Immundefizienz" anlässlich des 60. Geburtstages von Prof. Dr. Reinhold E. Schmidt, Hannover, GER

Dec. 20, 2011 Löhning, M., Generation, maintenance and reprogramming of immunological memory, Immunology Seminar Series, Erlangen, GER

Invited Talks 2012

Jan., 2012 Nedospasov, S., Functions of TNF and lymphotoxin produced by distinct cellular sources., Imperial College, London, GBR

Jan., 2012 Nedospasov, S., Physiological functions of TNF and lymphotoxin in immunity, University of Birmingham, Birmingham, GBR

Jan. 9, 2012 Fillatreau, S., Intrinsic TLR signaling and regulatory functions of B cells in autoimmune and infectious diseases TT, HENGSTBERGER Symposium 2012, Heidelberg, GER

Jan. 19, 2012 Baumgrass, R., Regulatory T cell induction by manipulation of transcription factors, SFB 650 Retreat, Berlin, GER

Jan. 19, 2012 Rudolph, C., Induction and functionality of hepatic regulatory CD4+ T cells, EASL Monothematic Conference Immune Mediated Liver Injury, Stratford upon Avon, GBR

Jan. 24, 2012 Worm, M., The European Anaphylaxis Register - current status and implications for allergist, Dänische Gesellschaft der Allergologen, Kopenhagen, DNK

Jan. 25, 2012 Hiepe, F., Diagnostik und Therapie bei SLE, Qualitätszirkel "Interdisziplinäre Rheumatologie und Osteologie", Bremen, GER

Jan. 26, 2012 Radbruch, A., Wie uns das Immunsystem schützt, Lecture within the lecture series of The Berlin-Brandenburg Academy of Sciences and Humanities at high schools in Brandenburg, Frankfurt/Oder, GER

Jan. 26, 2012 Siede, J., The role of miRNA in t helper cell plasticity, ZIBI Retreat, Berlin, GER

Jan. 26, 2012 Pellet, E., GATA-3 regulation and stability in murine T helper cells, ZIBI Retreat, Berlin, GER

Jan. 26, 2012 Worm, M., Soforttypallergie: Klinische Darstellung anhand von Fallpräsentationen, 4. Allergie Akademie der DGAKI, München, GER

Jan. 27, 2012 Rudolph, C., Induction and functionality of hepatic regulatory CD4+ T cells, Immuco Graduiertenkolleg SFB633 Jahrestagung, Potsdam, GER

Feb. 1, 2012 Hiepe, F., Goldstandards in der Therapie des SLE, I. Update Systemischer Lupus Erythematosus, Dresden, GER

Feb. 3, 2012 Radbruch, A., Memory plasma cells, Invited lecture at Novartis, Basel, CHE

Feb. 3, 2012 Baumgrass, R., Facts and figures about the e:Bio call, E:bio Workshop, Berlin, GER

Feb. 3, 2012 Hamann, A., Epigenetic imprinting of functional specialization in T cells, INTERNATIONAL CONFERENCE ON T CELL DIFFERENTIATION AND PLASTICITY, Newport Beach, USA

Feb. 11, 2012 Worm, M., Bagatellisierung der Allergie in der Öffentlichkeit, Betreuung der Workshops, Allergie - eine Krankheit mit vielen Gesichtern, Allergologische Veranstaltung - Stallergenes GmbH, Berlin, GER

Feb. 18, 2012 Worm, M., Autoimmunerkrankungen der Kopfhaut, 25. Tagung der Berliner Dermatologischen Gesellschaft – Dermatosen am Kopf, Berlin, GER

Feb. 23, 2012 Liu, F., IL-27: key regulator of immunopathology during influenza, ZIBI Retreat, Berlin, GER

Feb. 23, 2012 Taddeo, A., Targeting autoreactive plasma cells in autoimmunity: a new treatment approch combining plasma cell and B cell depletion, 32nd European Workshop for Rheumatology Research, EWRR, Stockholm, SWE

Feb. 23, 2012 Hiepe, F., Refractory SLE patients respond to the proteasome inhibitor bortezomib, 32nd European Workshop for Rheumatology Research, EWRR, Stockholm, SWE

Feb. 23, 2012 Alexander, T., Helios+ FoxP3+ regulatory T cells are peripherally expanded in active systemic lupus erythematosus, 32nd European Workshop for Rheumatology Research, EWRR, Stockholm, SWE

Feb. 24, 2012 Worm, M., Abklärung von Nahrungsmittelallergien einschließlich Histaminintoleranz, DDG Kompakt 12, Berlin, GER

Feb. 24, 2012 Minden, K., German Experience: JIA Registers, International Master-class "Modern approaches to diagnostics & therapy of Rheumatic diseases in children", Moskau, RUS

Feb. 25, 2012 Minden, K., Long-term efficacy and safety of biologic therapies in JIA patients, XVI Congress of pediatricians, Moskau, RUS

Feb. 26, 2012 Niesner, R., FLIM Invesuigation of NADPH oxidase activation in neuroinflammation, Annual Meeting of the Biophysical Society, San Diego, GER

Mar. 1, 2012 Heine, G., Modulation humaner Lymphozyten durch Vitamin D *in vivo*., 24. Mainzer Allergieworkshop, Mainz, GER

Mar. 1, 2012 Nedospasov, S., Cellular sources of pathogenic and protective TNF in autoimmunity, Kennedy Institute of Rheumatology, London, GBR

Mar. 5, 2012 Vu Van D, Franke RK, Beier KC, Hutloff A., The role of the inducible costimulator ICOS for local T/B cell cooperation in a murine model of allergic airway înflammation., B Cell Forum, Bad Staffelstein, GER

Mar. 8, 2012 Minden, K., Die Kerndokumentation rheumakranker Kinder und Jugendlicher – ein Instrument der Versorgungsforschung und Qualitätssicherung, DGKJ-Kolloquium "Qualitätsmanagement in der Pädiatrie", Berlin, GER

Mar. 8, 2012 Zink, A., Biologics Registries in RA., Third International Immunology Summit, Prag, CZE

Mar. 8, 2012 Zink, A., Efficacy and safety of TNF antagonists ., Third International Immunology Summit, Prag, CZE

Mar. 10, 2012 Minden, K., Systemische Form der JIA - Neues aus der Kinderrheumatologie, 4. Dresdener Frühjahrsgespräche, Dresden, GER

Mar. 10, 2012 Hiepe, F., Lupus – eine Herausforderung mit neuen Perspektiven, 12. Rostocker Forum, Rostock, GER

Mar. 11, 2012 Fillatreau, S., Cytokine-producing B cells as programmers of immunity, 8th Spring School on Immunology, Ettal, GER

Mar. 11, 2012 Chang, H-D.; Radbruch, A., The molecular characterization of Hopx in pathogenic T helper type 1 cells, 8th Spring School on Immunology, Ettal, GER

Mar. 11, 2012 Radbruch, A., The resting immunological memory, 8th Spring School on Immunology, Ettal, GER

Mar. 13, 2012 Klotsche, J., Binary regression: The measures of total gain in positive and negative predictive value in evaluating the usefulness of a risk prediction model. Biometrisches Kolloqium, Berlin, 2012, 58. Biometrisches Kolloquium, Berlin, GER

Mar. 16, 2012 Hiepe, F., Update Rheumatologie: Klassifikations- und Remissionskriterien, 27. Tagung der Landesarbeitsgemeinschaft Rheumatologie und kooperatives Rheumazentrum Rheinland-Pfalz, Bad Kreuznach, GER

Mar. 16, 2012 Hiepe, F., Das Targeting autoreaktiver Plasmazellen – Ein neues therapeutisches Konzept?, 8. Immundiagnostisches Meeting, Essen, GER

Mar. 17, 2012 Hiepe, F., Genderaspekte beim Lupus erythematodes und anderen Vaskulitiden, 2. Berliner Symposium "Neurologie der Geschlechter, Berlin, GER

