Flow cytometric quantification of Hsp70 on the surface of circulating exosomes by applying the unique cmHsp70.1 monoclonal antibody

Ali Bashiri Dezfouli [1,*], Mina Yazdi [2], Gabriele Multhoff [1]

[1] Central Institute for Translational Cancer Research Technische Universität München (TranslaTUM), Department of Radiation Oncology, Klinikum Rechts der Isar, Einstein Str. 25, 81675 Munich, Germany
[2] Pharmaceutical Biotechnology, Department of Pharmacy, Ludwig-Maximilians-Universität (LMU), 81377 Munich, Germany
[*] Correspondence to: Ali Bashiri Dezfouli, PhD; E-mail: ali.bashiri@tum.de

Exosomes are membrane-enclosed lipid vesicles which are actively released by nearly all living cells into the extracellular milieu. These microvesicles are considered novel biomarkers in liquid biopsies, reflecting the molecular profile of the cells of their origin. Extracellular vesicles including exosomes play a role in cell-to-cell communications. The lumen of exosomes consists of a mixture of cytosolic proteins, DNA, RNAs, and micro RNAs, which are crucial in regulating communications between parent (i.e., tumor cells) and recipient cells (i.e., other tumor cells, immune cells, stem cells).
Exosomes derived from tumor cells contain large amounts of cytosolic heat shock proteins (HSPs), especially Hsp70 (the major stress-inducible Heat shock protein 70), which is highly overexpressed in tumor cells and is also presented on their plasma membrane. It has been reported that exosomal Hsp70 is a prominent molecule triggering inflammation and immunity-associated processes. Using the compHsp70 ELISA, we could demonstrate that elevated levels of exosomal Hsp70 in the circulation reflect a membrane Hsp70 positive status in tumor patients. Moreover, patients with high exosomal Hsp70 levels in the blood end up with a poor prognosis. Hence, measuring the protein content inside and outside tumor-derived exosomes can help predict tumor response and clinical outcomes.
For a more detailed characterization of tumor-derived exosomes, we have established a method that enables the quantification of surface-bound Hsp70 on exosomes by applying the unique cmHsp70.1 mAb using multiparameter flow cytometry (MACSQuant) together with exosome-specific markers such as tetraspanins (i.e., CD9, CD63, and CD81).

Innovative robust high-throughput basophil activation test (BAT) to reliably diagnose allergy

Jochen Behrends[1], Christian Schwager[2], Martina Hein[1], Thomas Scholzen[1], Skadi Kull[2], Theresa Walsemann[2], and Uta Jappe[2, 3]

[1] Core Facility Fluorescence Cytometry, Research Center Borstel, Leibniz Lung Center, Borstel, Germany
[2] Division of Clinical and Molecular Allergology, Priority Area Asthma and Allergy, Airway Research Center North (ARCN), German Center for Lung Research (DZL), Germany
[3] Interdisciplinary Allergy Outpatient Clinic, Department of Pneumology, University of Lübeck, Lübeck, Germany

Allergic disorders have become the most common chronic disease in industrialized countries. Therefore, an increased need exists to establish highly specific and sensitive high-throughput diagnostic tests that not just detect sensitizations but prove the existence of an allergy with the utmost probability. The routine application of the basophil activation test (BAT), which mimics the in vivo situation of an allergic reaction, is still hampered by multiple factors such as complexity, costs, and lack of automation of the operation procedure. In this study, a novel gating strategy, including FCεRIα, CD203c and CD63 for a robust high-throughput analysis of basophils with an excellent performance has been established using over 1,300 samples. No significant differences were found between automated and manually analyzed samples. In addition, automated analysis saved up to 90% of working time. Furthermore, the time frame for basophil activation measurement after blood donation has been extended considerably. Respective storage conditions were optimized, which was confirmed by a nationwide ring trial showing the robustness and the applicability of our BAT on a wide variety of flow cytometers. All in all, our optimizations overcame the hurdles that prevented the application of the BAT as high-throughput allergy diagnostic test in routine diagnostic laboratories, so far.

Automatic and non-overlapping elliptical gating of flow cytometric data with flowEMMi v2

Carmen Bruckmann

Helmholtz-Centre for Environmental Research

Flow cytometry has become a powerful technology for studying microbial community dynamics and ecology. These dynamics are tracked over long periods of time based on multiple community fingerprints consisting of subsets of cell distributions with similar cell properties. These subsets are highlighted by cytometric gates all of which assembling to a gate template when samples are compared over time or between sites. The template is usually created manually by the operator which is time consuming, prone to human error and dependent on human expertise. Manual gating thus

lacks reproducibility, which in turn might impact ecological downstream analyses such as various diversity parameters, turnover and nestedness or stability measures. We present a new version of our flowEMMi algorithm – originally designed for an automated construction of a gate template, which now generates non-overlapping elliptical gates

within a few minutes. Gate templates can be created for both single measurements and time-series measurements, allowing immediate downstream data analyses and on-line evaluation. Furthermore, it is possible to adjust gate sizes to Gaussian distribution heights. This automatic approach makes the gate template creation objective and reproducible. Moreover, it can generate hierarchies of gates. flowEMMi v2 is essential not only for exploratory studies, but also for routine monitoring and control of biotechnological processes. Therefore flowEMMi v2 bridges a crucial bottleneck between automated cell sample collection and processing, automated flow cytometric measurement and automated downstream statistical analysis.

