6th German Mass Cytometry User Forum

19. - 20. January 2023, Berlin

Abstract Booklet

masscytometry2023.de

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Dear friends of cytometry,

Dear friends of mass cytometry,

after two years of virtual meetings we are excited to welcome you to the 6th German Mass Cytometry User Forum onsite and virtual.

Our program features invited speakers, a tutorial, selected abstracts and highlights projects from German Mass Cytometry and Imaging Mass Cytometry sites. We are glad to bring back a poster session along with a dinner, providing rich opportunity for networking.

I am looking forward to an inspiring and fruitful 6th German Mass Cytometry User Forum!

Best wishes,

Henrik Mei

Thursday, January 19, 2023

9:00- 9:15	**Welcome: Henrik Mei**
9:15-11:15	**Getting started - Introduction to Mass Cytometry** Chairs: Désirée Kunkel, Sarah Warth
11.15-11.30	**break**
11.30-12.15	**Ermelinda Porpiglia, Aarhus, Denmark** Chair: Henrik Mei
12:15-12:35	**Product feature - Standard BioTools by Melissa Klug**
12:35-14:00	**Lunch & networking**
14:00-15:30	**Data Analysis:** Chairs: Axel Schulz, Marie Burns Christian Busse, Heidelberg, Germany Rosario Astaburuaga-Garcia, Berlin, Germany News from Heidelberg, Felix Hartmann Short Talk by Jan Stuchly
15:30-16:00	**break**
16:00-17:30	**News From (1)…** Chairs: Selina Keppler, Claudia Peitzsch, Marc Rosenbaum ... Jena by Sabine Baumgart ... Berlin-DRFZ by Henrik Mei ... Munich by Selina Keppler ... Dresden by Sebastian Thieme ... Freiburg by Felix Roettele
17:30 - 21:00	**Poster sessions by participants** Chairs: Desiree Kunkel and Sarah Warth with wine and cheese

Friday, January 20, 2023

9:00-10:30	**2022: Imaging** Chairs: Bertram Bengsch, Déirée Kunkel Bernd Bodenmiller, Zurich, Switzerland Yvonne Scuiller, Brest, France Short Talk by Fabienne Birrer Short Talk by Juliette Krop
10:30-11:00	**break**
11:00-12:30	**News from (2)…** Chairs: Selina Keppler, Claudia Peitzsch, Marc Rosenbaum ... Berlin BIH at Charité by Adrian Huck ... Berlin-MPI for Molecular Genetics by Anika Rettig ... Erlangen Aleix Rius Rigau ... Ulm by Aoife Gahlawat Short Talk by Axel Schulz
12:30-13:40	**Lunch & networking**
13:40-14:00	**Product feature - Cytobank/Beckman Coulter** Chair: Henrik Mei Talk by Giulia Grazia
14:00-14:30	**Standard BioTools** Talk by Julia Eckl-Dorna
14.30	**Farewell and Poster Award** Henrik Mei

Thursday, January 19th

Getting started - Introduction to Mass Cytometry





Désirée Kunkel, Anika Rettig, Axel R Schulz, Henrike Salié, Sarah Warth

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Anika Rettig, Max-Planck-Institut für Molekulare Genetik, Berlin, Germany

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Henrike Salié, Clinic for Internal Medicine II , University Medical Center Freiburg, Freiburg, Germany

Sarah Warth, Core Facility Cytometry, Ulm University, Ulm, Germany

Our introduction to mass cytometry ensures that everyone is at the same level when talking about this technology. Four experts from the field tell you how mass cytometry works and how it can be used to examine cell suspensions and tissue sections. We will guide you through typical experimental workflows and share our experience with important aspects in the application of mass cytometry, such as metal conjugation, sample barcoding, spillover compensation and batch normalization. You will also learn about the advantages of Imaging Mass Cytometry (IMC) and how to establish a multiplexed antibody panel for it. This is complemented by an introduction to current concepts of data analysis, both for imaging and suspension mass cytometry. Following the introductory talks there will also be time to discuss individual questions concerning mass cytometry and its application.

Through the lens of CyTOF: resolving signatures of muscle stem cell aging one cell at the time

Ermelinda Porpiglia

Ermelinda Porpiglia[1,2], Thach Mai[2], Peggy Kraft[2], Colin A. Holbrook[2], Antoine de Morree[1,3], Veronica D. Gonzalez[4,5], Keren Hilgendorf[2], Laure Fresard[4], Angelica Trejo[4], Sriram Bhimaraju[2], Peter K. Jackson[2], Wendy J. Fantl[5] and Helen M. Blau[2]

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Skeletal muscle mass, strength and regenerative capacity progressively decline with aging. This is partly due to functional impairment of muscle stem cells (MuSCs), the key players in muscle regeneration. However, the mechanisms responsible for age- associated MuSC dysfunction remain elusive. A major barrier to gaining mechanistic insights into MuSC aging is the increased functional heterogeneity of the aged MuSC population, underscoring the need for single-cell studies. Here we capitalized on single- cell mass cytometry to resolve MuSC heterogeneity during aging and identified a dysfunctional MuSC subset, marked by high CD47 surface expression (CD47hi). Mechanistically, increased expression of U1 snRNA in aged MuSCs shifted the balance of CD47 mRNA isoforms, leading to increased levels of CD47 protein on the cell surface. Aged CD47hi MuSCs act via paracrine signaling, through secretion of thrombospondin-1, a ligand for CD47, to suppress the regenerative capacity of CD47lo MuSCs. Strikingly, in vivo thrombospondin-1 blockade restored the proliferative potential of aged MuSCs and enhanced muscle regeneration and strength in aged mice. These findings uncover an unexpected role for thrombospondin-1/CD47 signaling in aged MuSCs and suggest a novel therapeutic approach to improve muscle regenerative function in the elderly.

Product feature - Standard BioTools: Ready-to-use 45+ flow cytometry marker panels to measure activation, cytokine production and cytotoxicity from <300ul of whole blood

Melissa Klug

Standard BioTools, Munich, Germany

Designing flow cytometry panels to study cellular phenotypes and their function has always been hindered by the difficulty in designing high-dimensional flow cytometry panels and the amount of sample required to optimize and validate these panels. In this talk we will present our new range of ready-to-use 45+ marker panels for in-depth profiling of T, B, NK and myeloid cells starting from only 300 µl of whole blood or 3 x 10^6 of PBMC.

Our new flow cytometry panels are the only ones on the market that are ready-to-use and come with automated data analysis software, providing an end-to-end solution with proven run-to-run and site-to-site reproducibility. You can now profile cytokines, cytotoxic mediators, and other key functional epitopes all in one single tube, without wasting any time or money in validating and running your experiments.

Data Analysis - Cytometric Data and the FAIR Principles

Christian Busse

German Cancer Research Center (DKFZ), Heidelberg, Germany

Since they were first published in 2016, the FAIR Principles, i.e. the recommendations that scientific data by default should be Findable, Accessible, Interoperable and Reuseable, have become one of the key paradigms for research data management (RDM), the Open Data movement and various reproducibility initiatives. In this talk, I will give an introduction to what the FAIR Principles actually specify -- and what not -- and the benefits, opportunities and challenges that a FAIR data has from a practical perspective. We will then have a closer look at the current state-of-the-art of FAIRness of cytometric data and will close with an outlook to upcoming developments, both on the side of infrastructure as well as in the legal space.

Data Analysis - Investigation of divergent cell fate decisions after radiation-induced DNA-damage

Rosario Astaburuaga-García

Charité – Universitätsmedizin Berlin

Radiation therapy is the main treatment strategy for head-and-neck squamous cell carcinoma (HNSCC), and radioresistance of these tumours remains a major problem as it is not well understood. To get a better understanding of how therapy resistance emerges through differential signalling activity, we performed time-course mass cytometry (CyTOF) analyses of irradiated (6 Gy) and non-irradiated HNSCC cells. As a model system of intra-tumoral heterogeneity, we made use of the heterogeneous Cal33 cell line (parental), a radiosensitive (RS) and a radioresistant (RR) subclone and examined potential differences in signalling dynamics that could explain these divergent responses to irradiation. Cell cycle classification indicated a delay in cell cycle progression after irradiation. However, the cell cycle dynamics were largely comparable for the three cell lines studied, suggesting that their differential radiation sensitivity is not explicitly linked to distinct cell-cycle dynamics. By performing Louvain clustering on the phospho-protein expression from all cells across time points, we identified distinct signalling states that were present at early, transient, or late time points after irradiation. Interestingly, we found (1) an early signalling state (p53+) mainly present in damaged cells of the RR clone, and (2) a transient signalling state (p53+pERK1/2) mainly present in cells with residual DNA damage in RS clone. Following this transient pulse, the levels of cleaved Caspase-3 and pNF-κB increased in the RS subclone. This analysis allowed us to hypothesize plausible molecular mechanisms of resistance that are currently being tested via pharmacological inhibition of Chk1 and/or MEK.