Mar. 17, 2012 Westhoff, G., Mundgesundheit und Implantat-Versorgungbeim Sjögren-Syndrom Ergebnisse aus der Sjögren-Kohorte DRFZ & Charité, Sjögren-Tag, Berlin, GER

Mar. 19, 2012 Westhoff, G., Verlauf und Prognose der frühen Arthritis Course And Prognosis of Early Arthritis - CAPEA, Sjögren-Tag, Berlin, GER

Mar. 21, 2012 Hahne, M., CD34+ Hematopoietic Stem Cells accumulate in the initial inflammatory human fracture hematoma, IOF-ECCEO 2012, Bordeaux, FRA

Mar. 22, 2012 Dörner, T., Targeting B cells, DGfI / 1stSchool of Translational Immunology, Potsdam, GER

Mar. 22, 2012 Radbruch, A., Durchflusszytometrie – Advanced Technologies, DGfI / 1stSchool of Translational Immunology, Potsdam, GER

Mar. 26, 2012 Hamann, A., Topographical memory and epigenetics of homing receptors, Migration and Regulation: Managing immune-mediated diseases, Berlin, GER

Mar. 28, 2012 Fillatreau, S., B-regulatory cells, Advances in targeted therapies, Baveno, ITA

Mar. 28, 2012 Radbruch, A., MicroRNAs controlling expansion and survival of proinflammatory T cells, Advances in Targeted Therapies, Baveno, ITA

Mar. 31, 2012 Worm, M., Allergie gegen Weizen - Vom Säugling mit Ekzem bis zur FDEIA, Nahrungsmittelallergie-Seminar der WAPPA, Bergisch Gladbach, GER

April 1, 2012 Buttgereit, F., Moderne Therapie mit Glucocorticoiden, Berner Rheumatag, Bern, CHE

April 1, 2012 Alexander, T., Low baseline complement levels, autoantibody persistence and delayed thymic reactivation are risk factors for development of relapses after hematopoietic stem cell transplantation for refractory systemic lupus erythematosus, 38th Annual Meeting of the EBMT, Genf, CHE

April 14, 2012 Radbruch, A., Ausschaltung pathologischer immunologischer Effektorzellen – ein neues Therapieprinzip chronisch inflammatorischer immunvermittelter Erkrankungen, DGIM, Wiesbaden, GER

April 14, 2012 Listing, J., Einsatz von Biologika im Alter, DGIM, Wiesbaden, GER

April 14, 2012 Zink, A., Einsatz von Biologika im Alter, DGIM, Wiesbaden, GER

April 14, 2012 Zink, A., Rolle von Rauchen und anderen Lebensumständen bei rheumatoider Arthritis. Grundlagenforum: Umwelteinflüsse als Risikofaktoren rheumatischer Krankheiten: Was ist gesichert?, DGIM, Wiesbaden, GER

April 17, 2012 Hiepe, F., Immunologische Grundlagen rheumatologischer Erkrankungen: B-Zellen, DGIM, Wiesbaden, GER

April 15, 2012 Radbruch, A., The organisation of immunological memory, 7th ENII Spring School in Advanced Immunology, Alghero, ITA

April 15, 2012 Siede, J., The role of miRNA in t helper cell plasticity, 7th ENII Spring School in Advanced Immunology, Porto Conte, ITA

April 20, 2012 Worm, M., Nahrungsmittelallergie – Quo vadis?, Ideal Spring School 2012, Berlin, GER

April 22, 2012 Romagnani, C., Society for Natural Immunity, Asilomar, USA

April 25, 2012 Worm, M., Das GERtsche Anaphylaxie Register: Konsequenzen für die Praxis, Allergie – Symposium, Köln, GER

April 25, 2012 Radbruch, A., Immunological memory in chronic inflammation, The 56th Annual General Assembly and Scientific Meeting of the Japan College of Rheumatology: the 21st International Rheumatology Symposium, Tokio, JPN

April 26, 2012 Zink, A., Neue epidemiologische Daten zur rheumatologischen Versorgung, 7. Kongress des Berufsverbandes GERtscher Rheumatologen, Berlin, GER

April 27, 2012 Minden, K., Transition in die Erwachsenenmedizin - Was wird aus der TNF-Inhibitor-Therapie?, 15. Wörlitzer Konsensusgespräche, Wörlitz, GER

May 1, 2012 Buttgereit, F., RA chronotherapy with the new low dose modified-release prednisone, Israelischer Rheumatologenkongress, Tel Aviv, ISR

May 3, 2012 Hiepe, F., Klinik, Diagnostik und Therapie der Lupus-Nephritis, Fortbildung der Universitätsklinik für Nieren- und Hochdruckkrankheiten, Diabetologie und Endokrinologie, Magdeburg, GER

May 4, 2012 Radbruch, A., Memory plasma cells, DGfi / Guest symposium of the Deutsche Gesellschaft für Immunologie at the American Association of Immunologists 99th Annual Meeting 2012 (AAI 2012), Boston, USA

May 4, 2012 Hiepe, F., SLE – der komplexe Fall – Analyse und perspektive, B Cell Forum, Bremen, GER

May 4, 2012 Bacher, P., A high-sensitivity detection system for monitoring of rare antigen-specific naive and memory T helper cells, American Association of Immunologists, Boston, USA

May 11, 2012 Worm, M., Weizen – ein wichtiges Grundnahrungsmittel und häufiges Nahrungsmittelallergen, 13. Rheinischer Allergie-Roundtable, Alzey, GER

May 11, 2012 Radbruch, A., Plasmazellen bei SLE, Deutscher Lupus-Tag, Dresden, GER

May 12, 2012 Hiepe, F., Konventionelle Therapie von SLE-Organmanifestationen, 3. Deutscher Lupustag, Dresden, GER

May 18, 2012 Hiepe, F., B cells and plasma cells in (Auto)Immunity, Jahrestagung der Arbeitsgemeinschaft pädiatrische Immunologie, Ittingen, CHE

May 18, 2012 Tokoyoda, K., Resting T helper cell memory in bone marrow, 3rd International Synthetic Immunology Workshop, Kyoto, JPN

May 19, 2012 Liu, F., IL-27: key regulator of immunopathology during influenza, New Perspectives on Immunity to Infection, Heidelberg, GER

May 22, 2012 Worm, M., Anaphylaxie- eine Indikation für die Molekulare Allergiediagnostik?, Immuno-Day 2012, Hannover, GER

May 23, 2012 Mossakowski, A., Fibrillar network development in chronic neuroinflammation, CYTO 2012, Leipzig, GER

May 23, 2012 Niesner, R., NAD(P)H-FLIM investigation of NADPH oxidase activation in EAE, CYTO 2012, Leipzig, GER

May 23, 2012 Chang, H-D., Analysis of cytokine expression by flow cytometry, CYTO 2012, Leipzig, GER

May 23, 2012 Bacher, P., MACS-based antigen-reactive T cell enrichment (MACS-ART), CYTO 2012, Leipzig, GER

May 24, 2012 Hiepe, F., Non-renal flares in lupus nephritis patients – new options, 49th Congress of European Renal Association – European Dialysis and Transplant Association, Paris, FRA

May 30, 2012 Romagnani, C., Requirements for activation of Innate Lymphoid Cells (ILC), Invited Seminar, Jena, GER

June 1, 2011 Esplugues, E., Mount Sinai, School of Medicine, New York, USA

June 1, 2012 Worm, M., Nahrungsmittel -Anaphylaxie und IgE, Norddeutscher Allergie-Roundtable 2012, Rostock, GER

June 2, 2012 Gerhold, K., Transition aus der Sicht der Kinderrheumatologen, Leipziger Frühjahrstagung, Leipzig, GER

June 3, 2012 Winter, O., Maintaining humoral memory in alternative Multi-Component-Plasma-Cell-Niches (MCPN), Joint HKS and IMPAM Meeting, Berlin, GER

June 3, 2012 Alexander, T., Optimizing protocols for ImmunoReset in autoimmune diseases according to, Joint HKS and IMPAM Meeting, Berlin, GER

June 3, 2012 Weber JP, Fuhrmann F, Hutloff A., T follicular helper cells survive as long-term memory cells., Joint HKS and IMPAM Meeting, Berlin, GER

June 3, 2012 Chang, H-D., Adaptation of proinflammatory Th cells to chronic inflammation, Joint HKS and IMPAM Meeting, Berlin, GER

June 3, 2012 Tokoyoda, K., Resting T helper cell memory in bone marrow, Joint HKS and IMPAM Meeting, Berlin, GER

June 3, 2012 Bacher, P., Quantitative and Qualitative Modulation of human T cell memory by cross-reactivity, Hks + IMPAM, Berlin, GER

June 5, 2012 Hiepe, F., Targeting long-lived „memory“ plasma cells in antibody-mediated disease, 20th Annual Meeting of the Henry Kunkel Society, Berlin, GER

June 6, 2012 Daridon, C., Inhibition of B cell receptor signaling with epratuzumab and the effects of a-2,6-sialic acid removal., EULAR, Berlin, GER

June 6, 2012 Buttgereit, F., Impact of morning stiffness on work performance in people with rheumatoid arthritis, EULAR, Berlin, GER

June 6, 2012 Buttgereit, F., The key link between brain and the periphery: Glucocorticoids, EULAR, Berlin, GER

June 6, 2012 Buttgereit, F., Methotrexate versus methotrexate and adalimumab in early RA therapy – data from the HIT HARD study, EULAR, Berlin, GER

June 6, 2012 Fillatreau, S., Regulatory functions of activated B cells in autoimmune and infectious diseases, EULAR, Berlin, GER

June 6, 2012 Khodadadi, L., Studies o depletion of Long-lived plasma cells in autoimmune NZB/W mice, EULAR, Berlin, GER

June 6, 2012 Gerhold, K., Chronic Musculoskeletal Pain – Symptoms and Therapeutic Options, EULAR, Berlin, GER

June 6, 2012 Minden, K., Data collection for adolescents with arthritis: how to make it cool., EULAR, Berlin, GER

June 6, 2012 Minden, K., Transition for teenagers and young adults with arthritis: what does it need? Data collection for adolescents/young adults with arthritis: how to make it cool?, EULAR, Berlin, GER

June 6, 2012 Klotsche, J., Latent class analysis to understand the impact of recent-onset JIA on patient's perceived health-related quality of life., EULAR, Berlin, GER

June 6, 2012 Minden, K., Health-related quality of life of young adults with juvenile idiopathic arthritis treated with etanercept, results of the biologic register Jumbo., EULAR, Berlin, GER

June 6, 2012 Niewerth, M., Outcome of patients with oligoarticular onset of juvenile idiopathic arthritis: data from the German paediatric rheumatologic database - a longitudinal study., EULAR, Berlin, GER

June 6, 2012 Raab, A., Comorbidity profiles among adult patients with JIA who were treated with etanercept - results of the biologic register Jumbo., EULAR, Berlin, GER

June 6, 2012 Radbruch, A., TWISTing pathogenic T cell memory, EULAR, Berlin, GER

June 6, 2012 Sieper, J., NON ANTI-TNF BIOLOGICS IN AXIAL SPONDYLOARTHRITIS, EULAR, Berlin, GER

June 6, 2012 Listing, J., Successful control of disease activity and treatment with biologics increase the life expectancy in RA patients, EULAR, Berlin

June 6, 2012 Pattloch, D., Decreasing incidence of disability pensions in patients with rheumatoid arthritis in Germany, EULAR, Berlin, GER

June 6, 2012 Zink, A., Planning and conduct of observational studies, EULAR, Berlin, GER

June 6, 2012 Riemekasten, G., FUNCTIONAL AUTOANTIBODIES AGAINST VASCULAR RECEPTORS IN SYSTEMIC SCLEROSIS, EULAR, Berlin, GER

June 6, 2012 Hahne, M., Differential regulatory functions of HIF-1α and HIF-2α during angiogenesis of human microvascular endothelial cells (HMECs)., EULAR, Berlin, GER

June 8, 2012 Radbruch, A., Organisation and maintenance of immunological memory, DGVS Spring Conference 2012, Berlin, GER

June 8, 2012 Worm, M., Immunopathology and symptomatology of nutrition-based allergy are distinct from CD, DGVS Spring Conference 2012, Berlin, GER

June 9, 2012 Worm, M., Anaphylaxiemanagement heute und in der Zukunft, Allergie-Akademie, Eine Zeitreise durch die Allergologie, Berlin, GER

June 11, 2012 Chang, H-D., Introduction to flow cytometry, ZIBI Summerschool, Berlin, GER

June 13, 2012 Rudolph, C., Induction and functionality of hepatic regulatory CD4+ T cells, 6th German Meeting on Immune Regulation, Berlin, GER

June 18, 2012 Fröhlich, A., The Alarmin Interleukin 33 drives protective antiviral CD8 T cell responses, T cells subsets and function, Marburg, GER

June 19, 2012 Worm, M., Specific IgE profiles in patients with food-anaphylaxis” und “Network for online-registration of anaphylaxis: towards a European

registry of severe allergic reactions - current status, EAACI Congress, Genf, CHE

June 19, 2012 Worm, M., PDS 11 - Food allergy: anaphylaxis and diagnosis, Your title: Network for online-registration of anaphylaxis: towards a European registry of severe allergic reactions - current status, EAACI Congress, Genf, CHE

June 20, 2012 Khodadadi, L., Depletion of Long-lived plasma cells in autoimmune NZB/W mice, FOCIS, Vancouver, CAN

June 20, 2012 Sercan, Ö; Sgouroudis, E., The function and survival of protective and pathogenic memory CD4+ and CD8+ T lymphocytes, FOCIS, Vancouver, CAN

June 23, 2012 Worm, M., Anaphylaxiemanagement heute und in der Zukunft, Allergie-Akademie "Eine Zeitreise durch die Allergologie", Hamburg, GER

June 28, 2012 Zink, A., Data from biologics registeries, CME Course in Rheumatology, Berlin, GER

June 29, 2012 Worm, M., Workshop: Was können Pricktests leisten und wie werden sie optimal angewendet?, Summer School Charité, Berlin, GER

July 4, 2012 Minden, K., Transition in die Erwachsenenmedizin - was wird aus der Biologika-Therapie?, 7. Experten-Workshop, Berlin, GER

July 5, 2012 Romagnani, C., Requirements for activation of Innate Lymphoid Cells (ILC), Invited Seminar, Wien, AUT

July 9, 2012 Radbruch, A., MicroRNAs controlling expansion and survival of proinflammatory T cells, German-Italian workshop on miRNA and Immunity, Desanzo del Garda, ITA

July 12, 2012 Hiepe, F., Labordiagnostik bei Autoimmunerkrankungen, 10. Rheumatologische Sommerakademie, Maurach, GER

July 13, 2012 Fillatreau, S., Role of cytokine-producing B cells in the regulation of T cell-mediated inflammation, Cellular mechanisms and therapeutic concepts in transplantation and inflammation, Berlin, GER

JJuly 14, 2012 Taddeo, A., Effect of anti-CD20 therapy on plasma cell populations and on the dynamics between plasma cells and B cells in murine SLE, The VI. Expert Workshop AR:O.S.A. VI: Aktuelle Rheumatologie - Outcome, Science, Advances, Berlin, GER

July 16, 2012 Hiepe, F., Long-lived Plasma Cells: A Barrier to Transplantation, 24TH INTERNATIONAL CONGRESS OF THE TRANSPLANTATION SOCIETY, Berlin, GER

July 26, 2012 Worm, M., Schwere anaphylaktische Reaktionen: Unterschiede zwischen Kindern und Erwachsenen bei Auslösern und klinischem Verlauf, 23. Fortbildungswoche für praktische Dermatologie und Venerologie, Berlin, GER

Aug. 1, 2012 Nedospasov, S., Reverse genetics and anti-cytokine therapy., Novosibirsk University, Novosibirsk, RUS +