News from Heidelberg - Establishment of the multiplexed ion beam imaging (MIBI) technology for high-dimensional spatial proteomics

Felix Hartmann

German Cancer Research Center (DKFZ), Heidelberg, Germany

Multiplexed ion beam imaging (MIBI) is a novel technology that allows high-dimensional proteomic imaging. In MIBI, antibodies conjugated to metal isotopes are quantified via time-of-flight mass spectrometry to visualize the spatial distribution of up to 40 proteins in parallel. MIBI technology provides a unique combination of advantages: analysis of the spatial distribution of cells in tissues to identify tissue structure, multicellular communities, and cellular interactions; quantification of proteins and posttranslational modifications; adjustable resolution down to sub-cellular level; no autofluorescence background; the dynamic range spans five orders of magnitude; tissue staining imaging of all targets is performed in a single step, and compatibility with a wide range of samples, including fixed-frozen and formalin-fixed paraffin-embedded (FFPE) tissue. We have recently established the MIBI technology at the German Cancer Research Center (DKFZ) in Heidelberg and are now using this technology to quantify cellular metabolism in the human tumor microenvironment.

Short Talk: Interactive interrogation of the topology of high dimensional single cell datasets

Jan Stuchly

T. Kalina[1], D. Novák[2,5], N. Brdičková[1], D. Kužílková[1], S. Scaramuzzino[3], A. Iksi[4], H. Luche[3], J. Stuchlý[1]

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Introduction

With the onset of highly complex single-cell technologies, such as mass cytometry or CITE-seq, the investigation of cell-to-cell relationships going beyond the simple notion of populations came into prominence. The most apparent manifestation of the effort in this line of research is the large number of trajectory inference (TI) techniques developed in the last couple of years [1]. However these powerful tools seem to fail to establish themselves in standard workflows of biological research let alone in the clinical diagnostics. One reason for this lack of application is that existing TI tools offer close to no interaction of the researcher with the analysis process. We believe that feedback loops where the researcher learns to understand the particularities of the sample is crucial for the exploratory analysis of

unknown data.

Aims

We aimed to develop and to extensively test a TI approach designed to tackle large datasets, significant noise, technical artifacts and unequal distribution of cells along the time axis. This approach must offer a comprehensive language describing the geometry and the underlying dynamics of the data directly in the high dimensional space without the need of dimensionality reduction. At the same time this approach must offer sufficient interaction with the analytical process and allow the researcher to get to know the data while having a solid foundation of reproducible computational representation of the topological features.

Methods

We based our approach on the k-nearest neighbor graph representation and witness complex triangulation [2] and applied the tools of algebraic topology [3] and discrete differential geometry [4]. We applied our approach on 3 human thymi & PBMC measured by 32 parameters mass cytometry panel and on WT/transgenic CITE-seq dataset of mice thymi.

News from Jena - Pros and Cons of using iodinated Hoechst reagent to detect single nucleated cells in suspension mass cytometry

Sabine Baumgart

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Cell identification in mass cytometry relies on their sufficient labeling with a metal-labeled probe, which is routinely achieved by post-permeabilization staining with an iridium-labeled DNA intercalator. The detection of doublets is usually achieved by using the DNA content and event length but this practice is not sufficient.

Hoechst dyes are commonly used in flow cytometry to detect live nucleated cells due to its cell membrane permeant properties. Here, we introduce the permeabilization-free detection of nucleated cells using iodinated Hoechst-33258 (127I-Hoechst) by mass cytometry. 127I-Hoechst specifically stains live nucleated cells, and can successfully be combined with antibody staining in a single incubation step. We show that 127I-Hoechst can be applied to human whole blood and peripheral blood mononuclear cells (PBMC).

Preliminary data show a rather limited discrimination of doublet cells when using 127I-Hoechst as an alternative to intercalator-based detection of nucleated cells in order to reduce hands-on time for sample. However, when using 127I-Hoechst in addition to iridium-containing DNA intercalator the differentiation of doublets from single cells is more effective. Further advantages in discrimination of cells can be observed in more heterogenous samples such as urine.

Fluorescence microscopy confirmed that Hoechst maintains its fluorescent properties after iodination. Therefore, 127I-Hoechst can potentially be used

for cross-platform identification of nucleated cells in flow and mass cytometry. Together, we propose 127I-Hoechst to include into mass cytometry assays to improve the detection of single nucleated cells.

News from DRFZ - Vaccinology meets mass cytometry - Identifying baseline predictors of vaccination outcome

Henrik Mei

Axel Ronald Schulz[1], Addi J. Romero-Olmedo[2], Lisa-Marie Diekmann[1], Svenja Hochstätter[3], Dennis Das Gupta[2], Heike Hirseland[1], Daniel Staudenraus[2], Bärbel Camara[2], Carina Münch[3], Véronique Hefter[3], Siddhesh Sapre[3], Christian Keller[3], Michael Lohoff[2] and Henrik E. Mei[1]

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Vaccination efficiently protects from severe consequences of infection. However, little is known about immunological determinants of vaccination success in humans, specifically in groups with variable/poor vaccination response. We deeply profiled blood leukocytes by mass cytometry in 55 older (>80 years) and 44 younger adults (20–53 years) before receiving at least two doses of BNT162b2 mRNA vaccine and correlated the data with SARS-CoV-2-specific T and B cell vaccination response. Vaccination responses expectedly varied stronger among older compared to younger individuals, including individuals without detectable T and B cell immunity (Romero-Olmedo & Schulz et al., Nat Microbiol 2022, Lancet Infect Dis 2022). First analyses of mass cytometry data reproduced known features of immune ageing in senior adults, such as decreased frequencies of naive CD4+ CD31+ recent thymic emigrants, gamma-delta T cells, and plasmacytoid dendritic cells. From ongoing correlation analyses, we aspire the identification of immune signatures predicting the success of vaccination that can be translated into clinical practice, i. e., to identify individuals that may require additional doses or differently formulated vaccination to achieve protection. We expect that immune signatures will provide hints for the immune mechanisms underlying the magnitude and durability of the vaccination response, thus providing a basis for tailoring next generation vaccines and promote precision vaccination.

News from Munich - Metabolic profiling of murine regulatory T cells in the tumor microenvironment

Marc Rosenbaum & Selina Keppler

Klinikum rechts der Isar, Institut für Klinische Chemie und Pathobiochemie

Our work focuses on establishing a CyTOF workflow for metabolic profiling of regulatory T cells (Tregs) in mouse tumor models. Using the MC38 subcutaneous tumor model in the Foxp3IRES-Cre or Foxp3eGFP-Cre-ERT2 mouse strains, we established a CyTOF panel to detect the metabolic profile of intratumoral Tregs and other tumor-infiltrating immune cell populations. We included antibodies against

enzymes of the glycolysis and pentose phosphate pathway, tricarboxylic acid cycle, oxidative phosphorylation, fatty acid oxidation, glutamine metabolism as well as markers for Treg activation and proliferation. This CyTOF panel allows us to not only acquire information about the expression level of rate-limiting enzymes and crucial factors in cellular metabolism of intratumoral Tregs, but also to connect them to their activation and proliferation state.

Keywords: mouse CyTOF panels, regulatory T cells, tumor microenvironment, metabolic profiling

News from Dresden - High-dimensional immunophenotyping to identify resilience mechanisms against Sars-COV2 in families

Sebastian Thieme

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The Sars-COV2 pandemic poses an unprecedented challenge to society and health care. The development of new vaccines and effective treatment strategies is instrumental in overcoming the crisis. Despite the novelty of the pathogen, some individuals resisted infection or developed only mild symptoms without long-term consequences. We hypothesize that resilience mechanisms exist that depend on age and immunologic experience. We also propose that these mechanisms can be analyzed in a systems immunology approach by mass cytometry.

The Children's Hospital Dresden recruited a sample of families in the context of the Sars-COV2 pandemic, in which the infection status of each family member was recorded. Clinical and epidemiological data relevant to the infection event as well as blood samples were collected. Within this sample, we focused on families in which SARS-CoV2 transmission from one family member to others did not occur despite close contact. We presume that within these families individual immune responses prevented the disease.

To characterize the specific resilience mechanisms, peripheral blood cells were stimulated with different peptide mixes specific for Sars-COV2 and common cold coronaviruses, respectively, stained with broad-ranged panel and analyzed by mass cytometry. By immunophenotyping, we aim to identify the underlying immune networks that provide SARS-CoV2 resilience.

News from Freiburg -Spatial immunological analysis of novel immune-mediated liver diseases during the pandemic

Felix Röttele

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The liver is the central metabolic organ and a gatekeeper for detoxification of intestinal metabolites arriving via the portal vein and as an immunologic organ it is able to maintain tolerance against food-associated antigens. However, in many liver diseases, this tolerance is overcome and significant inflammation can occur. During the pandemic, several new liver disease manifestations have been noted. Autoimmune-like liver disease following mRNA vaccination against SARS-CoV2 dominated by CD8 T cell infiltrates has been observed as a rare side effect1. Additionally, in 2022 a rise in cases of severe hepatitis in children of unknown origin has been noted that is currently etiologically unexplained2. Several hypotheses are discussed, such a connection to HAdV infections in combinations with a second viral infection or exuberant post-COVID immunity. To understand the underlying histopathological correlates, we used imaging mass cytometry (IMC) on 12 formalin-embedded liver tissue sections of kids with an etiologically unknown acute hepatitis in collaboration with a network of Austrian and Turkish investigators. Our results indicate different immunological manifestations of liver disease in this group of pediaric patients suggesting different possible etiologies, with one group dominated by CD8 T cell infiltrated possibly associated with a post-COVID19 setting.

Poster Abstracts

Algorithmic stratification of autoimmune diseases and reclassification of single patients based on blood immune cell signatures

Vera Bockhorn

Vera Bockhorn, Marie Burns, Axel Schulz, Sebastian Ferrara, Henrik Mei, Andreas Grützkau
German Rheumatism Research Center Berlin, a Leibniz-Institute, Berlin, Germany

Autoimmune inflammation may involve different molecular pathways, and targeted treatments are not equally effective across different rheumatic diseases. Horizontal treatment transfer is an important promise of precision medicine, however we lack features distinguishing patients eligible for such treatment.

Based on mass cytometry data of blood leukocytes from healthy controls (n = 50), patients with rheumatoid arthritis (n = 8), systemic lupus erythematosus (n = 23), and ankylosing spondylitis (n = 18) we attempt to identify cytometric signatures specific for the different diseases, and of single patients with immune signatures similar to that of different conditions, i.e. individuals potentially eligible for horizontal treatment transfer.

We employed the Random Forest algorithm which supports multi-class classification and training data with varying group size, and resampled our data 1000 times to cross-validate our results.

We identified a preliminary signature correctly clas-

sifying samples to respective sample group with approx. 80% accuracy based on five features, comprising frequencies of IgM memory B cells, basophils, pSTAT1 of CD8 T cells, and levels of phosphorylated S6 protein in naïve T cells and basophils. Notably single patients were stably classified outside their group, indicating similarity of their leukocyte signatures to distinct patient groups. For example, two SLE patients consistently grouped with RA patients, suggesting that these patients may benefit from targeted treatments successfully established for the treatment of RA.

Next steps will include method validation on larger patient cohorts and integration of clinical data.

We show that cytometric fingerprinting combined with machine learning has the potential to distinguish autoimmune diseases, and re-classifies single patients who may benefit from horizontal treatment transfer.

Differential compartmentalization of myeloid cell phenotypes and responses towards the CNS in Alzheimer's disease

Camila Fernández Zapata

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Myeloid cells are suggested as an important player in Alzheimer´s disease (AD). However, its continuum of phenotypic and functional changes across different body compartments and their use as a biomarker in AD remains elusive. Here, we perform multiple state-of-the-art analyses to phenotypically and metabolically characterize immune cells between peripheral blood (n = 117), cerebrospinal fluid (CSF, n = 117), choroid plexus (CP, n = 13) and brain parenchyma (n = 13). We find that CSF cells increase expression of markers involved in inflammation, phagocytosis, and metabolism. Changes in phenotype of myeloid cells from AD patients are more pronounced in CP and brain parenchyma and upon in vitro stimulation, suggesting that AD-myeloid cells are more vulnerable to environmental changes. Our findings underscore the importance of myeloid cells in AD and the detailed characterization across body compartments may serve as a resource for future studies focusing on the assessment of these cells as biomarkers in AD.

Diffcyt-based extraction of early immune signature associated with severe courses of COVID-19

Sebastian Ferrara

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Considering that COVID-19 can become life-threatening or even fatal, early diagnosis and prediction of severe courses requiring special clinical care are needed. We here used mass cytometry data of blood leukocytes obtained after hospital admission of COVID-19 patients (1,2), to determine early fea-

tures associated with severe courses of COVID-19. For this, we implemented a workflow starting with FlowSOM clustering and extraction of cell frequencies and signal intensities in OMIQ.ai, followed by a modified diffcyt protocol in R (2) permitting the analysis of the same cytometric markers for clustering and per-cluster expression analysis. Using linear and generalized linear mixed models, we extracted a signature of severe disease comprising expansions of immature neutrophils, plasmablasts, activated T cells, besides diminished numbers of KLRG1+CD27+ NK and naïve B cells, and enhanced LAG3 expression by activated and terminally differentiated T cell subsets, consistent with inhibited T cell reactivity in immune synapses. Hierarchical clustering revealed two groups of patients with poor outcome, one characterized by a combined plasmablast / CD16+ T cell signature most strikingly detectable in early diseasehood, and another group with persistently expanded, terminally differentiated CD27- CD8 T cells, altogether suggesting the presence of distinct immunopathological axes involved in severe COVID-19. Based on our findings yet pending confirmation in validation cohorts, we envisage the development of a diagnostic cytometric test for the prediction of severe COVID-19 to enable timely treatment and clinical care.

Role of IL-36RA mutations in Crohn's disease

Julia Hecker

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IL-36 signaling has been recently identified as important factor in maintaining gut homeostasis and in the development of fibrosis. However, it is unclear how IL-36-signaling contributes to the development of intestinal inflammation and which cell types are involved in this process.

We identified in a patient with a severe course of Crohn's disease (CD) and consecutive of fibrosis a heterozygous missense mutation in the IL-36 receptor antagonist (IL-36RA) (p.Ser113Leu). By applying mass cytometry we performed an in-depth characterization of peripheral blood mononuclear cells (PBMCs) of the IL-36RA mutation carrying patient and identified cell subsets responding to IL-36 stimulation. Our data revealed that IL36α stimulation mainly induced TNFα and IL-6 expression in myeloid cells as well as IFNγ expression in NK cells but did not affect T and B cells. Furthermore, we found in the IL-36RA mutated patient an increased frequency of TH17 cells in PBMCS and detected increased levels of IL-36RA, IL-18 and IL-23 in the serum.

We also observed an enrichment of IL-36RA mutations in CD patients, as we identified in an available whole exome sequencing dataset of inflammatory bowel disease (IBD) patients and controls, two more CD patients with IL-36RA mutations (p.Leu133Ile, p.Pro76Leu).

Finally, we overexpressed the identified mutations in HEK cells and produced recombinant mutated proteins to study the effect of the mutations on protein expression and function. First results show that two mutations (p.Ser113Leu and p.Pro76Leu) lead to a reduced protein expression and at least one mutation (p.Ser113Leu) has a reduced capacity to antagonize IL36 stimulation.

Mass cytometry technology: Simultaneous analysis of blood and salivary glands of Sjögren's syndrome patients.

Patrice Hemon

Service immunology, Hyperion Core Facility, Brest, France

Background: Primary Sjögren syndrome (pSS) is a systemic autoimmune disease with chronic lymphocytic inflammatory mechanisms affecting salivary glands and other mucosa associated lymphoid tissue. Several studies investigate some subsets of immune cells but not so exhaustively.

Objective: To assess the distribution and phenotype of immune cells in blood and salivary glands of pSS patients.

Materials and Methods: 40 pSS patients characterized by the presence or absence of anti-SSA autoantibodies and 30 healthy donors were included in this study. On fresh whole blood a CyTOF analysis was done with the Fluidigm Kit, Maxpart Direct Immune associated with 7 additional markers. A panel of 37 markers was designed for salivary gland immune cell assessment by imaging mass cytometry (IMC).