Aug. 30, 2012 Worm, M., Medizinische Grundlagen zu pseudoallergischen Erkrankungen" und "Medizinische Grundlagen und Krankheitsbilder pseudoallergischer Erkrankungen mit Fallvorstelllungen, Vorträge mit Fallvorstellungen bei Helios Klinikum Emil v. Behring, Berlin, GER

Aug. 31, 2012 Taddeo A, Hoyer BF, Kodadadi L, Chang H-D, Radbruch A, Hiepe F, disease-specific requirements, DGRh, München, GER

Sep. 1, 2012 Buttgereit, F., Riesenzellarteriitis (RZA): Aktuelle Behandlungstrategien, DGRh, Bochum, GER

Sep. 1, 2012 Buttgereit, F., Aktuelle Therapiekonzepte: Vaskulitiden, DGRh, Bochum, GER

Sep. 1, 2012 Nedospasov, S., Functions of TNF and lymphotoxin, as defined in engineered mice, University of Edinburgh, Edingburgh, GBR

Sep. 2, 2012 Radbruch, A., Immunological memory in mouse and man, 4th European Veterinary Immunology Workshop, Edinburgh, GBR

Sep. 5, 2012 Fillatreau, S., Role of activated B cells in the regulation of autoimmunity, ECI, Glasgow, GBR

Sep. 5, 2012 Winter, O., Enhanced megakaryopoiesis as a pro-pathogenic factor in a mice model for Systemic Lupus Erythematosus, ECI, Glasgow, GBR

Sep. 5, 2012 Helmstetter, C., Quantity of IFN-g expression is stable in T helper 1 cells, ECI, Glasgow, GBR

Sep. 5, 2012 Kruglov, A., Soluble LTα3 produced by innate lymphoid cells regulates IgA production in the gut, ECI, Glasgow, GBR

Sep. 5, 2012 McGrath, M., Mobility of resting bone marrow Th cells, ECI, Glasgow, GBR

Sep. 5, 2012 Sercan, Ö., CD8+ memory T cells are resting in murine bone marrow, ECI, Glasgow, GBR

Sep. 5, 2012 Tokoyoda, K., CD69 and CD49b regulate the establishment and maintenance of T helper cell memory, 3rd ECI, Glasgow, GBR

Sep. 5, 2012 Radbruch, A., The resting and the restless immunological memory, ECI, Glasgow, GBR

Sep. 5, 2012 Rudolph, C., Induction and functionality of hepatic regulatory CD4+ T cells, ECI, Glasgow, GBR

Sep. 6, 2012 Hiepe, F., Labordiagnostik bei Autoimmunerkrankungen, 11. Rheumatologische Sommerakademie, Potsdam, GER

Sep. 8, 2012 Radbruch, A., Fortschritte in der Immunologie: vom Ratespiel zum systemischen Verstaendnis, Strategien der lebenswissenschaftlichen Forschung und institutionelle Umsetzung; Vorlesung zur Verabschiedung von Prof. Martin Zeitz, Berlin, GER

Sep. 13, 2012 Minden, K., Impfungen und Infektionsprophylaxe bei Kindern mit rheumatischen Erkrankungen, DGKJ, Hamburg, GER

Sep. 13, 2012 Raab, A., Erste Symptome und Diagnosefindung: Ergebnisse der Inzeptionskohorte für neu diagnostizierte Patienten mit juveniler idiopathischer Arthritis (ICON), Monatszeitschrift für Kinderheilkunde, DGKJ, Hamburg, GER

Sep. 13, 2012 Worm, M., Kontaktekzeme bei Kindern, DGKJ, Hamburg, GER

Sep. 19, 2012 Mei, HE., IgA-secreting plasmablasts with a mucosal phenotype contribute to the expansion of plasmblasts in the blood of patients with SLE., DGRh Kongress 2012, Bochum, GER

Sep. 19, 2012 Hahne, M., Angiogenesis of HMECs is impacted by two HIFα isoforms and their distinct functions, DGRh Kongress 2012, Bochum, GER

Sep. 19, 2012 Hiepe, F., Aktualisierte Therapieempfehlungen zum Einsatz von Mycophenolat mofetil beim SLE, DGRh Kongress 2012, Bochum, GER

Sep. 19, 2012 Hiepe, F., SLE: Neue Therapiemöglichkeiten, DGRh Kongress 2012, Bochum, GER

Sep. 19, 2012 Alexander, T., Stammzelltransplantation bei SLE, DGRh Kongress 2012, Bochum, GER

Sep. 19, 2012 Niewerth, M., Transition in der pädiatrischen Rheumatologie, DGRh, Bochum, GER

Sep. 19, 2012 Gerhold, K., Subjektive Krankheitslast bei Kindern mit chronischen idiopathischen muskuloskeletalen Schmerzen - Multilevel-Analyse einer prospektiven longitudinalen Beobachtungsstudie, DGRh Kongress 2012, Bochum, GER

Sep. 19, 2012 Klotsche, J., Anwendung statistischer Methoden zur Beurteilung der klinischen Bedeutsamkeit eines Vorhersagemodells, DGRh Kongress 2012, Bochum, GER

Sep. 19, 2012 Minden, K., Langzeitsicherheit von Etanercept bei Patienten mit juveniler idiopathischer Arthritis (JIA), DGRh Kongress 2012, Bochum, GER

Sep. 19, 2012 Niewerth, M., Wo und wie werden neu erkrankte Patienten mit juveniler idiopathischer Arthritis (JIA) versorgt?, DGRh, Bochum, GER

Sep. 19, 2012 Raab, A., Krankheitsverlauf bei systemischem Beginn der juvenilen idiopathischen Arthritis (soJIA), DGRh, Bochum, GER

Sep. 19, 2012 Raab, A., Use of Etanercept in the treatment of juvenile dermatomyositis: case reports, DGRh, Bochum, GER

Sep. 19, 2012 Radbruch, A., Pathological memory, DGRh, Bochum, GER

Sep. 19, 2012 Listing, J., Mortalität unter Biologika im Vergleich zu konventioneller Therapie, DGRh, Bochum, GER

Sep. 19, 2012 Zink, A., Krebs und Infektionen – Was ist der aktuelle Stand nach den Daten der europäischen Register?, DGRh, Bochum, GER

Sep. 19, 2012 Huscher, D., FFbH im Wandel der Zeit – Zeit für einen Wandel?, DGRh, Bochum, GER

Sep. 19, 2012 Huscher, D., Entwicklungen in Behandlung und Outcome bei ankylosierender Spondylitis in der Routineversorgung zwischen 2000 und 2010, DGRh, Bochum, GER

Sep. 19, 2012 Riemekasten, G., Treatment pattern of digital ulcers in various European countries – Findings from the DUO registry, DGRh, Bochum, GER

Sep. 19, 2012 Enghard, P., Urinary T cells identify SLE patients with proliferative Lupus nephritis and may be used to monitor treatment response, DGRh, Bochum, GER

Sep. 19, 2012 Scheffold, A., DGRh congress, Bochum, GER

Sep. 20, 2012 Fischer, T., Gerl, V., IDENTIFICATION AND CHARACTERIZATION OF A „MYELOID-LIKE“ SUBPOPULATION OF PLASMACYTOID DENDRITIC CELLS IN SLE, DGRh, Bochum, GER

EULAR, Annual European Congress of Rheumatology
DGRh, Annual German Congress of Deutsche Gesellschaft für Rheumatologie
ECI , European Congress of Immunology

Sep. 21, 2012 Zink, A., Zahnverlust ist bei Früharthritis-Patienten mit anhaltend höherer Krankheitsaktivität assoziiert – daran ändert auch der Verlust des letzten Zahnes nichts Ergebnisse aus der Früharthritis-Kohorte CAPEA, DGRh, Bochum, GER

Sep. 26, 2012 Worm, M., Schwere allergische Reaktionen unter besonderer Berücksichtigung von Arzneimittel-Allergien, 43. Recklinghäuser Forum, Recklinghausen, GER

Sep. 27, 2012 Niewerth, M., Transition – Brücken ins Erwachsenenalter, 50. Jahrestagung der Österreichischen Gesellschaft für Kinder- und Jugendheilkunde, Salzburg, AUT