Results: We quantified the frequency of circulating immune cells in pSS patients and healthy donors (HD) using mass cytometry. The distribution of immune cell subsets like NK cells, CD8 T cells but also B cells were altered in patients compared to HD. Additionally, we found that the alterations were strongly associated with the presence of anti-SSA autoantibodies in pSS patients. Furthermore, we analyzed salivary glands using IMC and identified an increase of immune cells, and especially subsets of CXCR3 B cells and T cells which can explain the altered distribution of immune cells in blood.

Conclusion: Our study shows the abnormal distribution of circulating immune cells in pSS patients which can be explained by the migration of these cells in mucosa associated lymphoid tissue, like salivary gland. Given the complexity of the immune cell phenotype, the mass cytometry instrument is strongly convenient to simultaneously analyze blood and tissue in patients. Our results justify further research into the identification of immune cell subsets using this technology.

High-dimensional mass cytometric analysis identifies disease-associated changes in the composition of the tissue-resident immune system in emphysema

Li Jia

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Chronic obstructive pulmonary disease (COPD) is a chronic inflammatory respiratory disease, and cigarette smoking is the main risk factor for its development. COPD is characterized by progressive and chronic inflammation, which may cause loss of alveolar tissue, known as emphysema. COPD patients have an impaired lung function, which severely impacts the quality of life, and COPD is associated with significant mortality.

We aim to assess alterations in pulmonary immune cell populations in emphysematous lung tissue

compared to control lung tissue using high-dimensional mass cytometry (cytometry by time-of-flight; CyTOF) analysis. Using imaging mass cytometry (IMC), we further confirmed the diseased-related cell populations in situ and explored alterations in the cellular spatial interactions in emphysema. Ficoll-separated single cells from fresh non-emphysematous control lungs (n=5) and emphysematous lungs (n=15) were isolated, and stained using 2 CyTOF panels comprising 40 (lymphoid markers) /38 (myeloid markers) antibodies. We next employed Hierarchical Stochastic Neighbor Embedding (HSNE) analysis on single, live CD45+ cells in Cytosplore, to obtain a global and detailed overview of the immune cell composition in human emphysematous and non-emphysematous lung tissue. Snap-frozen non-emphysematous control lung (n=3) and emphysematous lung (n=9) tissue sections were then immune-stained using a 36-antibody -IMC-panel to visualize the spatial distribution of immune populations in situ.

We identified major immune lineages, including CD4+ T, CD8+ T, ILC and B cells within emphysematous and non-emphysematous lung tissue. Zooming into the T cell populations, we observed higher levels of CD4+ T central memory and CD8+ T central memory cells in emphysematous lungs. Furthermore, multiple CD4+ T and CD8+ T central memory cell clusters

Early histopathological changes of the salivary glands associated with the development of primary Sjögren´s Syndrome

Luis E. Muñoz

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Background/Purpose

Primary Sjögren's syndrome (PSS) is a chronic inflammatory disease affecting exocrine glands causing dry eyes and mouth along with various systemic manifestations. Autoantibodies against nuclear proteins (SSA1, SSB, N-terminus peptide of the M3 muscarinic receptor (M3R)) are hallmark in this disease. Although a glandular epithelial deregulation is thought to be the critical initiator of PSS pathogenesis, the precise initiating events are yet to be identified. Neutrophil extracellular traps (NETs) are commonly found in mucosal surfaces and glandular ducts. Its aggregation to larger clusters can interfere with the secretion flow. The goal of this work is to quantify the rheological properties of saliva in mice under secretory stress and to evaluate the early epithelial changes by Imaging Mass Cytometry (IMC).

Methods

We induced the accumulation of NETs in the salivary ducts by the stimulation of salivary secretion with specific beta-adrenergic agonists in the hypercalcemic mouse. Transient hypercalcemia was induced by i.p. injection of calcium gluconate (300µmol/Kg). After 30 minutes abrupt salivary secretion was induced by a single doses of the β1-adrenergic agonist denopamine (14µmol/Kg) or the β2-adrenergic agonist fenoterol (1.4 mg/Kg). The viscosity and other rheological parameters of collected saliva were immediately analysed in a capillary extensional rheometer (CaBER). Histopathological changes were characterized by image mass cytometry of submandibular glands (SMG). Autoantibodies titers after a recovery phase of 8 weeks against SSA1, SSB and M3R autoantigens were measured by ELISA.

Results

ß-adrenergic stimulation of hypercalcemic mice caused the enlargement of lumen areas of serous acini suggestive of an obstructive sialadenitis. An absolute loss of epithelial serous persisted until the end of the recovery phase. The analysis of the rheological properties of murine saliva displayed an increased viscosity under secretory stress induced by beta-adrenergic stimulation. Analysis by IMC revealed fibroblasts accumulation and an impairment of the interaction between mucous and serous cells. NETs markers expressed in ducts and serous acini suggest that NETs or NETs-remnants influence rheological properties of secreted saliva. A strong downregulation of the expression of TGF-ß in the mucous acini accompanied by the upregulation of the phosphorylated ribosomal protein S6 (Ps6) and the structural protein MIST1 was also observed. This pattern of protein expression suggests an enhanced protein synthesis in the mucous compartment. After the recovery from obstructive sialadenitis mice developed autoantibodies against SSA1, SSB and M3R.

Conclusion

NETs are able to induce serous epithelial cell damage by altering the rheological properties of the secreted saliva. The reaction of the mucous compartment leads to the activation of the mTOR pathway and concomitant accumulation of fibroblasts accompanied by the impairment of mucous-serous interaction. These changes preceded the appearance of Sjögren's Syndrome-like manifestations in our murine model.

Radiotherapy has the potential to prevent T cell exhaustion and E-Cadherin-loss mediated metastasis in a murine breast cancer model

Clara Reichardt

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The World Health Organization (WHO) estimates cancer to be one of the greatest burdens on humanity, and breast cancer is one of the most prevalent types of disease. To estimate survival, the amount and ratio of immune cells such as lymphocytes and neutrophils in the tumor microenvironment (TME) have become an independent prognostic marker. However, there is limited data on the modulation of the immune system, especially neutrophils. Our recent in vivo data from image mass cytometry (IMC) shows high numbers of infiltrating monocytes and neutrophil extracellular trap (NET)-associated factors in untreated breast tumors, which is associated with an immunosuppressive microenvironment. However, irradiated tumors exhibit increased neutrophil infiltration while NET release decreases, possibly promoting an immunogenic phenotype of the tumor. Furthermore, the balance between cytotoxic T cells and T helper cells in irradiated tumors shifts toward a pro-inflammatory microenvironment and E-cadherin expression has been found to be maintained by radiotherapeutic treatment, possibly via the TGF-β/SMAD axis. Because loss of E-cadherin is a fundamental factor in metastasis through epithelial-to-mesenchymal transition (EMT), radiotherapy has the potential to prevent EMT-driven metastasis and a NET-promoted anti-inflammatory microenvironment in our murine breast cancer model.

CyTOF-based detailed characterization of virus specific CD4+ T cells after cure from severe COVID-19 without specific humoral immunity.

Addi Josua Romero-Olmedo

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While studying immunity after vaccination with the BNT162b2 vaccine, we noted a B-cell depleted (4-doses of anti-CD20, Rituximab), 54-year-old patient with hairy-cell leukemia who survived COVID-19 without specific humoral immunity. We characterized his spike-reactive CD4+ T cells, in comparison to four non-leukemic, B-cell competent control subjects of similar age, time of SARS-CoV-2 infection, and disease severity. Contrary to the absence of his B cells or detectable antibodies against SARS-CoV-2, the four controls presented with antibody amounts against SARS-CoV-2 similar to a 1-dose vaccinated cohort. Flow cytometry analysis revealed 2-10-fold more spike-reactive ($CD40L^+IFN\gamma^+$) $CD4^+$ T cells than the four B-cell-competent controls, and a frequency similar to that of a 3-dose vaccinated cohort. Remarkably, IFNγ per cell increased over time within these spike-reactive cells.

Using mass cytometry (45-marker antibody panel), we meticulously characterized the spike-reactive T cells of the patient and his four controls. The patient displayed enhanced frequencies of cytokine-producing CD4+ T cells. In all donors expanded spike-reactive $CD4^+$ T cells were high producers of Th1 cytokines, IL-21, and GM-CSF, compared to very few cytokines indicative of Th2, Th9, or Th17 cells. Then, we analyzed antigen-reactive cells showing very high limit of detection and restriction to SARS-CoV-2-spike-stimulated conditions ($CD40L^{hi}$). We identified, mainly in the patient, a $CD40L^{hi}CD4^+$ cell subset displaying the remarkable expression of various CCRs and RANKL. This subset possessed single-cell co-expression of CCR7, CXCR3, and CD45RA, characteristic of T memory stem (T_{SCM}) cells. Within his spike-reactive T_{SCM-}like cells, about 10-20% secreted IFNγ, GM-CSF, and TNFα, a frequency similar to the average $CD40L^+$ cell population. After B-cell reappearance, the patient received two doses of BNT162b2, and developed anti-spike IgG. Vaccination caused a rise of spike-specific antibodies, but also an almost lost of T_{SCM-}like cells and considerable reduction of $CD45RA^-CCR7^+$ T-effector-memory cells.

Summarizing, we present expansion of unusual poly-chemokine receptor-positive T_{SCM-}like cells in a B cell-depleted leukemic patient after surviving severe COVID-19. While these particular cells might be hampered from terminal differentiation in the absence of B cells, they might instead promiscuously express an array of CCRs for quick invasion of different tissues, if confronted by a novel viral challenge.

Landscape of exhausted and tissue resident memory T cells in the HCC TME revealed by highly-multiplexed mass cytometry

Henrike Salié

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Introduction

Despite the advent of immune checkpoint inhibition in the treatment of advanced hepatocellular carcinoma (HCC), the immunobiology of HCC remains incompletely understood. PD-1 can be expressed by exhausted (TEX) and tissue resident memory (TRM) CD8 T cells which are both present in the HCC TME but their roles in HCC remained unclear. Therefore, we comprehensively dissected TEX and TRM phenotypes and function in the HCC microenvironment utilizing multi-parametric flow and imaging mass cytometry.

Patients and methods

Lymphocytes were isolated from the blood, tumor and surrounding tissue of systemic therapy naive HCC patients (n=30) and mass cytometry was applied to thoroughly dissect TEX and TRM phenotype and function with respect to clinical parameters. Additionally, biopsies from HCC patients subsequently undergoing anti-PD-1 treatment (n=10) were collected and subjected to imaging mass cytometry (IMC) analysis. Lastly, we performed in vitro culture assays with an anti-PD-1 antibody.

Results

TEX (PD-1+CD39+) co-expressed multiple additional inhibitory molecules (e.g., TIGIT, Lag-3, CD39) and exhaustion-related transcription factors (e.g., Tox, Eomes) in comparison to CD103+ TRM or CD103+PD-1+ TRM. Of note, expression of TRM markers CD69 and CXCR6 that were co-expressed by CD103+ TRM could also be found on TEX cells. We observed a higher polyfunctionality (e.g., IFN-γ, TNF, IL-2 production) of CD103+ PD-1- or CD103+PD-1+ TRM upon stimulation compared to non-CD103 expressing CD69+ and CXCR6+ CD8 T cells. Lowest functionality was observed for TEX cells expressing CD39. Spatial analysis using IMC identified an abundance of CD103+ CD8 T cells in some patients with prolonged progression-free survival, in line with data from suspension mass cytometry. In patients undergoing anti-PD-1 treatment we observed a higher abundance of CD8 T cells, CD103+ TRM and PD-1+CD39+ TEX at baseline in patients that achieved a stable disease outcome compared to progressors. Interestingly, the TRM/TEX ratio positively correlated with overall survival. In vitro analysis of HCC TIL function and response to anti-PD-1 blockade identified a higher functionality of TRM compared to TEX at baseline but significant increase of TEX function after anti-PD-1 treatment.

Conclusion

Our findings identify divergent roles for TEX and TRM in the HCC TME. While CD103+ TRM are functionally more competent and linked to favourable clinical outcomes, prevalence of TEX is detrimental. In the context of anti-PD-1 treatment, a higher baseline TRM/TEX ratio may represent a novel biomarker for response to immunotherapy.

Highly multiplexed imaging of the oesophageal adenocarcinoma TME reveals correlates of neoadjuvant treatment response

Henrike Salié

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Introduction

Locally advanced oesophageal adenocarcinoma (EAC) is frequently treated with neoadjuvant chemotherapy prior to surgical resection but response rates remain limited. Currently no biomarkers are available to predict therapy response and the role of the anti-tumor immune response remains incompletely understood.

Patients and methods

We included locally advanced EAC patients from the MEMORI trial (n=15). Patients received neoadjuvant chemotherapy and were grouped into responders (RE) or non-responders (NR) after the first cycle of chemotherapy using PET-CT. NR were switched to neoadjuvant radiochemotherapy. Tumor samples were obtained and analysed using imaging mass cytometry (IMC) at diagnosis, after the first cycle of chemotherapy and following resection. Single cell-level data was obtained after segmentation and cell types were identified by clustering and hierarchical gating to assess immune dynamics and potential biomarkers of response or resistance to neoadjuvant therapy.

Results

We identified 28 cell types dynamically changing during the trial in the EAC tumor microenvironment, including tumor, stromal and immune cell subsets. Specifically, marked changes in immune composition were noted between RE and NR. CD4 T cell counts dropped significantly in NR whereas T cell infiltration was maintained in RE. Additionally, we observed a strong decrease in activated, cytotoxic and non-naïve CD8 T cells in NR patients while RE maintained CD8 T cell activation during therapy. Interestingly, the ratio between cytotoxic GranzymeB+ to PD-1+ CD4 and CD8 T cells was higher in RE compared to NR at all timepoints and the ratio was lost progressively in patients with poor response to neoadjuvant treatment.

Conclusion

In sum, in-depth spatial analysis of the tumour microenvironment of EAC patients identifies correlates of treatment response and failure to standard-of-care neoadjuvant therapy. Our data highlights the quantitative and qualitative composition of the T-cell compartment as potential biomarkers and mediators of treatment response of EAC and suggests a high potential for immune-based treatment strategies.

Identification of CD14+CD8+ intrahepatic T cells interacting closely with CD14+ mononuclear phagocytes in specific microanatomic niches in the healthy liver

Marius Schwabenland

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Background

CD14 is the coreceptor for TLR-4 mediated LPS recognition and expressed by many myeloid cells. As a product of gut microbiota, LPS may enter the liver via the portal vein. While expression of CD14 by myeloid cells is well characterized, it remained unclear if intrahepatic CD8 T cells also utilize CD14 to sense LPS and if so, where and how CD14 is acquired. Here, to validate and expand on prior work using ex vivo cell suspension profiling and in vitro cultures, we utilized imaging mass cytometry to analyze the expression of CD14 on intrahepatic CD8 T cells and other immune and parenchymal cells, and fine-mapped their microanatomical localization.

Methods

Imaging mass cytometry (IMC) was performed on macroscopically healthy liver biopsies (2 sections from 3 donors) using a dedicated (16-parameter) liver panel. The IMC data was quality-controlled, spillover correction performed and single-cell segmentation was performed using imctools. Phenograph clustering revealed all major intrahepatic parenchymal and immune cell populations. To identify liver zonation, we developed a liver zonation index that integrated liver zonation marker Cytochrome P450 1A2 (CYP1A2) (highest expression around the central vein in liver zone 3 (pericentral zone), lowest in liver zone 1 (periportal zone)) using a macro for use in ImageJ that applied a Gaussian Blurr filter in CYP1A2 channel to calculate a Liver Zonation Index per image pixel. Segmented cells were analyzed with respect to liver zones.

Results

We identified CD14+CD8+CD3+T-cells in all sections analyzed with CD14+ cells representing 20% of total CD8+CD3+T-cells. Spatial analysis revealed that CD14+CD8+T-cells were preferentially found in zones 2 and 1, where the highest LPS gradients are expected. Neighbor analysis revealed that CD14+CD8 T cells were frequently found adjacent to activated myeloid cell populations. Finally, we studied the distance from each CD14+CD8+ and CD14-CD8+ T-cells to the closest mononuclear phagocyte (MNP) expressing CD14. Clearly, CD14+CD8+ T-cells were significantly closer to MNPs than CD14-CD8+ T-cells, raising the possibility of acquisition of CD14 by CD8+ T cells via trogocytosis.

Conclusion/Discussion

Our analysis highlights a subset of CD14+CD8+ T-cells with a preferential localization in hepatic zones exposed to higher LPS gradients in proximity to MNPs. Myeloid licensing of CD8+ T-cells to bacterial recognition via CD14 transfer may represent a novel mechanism to fine-tune liver immunity and tolerance.