Sep. 27, 2012 Worm, M., Wird die Notfallbehandlung der Anaphylaxie leitliniengerecht durchgeführt? Daten aus dem Anaphylaxie-Register und Implikationen für die Praxis, 50. Jahrestagung der Österreichischen Gesellschaft für Kinder- und Jugendheilkunde, Salzburg, AUT

Sep. 30, 2012 Baumgrass, R., How T cells help, 4th Autumn School "Current Concepts in Immunology" of the German Society for Immunology (DGfI), Bad Schandau, GER

Sep. 30, 2012 Radbruch, A., Regulation and imprinting of gene expression in the immune system, 4th Autumn School "Current Concepts in Immunology" of the German Society for Immunology (DGfI), Bad Schandau, GER

Sep. 30, 2012 Baumgrass, R., Challenges of systems biology, 8th Workshop Molecular Interactions, Berlin, GER

Oct. 5, 2012 Fillatreau, S., Roles of the antibody-independent functions of activated B cells in the regulation of autoimmunity, International Symposium on Regulators of the Humoral Immune Response, Erlangen, GER

Oct. 10, 2012 Radbruch, A., Cytometric monitoring of immunity and inflammation, Annual meeting of the German Society for Cytometry (DGfZ), Bonn, GER

Oct. 11, 2012 Romagnani, C., Requirements for activation of Innate Lymphoid Cells (ILC), GKsfb620, Freiburg, GER

Oct. 11, 2012 Worm, M., Versorgungslage des Patienten mit Insektengiftanaphylaxie, 7. Deutscher Allergiekongress, München, GER

Oct. 12, 2012 Zink, A., Versorgungsforschung in der Rheumatologie – Was bringt sie den Patienten?, Welt-Rheumatag, Berlin, GER

Oct. 12, 2012 Worm, M., Diagnostik der Nahrungsmittelallergien, 7. Dtsch Allergiekongress, München

Oct. 13, 2012 Hiepe, F., Neue Therapieoptionen bei SLE, Weltrheumatag 2012, Göttingen, GER

Oct. 16, 2012 Radbruch, A., Molecular adaptations of proinflammatory Th memory/effector cells to chronic inflammation, The 34th Naito International Conference: "Infection, Immunity and their Control for Health: Barrier and Vaccine for Infection and Immunity", Sapporo, JPN

Oct. 18, 2012 Worm, M., Schwere allergische Reaktionen durch Nahrungsmittel. Was kann ich - was muss ich tun!?, Vortrag für Bezirksamt Spandau zum Motto Spezialisten informieren, Berlin, GER

Oct. 25, 2012 Fröhlich, A., Cytokine-mediated regulation of antiviral CD8 T-cell responses: lessons from LCMV, House Seminar Bernhard-Nocht-Institut für Tropenmedizin, Hamburg, GER

Oct. 27, 2012 Nedospasov, S., Cellular source and molecular form of TNF defines its pathogenic and protective functions during autoimmune arthritis, Annual Conference of Society of Leukocyte Biology, Maui, USA

Oct. 29, 2012 Worm, M., Anaphylaxis – elicitors, risk factors and management, 5. Internationale Fresenius Konferenz zum Thema Food Allergens, Mainz, GER

Nov. 2, 2012 Zink, A., Safety update from the German and the European registries, Experienca program: A journey into RA management and costimulation experience, Berlin, GER

Nov. 3, 2012 Hiepe, F., Novel treatment strategies for SLE and lupus nephritis, EXPERIENCIA, Berlin, GER

Nov. 9, 2012 Romagnani, C., Human RORgt+ Innate Lymphoid Cells (ILC), Innate Immunity Symposium, Coimbra, PRT

Nov. 9, 2012 Sieger N., Fleischer S., Reiter K., Mei H.E., Shock A, Burmester G.R., Daridon C, Dörner T, Targeting CD22 by epratuzumab potentially raises the threshold of BCR activation., ACR/ARHP, Washington, D.C., USA

Nov. 9, 2012 Buttgereit, F., Are You Using Your Father's Prednisone to Treat Your Mother's RA? New Understanding of a Familiar Therapy, ACR/ARHP, Washington, D.C., USA

Nov. 9, 2012 Hahne, M., The bioenergetic role of HIF-1 and HIF-2 during angiogenesis of human microvascular endothelial cells, ACR/ARHP, Washington, D.C., USA

Nov. 9, 2012 Winter, O., Numbers of Splenic Long-Lived Plasma Cells in Autoimmune and Pre-Autoimmune Lupus Mice Are Linked to a Hyper-Responsive Variant of the Thrombopoietin Receptor and Enhanced Megakaryopoiesis, ACR/ARHP, Washington, D.C., USA

Nov. 9, 2012 Weiss, A., Analysis of clinical, crp- and mri-responses to tnf-blockade in axial spondyloarthritis patients with short vs long symptom duration., ACR/ARHP, Washington, D.C., USA

Nov. 9, 2012 Klotsche, J., Improvement in health-related quality of life for children with juvenile idiopathic arthritis after start of treatment with etanercept, ACR/ARHP, Washington, D.C., USA

Nov. 9, 2012 Klotsche, J., Perceived health-related quality of life and its determining factor in children with recent-onset JIA., ACR/ARHP, Washington, D.C., USA

Nov. 9, 2012 Minden, K., Long-term safety of etanercept in patients with juvenile idiopathic arthritis (JIA)., ACR/ARHP, Washington, D.C., USA

Nov. 9, 2012 Westhoff G, de Pablo P, Dietrich T, Schett G, Zink A., The impact of periodontal disease on early inflammatory arthritis persists even after all teeth are lost., ACR/ARHP, Washington, D.C., USA

Nov. 14, 2012 Pellet, E., GATA-3 regulation and stability in murine T helper cells, InterGeR conference, Athen, GRC

Nov. 16, 2012 Hiepe, F., Zelltherapie bei systemischen Autoimmunerkrankungen, Paul-Martini-Symposium, Berlin, GER

Nov. 16, 2012 Alexander, T., Cell Therapy in Systemic Lupus Erythematosus, EBMT Autoimmune Diseases and Immunobiology Working Parties Joint Educational Meeting, Paris, FRA

Nov. 17, 2012 Worm, M., Notfallbehandlung der Insektengiftanaphylaxie, Symposium zur Insektengiftallergie, München, GER

Nov. 20, 2012 Polansky, J., Epigenetics of inflammatory T cells - features, functions and implications for the clinics, DEEP kick-off Meeting, Überherrn, GER

Nov. 23, 2012 Radbruch, A., Systemic understanding of chronic inflammation, French-German Bilateral Workshop: Perspectives of Systems Biology – from Modelling to Therapy of Complex Diseases, Berlin, GER

Nov. 23, 2012 Hiepe, F., Neue therapeutische Targets bei Kollagenosen und Vaskulitiden, Fortbildungsveranstaltung, Potsdam, GER

Nov. 24, 2012 Minden, K., Die juvenile idiopathische Arthritis (JIA), Seminar Uveitis -Charité Universitätsmedizin Berlin, Berlin, GER

Nov. 25, 2012 Radbruch, A., Protective and pathogenic immunological memory, XXXVIII Annual Meeting of the Portuguese Society for Immunology, Porto, PRT

Nov. 28, 2012 Radbruch, A., Das immunologische Gedächtnis, Jahrestagung der Leibniz Gemeinschaft, Berlin, GER

Nov. 28, 2012 Baumgrass, R., NFATc2-dependent switch decisions, TR 52 Meeting, Mainz, GER

Nov. 30, 2012 Worm, M., Anaphylaxis – situation in german speaking countries, Summit on Anaphylaxis in children and adolescents in the community, Madrid, ESP

Dec. 1, 2012 Buttgereit, F., Modern glucocorticoid therapy, Internationales PMR-Meeting, Chelmsford, GBR

Dec. 1, 2012 Buttgereit, F., New glucocorticoids on the horizon – teaching old drugs new tricks, Tagung der slovakischen Rheumatologen, Piestany, SVK