Molecular landscape analysis of B cell subsets in IgG4-RD with mass cytometry

Lena Teichert

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IgG4-related disease (IgG4-RD) is an immune-mediated fibroinflammatory disease characterized by sclerosing lesions at different sites, abundance of IgG4+ plasma cells in affected tissue, and, in many cases, elevated serum IgG4 levels. The pathophysiology of this condition is poorly understood, although there is evidence that it is antigen-driven and that CD4+ cytotoxic T cells and B cells, especially plasmablasts, play an important role in driving the disease. So far, only few alterations in B cell composition have been described in IgG4-RD using flow cytometry. Similar to previous results, we found significantly increased levels of circulating plasmablasts (6.89% vs. 3.62% among CD19+ B cells, p=0.013) in a cohort of 22 IgG4 patients, while the levels of circulating transitional B cells (0.82% vs. 2.95%, p<0.001) and circulating unswitched memory B cells (8.35% vs. 16.26%, p<0.001) were significantly decreased compared to age-matched healthy controls. Here, we want to take it a step further and investigate the in-depth composition of B cell subsets in peripheral blood of IgG4-RD patients and healthy controls with mass cytometry in correlation with the phosphorylation status of signal transducer and activator of transcription (STAT) proteins on a per cell basis. These data will be put in relation to each other and with serum cytokines and various clinical parameters to create a molecular landscape of B cell disturbances in IgG4-RD. These findings will contribute to a broader understanding of the disease pathophysiology and may provide insights into the heterogeneity of immune signatures, potentially enabling personalized therapeutic approaches in the future.

Spatial composition of decidual immune cell in oocyte donation pregnancies in relation to fetal-maternal HLA incompatibility

Xuezi Tian

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Introduction

Oocyte donation (OD) pregnancies are related to a higher degree of fetal-maternal HLA mismatching and a higher risk of complications compared to naturally conceived pregnancies. Nevertheless, many OD pregnancies maintain healthy until term. We hypothesize that in OD pregnancies with high HLA dissimilarity, the immune response at the fetal-maternal interface (the decidua) is divergent to maintain a healthy state. Here we focus on myeloid cells as we previously found these cells to be highly frequent in the decidua and they are essential in maintaining a healthy pregnancy.

Methods

We performed imaging mass cytometry (IMC) using a 42-antibody panel on decidua tissues of 8 uncomplicated singleton OD pregnancies. Single cell

masks were created using cell segmentation. Child and mother were typed for HLA-A, -B, C, -DRB1, and - DQB1, and fetal-maternal HLA mismatches were calculated. Based on the number of HLA mismatches, samples were separated into a semi-allogeneic group (≤5 HLA mismatches, n=4) and a fully allogeneic group (>5 mismatches, n=4).

Results

Myeloid cells represented the most abundant (~60%) immune cell population in the decidua. Thirteen phenotypically distinct subclusters were identified within the myeloid cell lineage. The IMC gave a possibility to study the microenvironment of each cell. The fully allogeneic group showed a higher frequency of maternal myeloid cells in the maternal T cells microenvironment than the semi-allogeneic OD group ($p<0.050$). Most notably, a higher extent of interaction between CD163+CD206+HLA-DR- myeloid cells and CD4+ T cells was observed in the fully allogeneic group ($p<0.050$).

Conclusions

Our results show the phenotypic diversity of decidual myeloid cells and their prominent frequency in uncomplicated OD pregnancies. By interacting with T cells, decidual myeloid cells might perform immune regulatory functions to compensate for the higher fetal-maternal HLA mismatch load in OD pregnancies.

Deciphering the connection between early response to therapy and signaling pathways activity in childhood T-cell acute lymphoblastic leukemia cells using mass cytometry

Adela Vavrova

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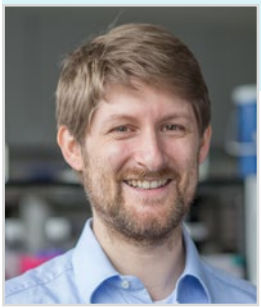
T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive malignant disease arising from T-lymphoblasts. While T-ALL accounts for only 15% of childhood ALL and at diagnosis the prognosis is comparable to other childhood ALL subtypes, 30% of patients undergo relapse with a dismal prognosis. Therefore targeted therapy of resistant and high-risk pediatric T-ALLs is urgently needed and together with precision medicine tools offers a hope for better therapy outcome. Moreover, intrinsic heterogeneity of T-ALL cells requires single-cell resolution.

To address this topic, we used a mass cytometry panel (38 parameters) to study signal transduction pathways such as the JAK-STAT, PI3K-AKT-mTOR and MEK-ERK pathways initially in 16 diagnostic T-ALL primary samples and investigated the in vitro response of cells to Interleukin-7 (IL-7) and the inhibitor BEZ-235. We found that perturbation induced by the ex vivo administration of either IL-7 or BEZ-235 reveals a high degree of exclusivity with respect to the phospho-protein responsiveness to these agents. Moreover, we found a tendency between ex vivo IL-7 responsiveness and in vivo prednisone responsiveness. To build on this findings we slightly modified the mass cytometry panel (44 parameters), we added markers for a deeper description of the PI3K-AKT-mTOR and apoptotic pathway and investigated a mini-cohort of 7 T-ALL samples obtained at diagnosis and during the early phase of treatment (day 8 and day 15). Our preliminary data suggest a very heterogeneous pattern between individual T-ALL patients in terms of up- or down-regulation of various signaling pathways. On the other side, several observations were characteristic for patients with respect to their in vivo prednisone responsiveness. For example, at diagnosis we observed a higher phosphorylation of Akt in patients with an in vivo prednisone poor response compared to patients with in vivo prednisone good response. This difference was maintained at day 8 and it was lost at day 15. Furthermore, we investigated the presence of pro- and anti-apoptotic proteins at time of diagnosis. Based on expression of pro-apoptotic (Bim

and cleaved caspase 3) and anti-apoptotic (MCL-1, pBAD and BCL-2) proteins the patient samples formed two separate clusters in hierarchical clustering analysis using Euclidian distance metrics. The clusters correspond with patients´ in vivo prednisone response.

In conclusion, we demonstrated the feasibility and power of mass cytometry single-cell profiling of signaling pathways in childhood T-ALL offering novel and targetable insights into the complexity of this disease.

Friday, January 20th



Imaging Session - Highly multiplexed imaging of tissues with subcellular resolution by imaging mass cytometry.

Bernd Bodenmiller

University of Zurich, ETH Zurich, Switzerland

Cancer is a tissue disease. Heterogeneous cancer cells and normal stromal and immune cells form a dynamic ecosystem that evolves to support tumor expansion and ultimately tumor spread. The study of the tumor ecosystem and its cell-to-cell communications is thus essential to enable an understanding of tumor biology, to define new biomarkers to improve patient care, and ultimately to identify new therapeutic routs and targets.

Highly multiplexed image information of tumor tissues is essential to understand this system. Such multiplexed images will reveal which cell types are present in a tumor, their functional state, and which cell-cell interactions are present. To enable multiplexed tissue imaging, we developed imaging mass cytometry (IMC). IMC is a novel imaging modality that uses metal isotopes of defined mass as reporters and currently allows to visualize over 50 antibodies and DNA probes simultaneously on tissues with subcellular resolution. In the near future, we expect that over 100 markers can be visualized. We applied IMC for the analysis of breast cancer samples in a quantitative manner. To extract biological meaningful data and potential biomarkers from this dataset, we developed a novel computational pipeline called histoCAT geared for the interactive and automated analysis of large scale, highly multiplexed tissues image datasets. Our analysis reveals a surprising level of inter and intra-tumor heterogeneity and identify new diversity within known human breast cancer subtypes as well as a variety of stromal cell types that interact with them.

In summary, our results show that IMC provides targeted, high-dimensional analysis of cell type, cell state and cell-to-cell interactions within the TME at subcellular resolution. Spatial relationships of complex cell states of cellular assemblies can be inferred and potentially used as biomarkers. We envision that IMC will enable a systems biology approach to understand and diagnose disease and to guide treatment.

Imaging Session - Development of new analysis tools for images generated by the Hyperion mass cytometer

Yvonne Scuiller

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Lymphocytes B et Autoimmunité (LBAI), UMR 1227, Université de Bretagne Occidentale, INSERM, CHRU Morvan, BP 824, 29609 Brest

Immunohistochemistry and immunofluorescence are the techniques primarily used for the analysis of patient biopsies. With the identification of four to six markers at a time, these techniques make large-scale analysis difficult, yet they appear to be indispensable for future effective approaches to personalized immunotherapy of patients with diseases.