Dec. 1, 2012 Minden, K., Transition in der Rheumatologie - aktueller Stand, 17. Advents-Symposium, Sendenhorst, GER

Dec. 6, 2012 Worm, M., Chairperson und Speaker für Anaphylaxis Track - Causes of Anaphylaxis, 2012 WAO International Scientific Conference, Hyderabad, IND

Dec. 10, 2012 Bacher, P., Antigen-reactive T cell enrichment for high-resolution analysis of the human naive and memory T helper cell repertoire, DgfZ, Bonn, GER

Dec. 13, 2012 Radbruch, A., Protective and pathogenic immunological memory, Talk with the lecture series of Graduate School (GRK) 1043, Mainz, GER

Dec. 1, 2012 Nedospasov, S., Physiological functions of TNF and lymphotoxin produced by distinct cellular sources, Kantonsspital, St. Gallen, CHE

Dec. 1, 2012 Nedospasov, S., Physiological functions of TNF in experimental arthritis., National Cancer Institute, Bethesda, USA

Qualifications

Bachelor 2011

Theresa Bartossek, 2011, Gewinnung und funktionelle Charakterisierung von polyklonalen Antikörpern gegen wichtige Transkriptionsfaktoren/Signalwegs-moleküle bei der Induktion von regulatorischen T-Zellen, Universität Potsdam

Maria Jäpel, 2011, Transkriptionelle Regulation des Foxp3-Gens durch Bindung von NFATc2, Smad3 und TGIF am Promoter, Hochschule Lausitz, FH

Bachelor 2012

Enrico Fritsche, 2012, Investigating the role of BCL10 and NFAT, two key molecules in T-cell receptor signaling pathways during Th cell activation, Hochschule Lausitz (FH)

Alicia Niedzwiecka, 2012, Etablierung und Optimierung neuartiger Methoden zur Untersuchung von Protein-DNA Interaktionen, TU

Josephine Scholz, 2012, Stimulation mit nukleärem Extrakt zur Detektion autoantigenspezifischer CD4+-Zellen in Patienten mit Systemischen Lupus Erythematodes, FU Berlin

Sabina Schüngel, 2012, „Entwicklung von Algorithmen zur Verbesserung der räumlichen Auflösung in Intravitalmikroskopie“, TFH Wildau

Victoria Zarske, 2012, Die Expression von CD70, OX40L und Siglec-1 in dendritischen Zellen und deren Rolle im SLE, Hochschule Lausitz FH

Master 2011

Basak Burcu Cicek, 2011, Transcriptional Regulation of the IFNγ Gene in Human Natural Killer cells, Charité

David Cartoixa Cartoixa, 2011, Genome wide analysis of Th17 signature genes, University of Barcelona, ESP

Jenny Gerhard, 2011, Aktivierung der NADPH oxidase in Mikroglia bei NAD(P)H-basiertem FLIM, FU

Claudia Giesecke, 2011, Characterization of tetanus-specific human B effector cells in healthy donors and splenectomized individuals, Charité

Master 2012

Katharina Hecklau, 2012, Untersuchung zu stimulationsabhängigen Histonmodifikationen bei der Differenzierung von murinen naive Th Zellen, HU

Martin Karl, 2012, Die Rolle ausgewählter Transkriptionsfaktoren in der Induktion regulatorischer T-Zellen, Universität Potsdam

Elisabeth Müller (Kristoffersen) 2012, Characterization of IL-10-producing B cells, HU

Katarzyna Luda, 2012, Characterization of circulating plasma cells under therapy in RA, HU

Joan Röhl, 2012, Untersuchung zur Bindung wichtiger Transkriptionsfaktoren in Genbereichen von FoxP3, Universität Potsdam

Özlem Vural, 2012, Quantification and Phenotyping of Foxp3+CD4SP Treg from New Zealand Black and New Zealand White Mice by Flow Cytometry, TU

Diploma theses 2011

Aleksandre Beller, 2011, Vergleich der Genexpression in verschiedenen Plasmazellpopulationen, FU

Lina Burbat, 2011, Untersuchung der transkriptionellen Regulation von Zytokinen in aktivierten murinen T – Helferzellen, Universität Potsdam

Katharina Jörß, 2011, Einfluss eines Vitamin-D-Rezeptor-Agonisten auf die atopische Dermatitis im Mausmodell - Bedeutung regulatorischer T-Zellen, Charité

Stefanie Schmidt, 2011, Charakterisierung von Immunabnormalitäten bei Patienten mit Rheumatoider Arthritis – Beiträge zur Etablierung von Biomarkern für das individuelle Ansprechverhalten auf Rituximab-Therapie, TU

Frank Thormann, 2011, Einfluss diätetischer mehrfach ungesättigter Fettsäuren und Präbiotika auf die allergische Immunantwort in der Haut, Charité

Ralph Willebrand, 2011, Die Rolle von Camta_1 für die Funktion humaner regulatorischer T-Zellen, FU

Diploma theses 2012

Diploma 2012 Maysun Al Baz, 2012, Die Rolle von B-Zellen als antigenpräsentierende Zellen im entzündeten Gewebe, FU

Diploma 2012 Taisiya Bezhaeva, 2012, Role of T cell lineage-specific transcription factors in regulation of expression of genes from the TNF/Lymphotoxin locus, Charité

Diploma 2012 Stefanie Schmidt, 2012, Circulating plasma cell subsets in SLE, TU

MD theses2011

Kristina Beyer, 2011, Anaphylaxie im Notarzteinsatz – Ergebnisse aus zwei Jahren Datenerhebung in Berlin, Charité

Sabine Dölle, 2011, Neue Therapieansätze bei der Nahrungsmittelallergie und der atopischen Dermatitis, Charité

Tim Hollstein, 2011, Retinoic acid and B cells, Charité

Anastasia Mikuscheva, 2011, Role of Interleukin 6 in Breast Cancer Cell Growth in Bone, Charité

Nicole Wendt, 2011, Plazeboeffekt bei Atopie, Charité

MD theses2012

Fidan Barkhudarova, 2012, Diagnostic value and clinical laboratory associations of antibodies against recombinant ribosomal P0, P1, P2 proteins and their native heterocomplex in a Caucasian cohort with systemic lupus erythematosus, Charité

Claudia Rieder, 2012, CD4-T-Zell-Subsets im Urin als Biomarker der Lupus-Nephritis, Charité

Nadine Sieger, 2012, CD22 Ligation führt zu intrazellulärer Signalinhibition humaner B-Zellen, Charité

PHD theses 2011

Claudia Brandt, 2011, Molekulare Mechanismen der T Zellmodulation bei der Cyclosporin A-Therapie von PatientInnen mit atopischer Dermatitis, FU

Monique Fangradt, 2011, Adaptation von primären humanen Monozyten/Makrophagen an Hypoxie, TU-Berlin/Charité

Stefan Frischbutter, 2011, Die Dephosphorylierung des Adapterproteins Bcl-10 durch die Ser/Thr-Phosphatase Calcineurin ist essentiell für die Aktivierung des Transkriptionsfaktors NF-κB in Th-Lymphozyten, FU

Lotta Gäwert, 2011, Registerdaten zur Risikobewertung neuer Therapien am Beispiel der Biologikatherapie der rheumatoiden Arthritis. Die Bedeutung der Patientenangaben in der Erfassung unerwünschter Ereignisse, Charité

Kerstin Geldmeyer-Hilt, 2011, Vitamin D inhibits NF-kB activation in B cells and controls the humoral immune response, TU

Björn Hartmann, 2011, Vitamin D receptor activation modulates the allergic immune response, TU

Juliana Köck, 2011, Regulation und Kontrolle des Zytokins Interleukin-4 in T Helferlymphozyten, HU

Vicky Lampropoulou, 2011, TLR/MyD88 signaling in B cells suppresses T cell-mediated CNS autoimmunity, TU

Anna-Barbara Stittrich, 2011, Untersuchung der Funktion von microRNAs in T Helfer Lymphozyten, HU

Cindy Strehl, 2011, Analyse von Herkunft und funktioneller Aktivität humaner membranständiger Glucocorticoidrezeptoren, TU/Charité

Balint Szilagyi, 2011, Regulation of the Gut-Homing Receptor alpha4beta7 and the Chemokine Receptor CCR9, HU

PHD theses 2012

Randi K. Franke, 2012, Die Relevanz des induzierbaren Kostimulators ICOS für die Differenzierung und Aufrechterhaltung der follikulären T-Helferzellen *in vivo*, FU

Melanie Krüger, 2012, Die Rolle von TGIF1 in der peripheren T-Zellaktivierung und Differenzierung, FU

Anna Kuchmiy, 2012, Characterization of novel repoter mice to evaluate TNF expression *in vivo* and in vitro, Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, RUS

Francesca Liu, 2012, Role of regulatory T cells in influenza infection/ Role of IL27 in influenza infection, Universität Potsdam

Micha Schröter, 2012, Molecular regulation of homing receptors involved in T cell migration to inflammation and infection, HU

Dana Vu Van, 2012, Die Rolle des induzierbaren Kostimulators (ICOS) in der Kooperation von T- und B-Zellen im entzündeten Gewebe, FU

Legend:
HU - Humboldt Universität zu Berlin
TU - Technische Universität Berlin
Charité - Charité – Universitätsmedizin Berlin

Prizes and Awards

date	prize winner	award	awarded by
05.06.2011	Mir-Farzin Mashreghi, Anna-B. Stittrich	Wolfgang-Schulze-Forschungspreis	Deutsche Rheuma-Liga e.V.
17.06.2011	Andreas Radbruch	Carol-Nachman-Price	Stadt Wiesbaden
01.09.2011	Jan Broder Engler	Robert Koch Best Thesis award	Charité - Universitätsmedizin Berlin
03.09.2011	Hyun-Dong Chang	Rheumatology Foundation "inspiration award"	Rheuma-Stiftung, München, DGRh-Kongress
28.09.2011	Margitta Worm	Kanert Prize	Stiftung Kanert für Allergieforschung
29.09.2011	Henrik Mei	Hans Hench Prize	DGFI
02.11.2011	Edda Schulz	young scientists award 2011	Forschungsverbund Berlin
06.12.2011	Anna-Barbara Stittrich	Avrion Mitchison Prize for Rheumatologoy	Schering Stiftung
08.02.12	Koji Tokoyoda	Chiba University Advanced Science Award	Chiba University
18.04.12	Henrik Mei	Wolfgang-Schulze-Prize	Stiftung Wolfgang Schulze
20.09.12	Cindy Strehl	Chugai Science Award Rheumatology	

Photo: H.Kubenka, Wiesbaden

Carol Nachman Prize

The 2011 Carol Nachman Prize for Rheumatology, of 37.500 Euros, was jointly awarded to Andreas Radbruch and Désirée MFM van der Heijde, Leiden University, the Netherlands. The Nachman Prize, awarded by the city of Wiesbaden, is one of the most prestigious medical awards in Germany.

Wolfgang Schulze Prize

In 2012, Henrik Mei of the DRFZ was awarded the "Wolfgang Schulze" research award for his work on the effect of the drug Rituximab in patients with inflammatory rheumatic diseases. Left to right: H. Sörensen, E. Gromnica-Ihle, H. Mei, T. Dörner

Events

Regional, national and international meetings, workshops, symposia and other events organised or co-organised by DRFZ members

2011 (selected)

March 13 -18, 2011
7. Spring School on Immunology organized by the German Society for Immunology, DGfI, Andreas Radbruch, Tanja Duréz, Jacqueline Hirscher, Ettal

April 07, 2011
3. National Forum for Innovation Medicine: Focus Immunology 2011, Andreas Radbruch, Martin Zeitz, Steigenberger Hotel, Berlin

May 21, 2011
XXVI Congress of the International Society for the Advancement of Cytometry (ISAC), Baltimore, USA, Congress president: Andreas Radbruch

May, 28, 2011, Long night of science "How it works: research - understanding - healing. Jacqueline Hirscher, Andreas Radbruch, co-organised with Clinics of Rheumatology of Berlin: Charité, Immanuel Diakonie, Schlosspark-Klinik and the Patien-Organization Rheuma-Liga.

September 27, 2011
Joint Annual Meeting DGfI / SIICA, Riccione, ITA, Joint annual meeting of the german and the italian societies for immunology. Andreas Radbruch

October 03, 2011
German-Russian meeting on Tumor Biology (The 3rd in the series of bilateral German-Russian meetings in biomedical sciences), Sergei Nedospasow, Schloss Heigerloh (near Tubingen),

October 09, 2011
3rd Autum School organized by the German Society for Immunology, DGfI, Ria Baumgrass, Bad Schandau

October 12, 2011
World Arthritis Day. Together with the Rheuma-Liga Berlin the DRFZ welcomed patients and interested public. Jacqueline Hirscher, Andreas Radbruch, Rheuma-Liga

10.-11.11.2011
Site-Visit of the evaluation board appointed by the Leibniz Association, Posterssion at the DRFZ

12.10.11
Annaul meeting of the German Society for Cytometry, Zytometrie, Bonn, Hyun-Dong Chang

14.11.2011
Berlin Day of ImmunoSciences (RCIS) und RCIS Symposium, Alf Hamann, Charité, Berlin

29.11.2011,DRFZ
Birthday-Symposium and farewell party for Claudia Berek

06.12.2011
Hasinger Lecture, given by Peter Lipsky, USA
Mitchison Prize Winner: Anna-Barbara Stittrich (DRFZ)

2012 (selected)

February 23, 2012, 32nd European Workshop for Rheumatology Research, EULAR, Annual meeting, Falk Hiepe, Stockholm, SWE

March 11-16, 8th Spring School on Immunology of the German Society for Immunology, Co-Organiser Andreas Radbruch, Tanja Duréz, Jacqueline Hirscher, Ettal

April 1, 2012
38th Annual Meeting of the European Group for Blood and Bone Marrow Transplantation, EBMT, Annual meeting, Falk Hiepe, Genf, CHE

June 2, 2012
Long Night of Science, Andreas Radbruch, Jacqueline Hirscher, DRFZ, Berlin, GER

2012 (selected)

June 3-6, 2012
Joint Henry Kunkel Society and IMPAM meeting "Immunological Memory in Health and Disease", Andreas Radbruch, Eva Kreiss, Berlin

July 4, 2012, International Immunological Memory and Vaccine Forum (IIMVF), Chiba University, Andreas Radbruch, Chiba, JPN

Juli 6/7, 2012, EULAR Epidemiology Course, Angela Zink, DRFZ, Berlin

July 13-14, 2012, Workshop: Cellular mechanisms and therapeutic concepts in transplantation and inflammation, Michael P. Manns, E. Jaeckel, M. Zeitz, Alf Hamann, H-D.Volk, R. Förster, H. Haller, of SFB 621, 633, 650, 738, Berlin, GER

July 15-August 10, 2012, Consultation of universities in Ethiopia, Aderajew Waka (AG Hiepe), Gonder University and others, ETH

September 22-25, 2012, EMBO-Tagung, Angela Zink, Nizza, FRA

September 30 - October 5, 2012, 4th Autum School organized by the German Society for Immunology (DGfI), Ria Baumgrass, Bad Schandau, GER

September 30 - October 5, 2012, 8th Workshop Molecular Interactions, Ria Baumgrass (Workshop Leader), DGfI, Berlin, GER

October 12, 2012, Welt-Rheuma-Tag - "Aktiv gegen den Rheumaschmerz", co-organised by Gerd-Rüdiger Burmester, (Charité), Jacqueline Hirscher (DRFZ), Malte Andersch and Sandra Bluhm (Deutsche Rheuma-Liga Berlin), 90 patients as guests in the DRFZ, Berlin, GER

November 1, 2012, Vernissage "the art of memory". The first exhibition of members of the MPI and DRFZ. Part of the birthday celebration of Andreas Radbruch. Photographic artworks illustrated "memory" in a translational sense. DRFZ, Berlin, GER