The LBAI was then equipped with an imaging mass cytometer (IMC, Hyperion), allowing the simultaneous analysis of forty biomarkers on a single biopsy. The exploitation of this high dimensional information requires the development of powerful analysis software which integrates indispensable functions, and in particular the cellular segmentation from the images generated by the Hyperion which is a critical point for a good quality analysis.

A software including all the steps from pre-processing to image analysis has been developed in the laboratory. The key step, which is the cell segmentation, is managed by the optimization of a neural network, called U-Net, which is done automatically thanks to a selection of markers defined by the user.

Once the segmentation of the Hyperion data cells is obtained, a file in .csv format is generated. For each segmented cell, this file includes its centroid as well as the average gray intensity of each marker. From this file, our software allows to perform multiparametric analyses such as dimension reduction, clustering, manual gating, and neighborhood analysis. All those results are exportable in .csv format.

The next step in the development of the software is to include every tool usually used for downstream analysis such as dimension reduction, clustering, manual gating…

All these steps would be accessible through a single user-friendly graphical user interface.

Short talk - A 20-Target Imaging Mass Cytometry Panel for the Major Cell Populations in Mouse FFPE Liver Tissue

Fabienne Birrer

Fabienne Birrer, Tess Brodie and Deborah Stroka

Department of Visceral Surgery and Medicine, University of Bern, Inselspital, Bern University Hospital, Switzerland

Background: The liver is a complex organ including many unique immunological properties. It is comprised of about 80% parenchymal hepatocytes and non-parenchymal liver cells including Kupffer cells, stellate cells, liver sinusoidal endothelial cells (LSEC) and immune cells such as T- and B-cells, dendritic cells (DC), bone marrow derived macrophages (BMDMs) and natural-killer cells (NK). Local dysregulation of the immune system can be an underlying driver of liver disease, such as non-alcoholic fatty liver disease (NAFLD) and the more advanced state of non-alcoholic steatohepatitis (NASH). Underlying mechanisms, cell communications and possible targets for treatment are topics to continue to explore. In the liver, fluorescence imaging techniques are difficult due to auto fluorescence of the tissue and spectral overlap. Imaging mass cytometry (IMC) is therefore an excellent method to use for liver tissue, as it excludes background auto fluorescence and sections can be simultaneously stained with up to 40 antibodies. However, up to now, the development of a conventional IMC panel for mouse im-

mune cells in liver on formalin fixed paraffin (FFPE) tissue has not been published.

Methods: We designed and optimized a 20-target IMC antibody panel to identify the main cell types in the mouse liver from FFPE tissues. The antibody panel includes targets for the most abundant cell populations, as well as markers for cell activation, proliferation and cell segmentation. Purified antibody clones were first validated using conventional immunohistochemistry with a horse radish peroxidase detection and then conjugated to heavy metals. After, all targets were titrated on a tissue microarray containing lung, liver, spleen and colon tissue. Signals were acquired from two 1mm2 region of interests using the Hyperion imaging mass cytometer. Data was quality controlled in napari image viewer using the napari-imc plugin and then single cell data was extracted by segmenting the cells with DeepCell in the steinbock framework. Single cell analysis was performed in R with the imcRtools Data Analysis Workflow to create the dimensionality reduction plots, cell annotation and heatmaps.

Results: By staining WT, NAFLD and NASH liver tissue with the 20 marker IMC panel, we detected structural differences between conditions, using markers such as E-cadherin and CD206. Additionally, cell segmentation and downstream analysis revealed cell number differences between conditions. Comparing NASH to control tissue, we see a decrease in; B-cells, KCs and hepatocytes, an increase in; cholangiocytes, BMDM and LSECs and no differences in; neutrophils, endothelial cells and T-cells.

Discussion: With this IMC panel we will be able to study the immune cell changes during progression from NAFLD to NASH in mice in spatial context. The impact of this disease on cell composition and cellular communities in the liver will hopefully bring new mechanistic insights and a better overview of cell changes during disease progression. This will help to understand tissue architecture and cell communications in the liver, as well as important cell contributions in diseased states.

Short talk - Identification of novel cell cluster expressing CD38 and HLA-DR in chronic histiocytic intervillositis

Juliette Krop

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Pregnancy is a semi-allogeneic situation where the paternally inherited antigens of the fetus are tolerated by the maternal immune system. When the maternal immune system fails to achieve proper tolerance against fetal antigens pregnancy complications may arise. Chronic histiocytic intervillositis (CHI) is a rare histopathological lesion in the placenta, characterized by an abundant intervillous cell infiltrate of CD68-positive macrophages of maternal origin, accompanied by adverse pregnancy outcomes (e.g., still-birth, growth restriction). Currently, diagnosis of CHI can only made afterwards by pathological examination of the placenta. Due to the absence of a suitable clinical biomarker and the unknown cause, there is currently no substantiated treatment option available. In this study we aimed to phenotype the maternal intervillous immune cells and the fetal trophoblast cells they are in contact with using imaging mass cytometry.

We performed imaging mass cytometry (IMC), using a 42-marker panel on 3 dizygotic twin couples where one is affected with CHI and its twin is not affected. Cell segmentation was used to phenotype the intervillous immune cells. Next, we confirmed our findings on 11 CHI cases and 8 control samples using immunofluorescence and immunohistochemistry staining.

The intervillous immune cell composition of the unaffected twin resembled the maternal peripheral blood immune cell composition during pregnancy, as expected since maternal blood flows through the intervillous space. Intervillous immune cell numbers of the CHI affected twins are vastly different compared to their healthy twin. CD68+ mononuc-

lear cells numbers (unaffected mean: 7.8; affected mean: 122.1 cells per mm2) were increased, as were T cell numbers (1.1 vs 12.1 per mm2). When zooming into the myeloid compartment, six different myeloid clusters were apparent. The most prominent (55% of all myeloid cells) in CHI was cluster 2, which uniquely expressed CD38 and HLA-DR. Using immunofluorescence, we confirmed the presence of CD38+HLA-DR+ cells on all 11 CHI cases from the confirmation cohort. When mapping this cluster 2 back on the tissue we found that these cells cluster together and surround fetal trophoblast cells that show reduced CD39 expression compared to regions without cells from cluster 2 and controls.

We confirmed previous data that the CD68+ cell population, along with T cells, is increased in the intervillous space during CHI. Moreover, IMC identified a novel cluster of CD68+CD38+HLA-DR+ macrophages in the intervillous space of the twin with CHI. Since maternal intervillous blood is part of the maternal circulation and paired twin couples were studied here, it is likely that macrophages adhere and are activated locally in CHI. Further study should point if the CD68+CD38+HLA-DR+ cells can be found back in the maternal circulation and represent a potential biomarker of CHI. Furthermore, functional testing in this cell population could provide information on their effect on fetal trophoblast cells and if they should be enhanced or rather avoided in immune cell targeting therapies.

News from BIH at Charité - Mass Cytometry- based immune profiling of human Peyer's patches in inflammatory bowel disease

Adrian Huck

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Constant exposition of the immune system to environmental factors such as food antigens through Peyer´s patches (PP) in the ileum, is essential for oral tolerance development. Furthermore, mouse data indicate an important role of PP and other secondary lymphoid structures in maintaining the healthy balance of the mucosal immune system and prevent intestinal inflammation via T-cell activation and subsequent apoptosis. Yet, there are only few data regarding the immune cell composition of human PP in the state of inflammation. To study the T-cell compartment in particular, we combined flow and mass cytometry (CyTOF) as well as imaging mass cytometry (IMC) to analyze fresh samples of inflammatory bowel disease (IBD) patients. Using CyTOF, we were able to identify different T-cell subsets from single cell suspensions and identified differences between Crohn's disease patients in different stages (e.g. active inflammation, remission) and healthy controls. As spatial context is critical for the analysis of the analyzed cells within specific structures (lamina propria vs. lymphoid follicle), we performed IMC to resolve cell-cell interactions as well as the spatial distribution of distinct cell populations. By applying a background removal step, we could integrate samples from different patients and a combination of gating on major cell populations and subsequent unsupervised clustering of CD3+-cells facilitated the identification of specific T-cell subpopulations. In conclusion, by refining our established analysis pipeline to the specific characteristics of our samples, we were able to tackle typical problems of the analysis of dense lymphoid tissue using IMC.

News from Berlin MPI - Using IMC to understand treatment response

Anika Rettig

Gene Regulation and Systems Biology of Cancer (Yaspo lab), Max Planck Institute for molecular Genetics, Berlin

In our news from section, I will present the newly started 3TR project, which aims to identify the mechanisms of (non) response to auto-immune disease treatment. In the framework of this project, our group will analyse the tissue architecture and compare the immune composition of responders versus non-responders. The consortium involves partners from industry and academia across 15 countries and will focus on understanding response mechanisms of seven autoimmune, allergic and inflammatory diseases, starting with Lupus Erythematosus samples. Secondly, I will showcase how we used our in-house developed pipeline MICCRA to quantitatively analyse the response of melanoma patients to treatment with IMCgp100 within the framework of the MSTARS project

News from Erlangen - Deciphering the stromal compartment of immune mediated inflammatory diseases

Aleix Rius Rigau

Aleix Rius Rigau, Simon Rauber
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Stromal cells are the parenchymal cells of tissue and represent an extremely diverse cell type. Importantly, they show a high degree of spatial organization thus providing structure and compartmentalization to the tissue. Beyond providing mere structural support, it is increasingly recognized that „stroma" provides disease-relevant tissue „imprints" that modulate disease parameters and immune cell activation. Many of these chronic inflammatory diseases are characterized by an overshooting mesenchymal responses which is not tackled by the available immunosuppressive treatments.

We have set up mouse and human CyTOF and IMC panels, covering fibroblasts as well as endothelial and immune cells to study the stromal cell niche in the skin of patients with systemic sclerosis (Ssc), in the liver of cirrhosis and in the synovium of rheumatoid and psoriatic arthritis patients. We studied the interactions between fibroblasts, endothelial cells and immune cells in these diseases. We were able to describe the disease-specific interactome of endothelial cells in Ssc patients. Furthermore we traced the development of myofibroblasts in different tissues and fibrosis states. Finally, we uncovered a fibroblasts subpopulation which arises during resolution phases of arthritis.

Using CyTOF and imaging mass cytometry, in Erlangen we are investigating the diversity of stromal cell populations and their phenotypic changes in immune mediated inflammatory diseases to discover novel disease mechanisms and new therapeutic targets.

News from Ulm - Immune System Profiling Using Mass Cytometry in Metastatic Melanoma Patients Undergoing Radiotherapy

Aoife Gahlawat

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Radiotherapy can provoke tumor-specific immune responses and reshape the tumor immune microenvironment thereby eliciting beneficial effects both at local (in-field) and systemic (abscopal effects) levels. In this project, we aimed to characterize the perturbation of immune cells from peripheral blood mononuclear cells (PBMCs) after radiotherapy using the MaxPar antibody panel for mass cytometry (CyToF) combined with the streamlined Pathsetter analysis vs. an expanded set of antibodies and deep analysis using cutting-edge single cell analysis R-packages.

PBMCs were collected longitudinally, at baseline, 6 and 12 weeks and at the end of radiotherapy in a cohort of 21 metastatic melanoma patients with no-response to three months immune therapy with checkpoint blockade of the PD-L1/PD-1 and/or CTLA4 axis. For antibody staining, PBMCs were retrieved from liquid nitrogen and allowed to rest for 2-3 hours in fresh medium. On average, 1.5x 106 viable cells were labelled with the Maxpar Direct Immune Profiling Assay (MDIPA), which allows for the detection of 37 immune cell types, plus six additional T-cell markers (Tim3, CD69, LAG3, NKG2A, PD1 and TIGIT). Single-cell proteomics analysis was carried out via mass cytometry (CyTOF) for an average of 3x105 PBMCs.

For data analysis, we first used the Maxpar Pathsetter software which cleans up the data and assigns the 37 defined immune cell populations. Next, we took an unbiased approach by implementing R-packages for scRNAseq analysis to obtain signal per cell analysis. Here, we will report the outcomes of both approaches, comparing the results of using a predefined population or an unbiased holistic approach to identify potentially novel subpopulations of immune cells in response to radiotherapy.

Short talk: Tin sulfide nanoparticles (SnS-NP) as novel reporter tags for mass cytometry

Axel Ronald Schulz

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Current mass cytometers can detect up to 135 different isotopes in the range of 75 - 209 amu. However, this potential is only partially exploited, so that current applications are limited to approximately 60 parameters. Tin (Sn) has 10 natural, stable isotopes, six of which do not overlap with other reporter isotopes already used in mass cytometry. Despite its promise to increase the analytical capacity of mass cytometry, tin-based reagents remain unexplored until today. Here, we describe pilot studies on the application of elemental tin (II) sulfide nanoparticles (SnS-NP) as antibody tags in mass cytometry.

Stable SnS-NP, made from elemental Sn or isotopically enriched 117Sn and 118Sn, were synthesized in sizes ranging from 60 – 80 nm, and functionalized with carboxymethyl dextran. Several antibody conjugates were produced and tested on PBMC by mass cytometry revealing specific staining with CD8-SnS-NP, CD4-SnS-NP and HLA-DR-SnS-NP, with minimal to moderate background binding to non-target cells. Application of SnS-NP antibody conjugates was compatible with cell-surface staining protocols and multiplex-capable as demonstrated by the concurrent use of CD8-117SnS-NP and CD4-118SnS-NP. Finally, SnS-NP conjugates of β-2-microglobulin were successfully tested for live-cell sample barcoding.

In summary, we report the successful development and versatile application of SnS-NP-based reagents for mass cytometry, expanding the analytical capacity by two channels, with the potential for four more. We envision broad application of SnS-NP conjugates as reagents for cell marker detection and sample barcoding.

Product Feature - Cytobank /Beckman Coulter - Experience meets innovation- Automatic gating in the Cytobank platform

Giulia Grazia

Beckman Coulter

Manual gating has long been identified as a major source of variability for flow cytometric analysis. Centralized data analysis as well as standardized gating strategies and stringent standard operating procedures have been suggested as a means to increase the reproducibility, but are time-consuming. Machine learning-assisted analysis of cytometry data has proven advantageous; however, there is a lack of methods that allow users to automate their own user-defined gating strategy for their specific marker panel in a hypothesis-driven setting. Here, we present an automatic gating approach that allows users to train a model on their manual gating results on a small number of samples without requiring coding skills.

Standard BioTools - Immunophenotyping of blood and human airway samples by mass cytometry

Talk by Julia Eckl-Dorna

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Mass cytometry is a powerful method for mapping of complex cellular systems and is widely used for immunophenotyping in human blood. However, protocols for the preparation of human airway tissue samples such as bronchoalveolar lavage fluid (BALF) and nasal polyps (NPs) for mass cytometry are scarce. Thus, here we established and optimized a workflow for processing, labelling and acquisition of these samples. We found that combined enzymatic and mechanical processing led to better cell recovery as compared to treatment with collagenase or protease. Sample acquisition could be further improved by using DNAse but not by the usage of basic microbeads for the removal of cell debris. Using this protocol, we finally established a 31-marker mass cytometry antibody panel to identify immune cell subsets in blood, BALF and NP cells. With this panel we were also able to characterize a putative ILC2 population as well as an inflammatory NK cell subset in NPs. Thus, here we described an optimized workflow for mass cytometry-based analysis of human NP and BALF for future comparative analysis of different airway diseases.

Farewell and Poster Award

Henrik Mei

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Site map

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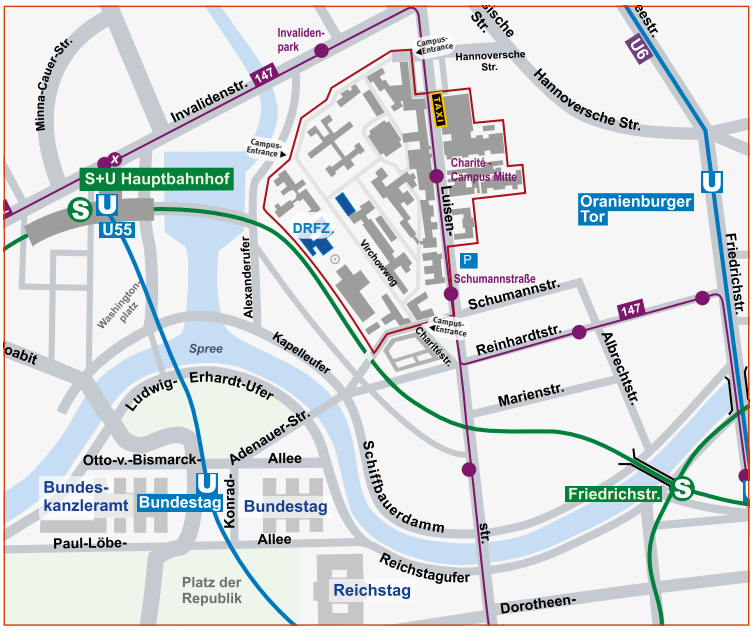

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