November 5, 2012, International Symposium in honor of the birthday of Andreas Radbruch "From cell sorting to immunological memory", Französischer Dom and DRFZ, Berlin, GER

November 16, 2012, EBMT Autoimmune Diseases and Immunobiology Working Parties Joint Educational Meeting, EBMT, Educational Meeting, Paris, FRA

December 4, 2012, Hasinger Lecture and Mitchison Prize Ceremony. Winner: Stefan Uderhardt. Andreas Radbruch, Christine Raulfs, Jacqueline Hirscher, DRFZ, Berlin, GER

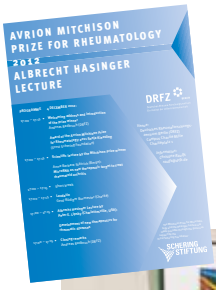
December 4-7, 2012, 19. "Charité - Trainingskurs Rheumatologie und Klinische Immunologie", Gabriela Riemekasten (sci. org.), training course for Rheumatologists, Berlin, GER

Immunological Memory in Health and Disease- Joint Henry Kunkel Society and IMPAM meeting

The annual Henry Kunkel Lecture was given by Louis Staudt , NIH, USA Photo: A.Sattler

The 200 international participants enjoyed the excellent talks and lively discussions during the 3-day meeting... Photo: A.Sattler

...as well as the wonderful boat trip through Berlin's City Centre Photo: A.Sattler



Hasinger Lecture and ...

In the 2012 Hasinger Lecture, Professor Mark Shlomchik (University of Yale, USA) gave insight into the role of B cells and myeloid cells in systemic lupus erythematosus.
from left: G.-R.Burmester (Charité), S. Kießling (Scientific Board of the Schering Foundation), M. Shlomchik, A. Radbruch (DRFZ)

... Mitchison Prize Ceremony

Dr. Stefan Uderhardt (University of Erlangen-Nuremberg) received the 2012 Avrion Mitchison Prize for Rheumatology for his work on the clearance of apoptotic cells under inflammatory conditions.
from left: G.-R.Burmester (Charité), S.Uderhardt, S. Kießling (Scientific Board of the Schering Foundation), A. Radbruch (DRFZ)

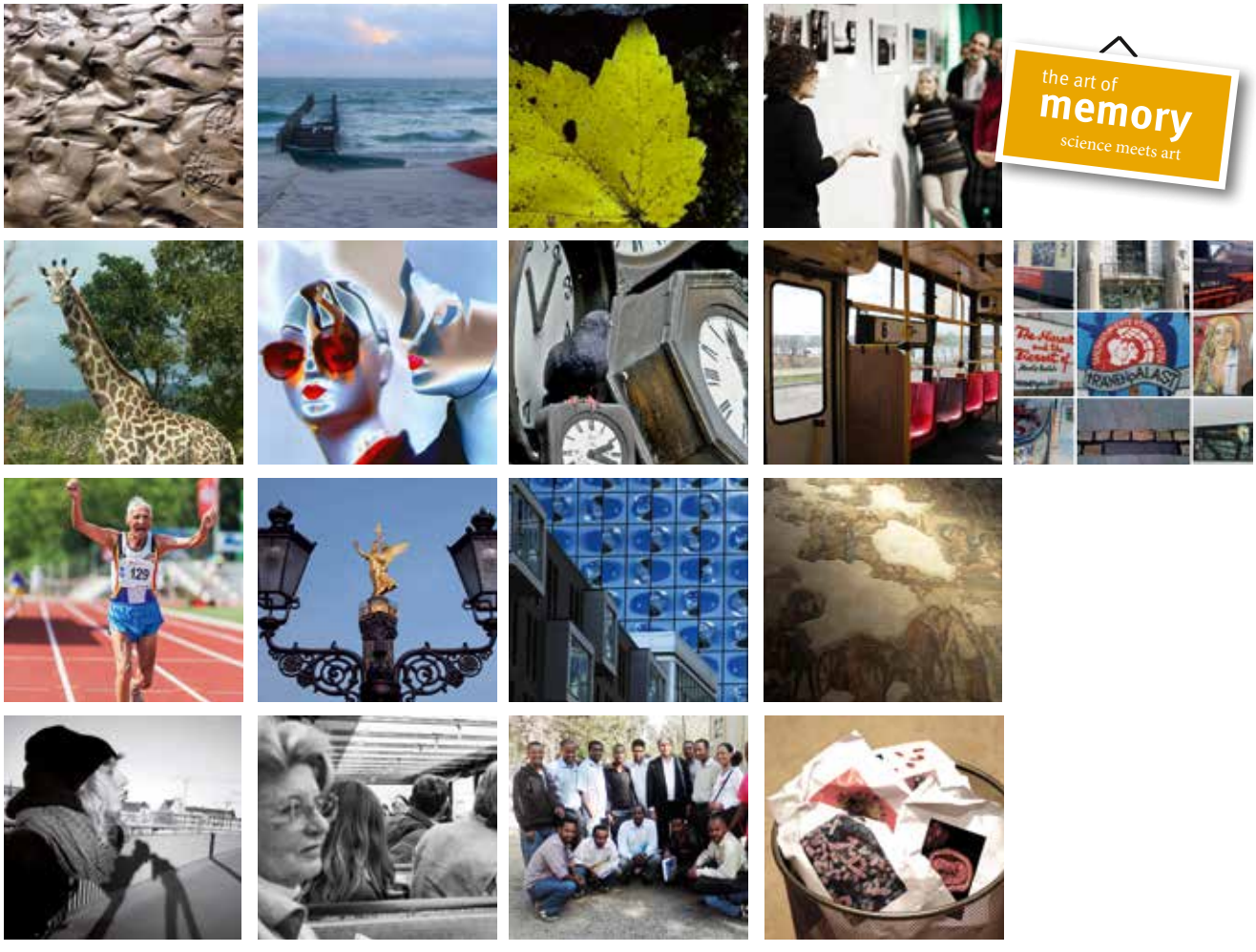
Impressions: Symposium *From Cell Sorting to Immunological Memory*

Left side: Französischer Dom at the Gendarmenmarkt, Berlin Mitte.
Below: Andreas Radbruch enjoyed the ceremony

from left:
Elimar Brandt, Avrion Mitchison and his wife Lorna and Hans Kröger - the first pioneers of the DRFZ

Science meets art: Vernissage "the art of memory".

Photographers from left: Marina Babic Cac, Claudia Berek, Zoltan Cseresnyes, Caterina Curato, Sven Dombrowski (MPI-IB), Joachim Grün, Andreas Grützkau, Martin Hahne, Thomas Häupl, Jacqueline Hirscher, Ute Hoffmann, Burkhard Ilchen (MPI-IB), Monika Killig, Christian Neumann, Dirk Schlienz, Aderajew Waka, Oliver Winter



Cover: Scientists at work

from left to right: Claudia Haftmann, Qingyu Cheng, Anja Weiß, Mir-Farzin Mashreghi, Ute Hoffmann, Toralf Kaiser, Andrey Kruglov , Bimba Hoyer and Atiene Hackebrocht, Laura Oehme

Photos: Jacqueline Hirscher

Imprint

Deutsches Rheuma-Forschungszentrum Berlin (DRFZ)
A Leibniz Institute
Charitéplatz 1, 10117 Berlin, Germany

Andreas Radbruch, Scientific Director
Petra Starke, Administrative Director

Phone	+49 (0)30 28460 280
Fax	+49 (0)30 28460 603
Contact	info@drfz.de

Coordination	Jacqueline Hirscher
Graphics	Diane Schad, Jacqueline Hirscher, Steffen Meixner
Translation	Christine Raulfs
Photographs	Jacqueline Hirscher, if not noted otherwise
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Sitemap

Legend: Campus Universitätsmedizin – Charité Berlin.
The building on the left side: Max Planck Institute for Infection Biology and Deutsches Rheuma-Forschungszentrum. On the right side: North wing of the Charité Department "Innere Medizin" with the DRFZ Epidemiology Group on the ground floor.