Activation-induced marker T cell assay for the *in vitro* assessment of sensitizing chemicals: a *p*-phenylene diamine and Brandrowski´s base study

Marina Aparicio-Soto[*1], ***Caterina Curato***[*1], Melanie Leddermann[1], Niko Himmeldirk[1], Maximilian Schäfer[1], Johanna Bachmeier[4], Wolfgang Bäumler[4], Andreas Luch[1,2,3], Katherina Siewert[1]

[1]Dermatotoxicology Study Centre, [2]Department of Chemical and Product Safety, German Federal Institute for Risk Assessment, Berlin, Germany
[3]Institute of Pharmacy, Freie Universität Berlin, Berlin, Germany
[4]Department of Dermatology and Allergology, University of Regensburg, Regensburg, Germany

[*]These authors share first authorship

Allergic contact dermatitis (ACD) is a T cell-mediated disease, but the development of T cell-based in vitro assays remains challenging. Here, we introduce activation-induced marker (AIM) T cell assays by applying *p*-phenylene diamine (PPD), a strong chemical sensitizer, and Bandrowski's Base (BB), an oxidation product of PPD. For both chemicals, the induced frequencies of allergen-specific T cells and the extent of TCR cross-reactivity remain elusive.
Using peripheral mononuclear blood cells (PBMC), we determined a concentration of 6 µM PPD or BB (dissolved in DMSO ≤0.5%) as non-toxic and non-interfering with multi parameter flow cytometry for 5 and 16 h incubation times. We then assessed frequencies of PPD- and BB-specific CD4+ and CD8+ memory T cells from allergic (n=7) and non-allergic (n=8-11) individuals using the activation markers CD154, CD137, OX40, and CD69, respectively.
As prior proliferation assays, we detected BB-specific CD154+CD4+ memory T cells in all donors (means allergic/non-allergic: 0.060%/0.014%, 16 h). In addition, we reached detection limits for PPD-specific T cells (0.010%/0.004%). Thus, both BB- and PPD-specific T cells may serve as allergy indicator, although PPD-induced epitope formation is rather slow and requires longer incubation time. We also detected increased PPD/BB-specific CD154+CD8+ memory T cell frequencies (16 h) in allergic donors. Increased cutaneous lymphocyte-associated antigen (CLA) co-expression indicated the involvement of PPD/BB-specific T cells in allergic immune responses, thus showing that these activation markers combined giving reliable allergy detection rates. In a preliminary TCR repertoires analysis, we did not detect overrepresentation of gene segments or CDR3 amino acids but considerable clonotype overlap in line with common PPD/BB epitope formation or TCR cross-reactivity.
In conclusion, this work represents the first application of AIM T cell assays to detect human-relevant organic chemical sensitizers. Being fast, sensitive and quantitative, AIM assays enable efficient optimization of assay conditions including epitope generation. Thus, they can serve to characterize chemical-mediated T cell activation and cross-reactivity for regulatory and diagnostic purposes.

A comparison of cytometric and substrate-based methods to understand the role of surface contacts for the biophysical properties of HEK293T cells

Venkata Dabbiru

Universität Greifswald

Cell physiological processes are accompanied by integral changes in their biophysical properties, e.g., during the gradual loss of cell-surface interactions and the resulting transition from adherent to suspended state. While an increasing number of reports are available investigating cells in the presence or absence of surface contacts, fewer studies focus on the transition itself. The reason is found in challenges mimicking this semi-adherent state and in the availability of corresponding methods to characterize the physical properties of cells like size, volume, deformation and elasticity. Here, we employed atomic force microscopy and real-time deformability cytometry to study human embryonic kidney 293T (HEK293T) cells attached to a surface, in suspension as well as passively adhered to a substrate using surface-tethering molecules. Our results demonstrate that seeding density alters cell size but does not impact on their stiffness. Furthermore, the loss of cell-surface interactions is linked to an increase in the cellular elasticity. Interestingly, we observe that the contribution of F-actin density to the cellular elasticity is minimal. Instead, geometrical features including circularity, volume and morphology are predominantly important for the biophysical properties of cells.

Construction of Functional Active Artificial Communities Based on Individual Cell Analyses and Ecological Theory

***Ruyu Gao*[1]**, Shuang Li[1], Susann Müller[1]

[1]Department of Environmental Microbiology, Helmholtz Centre for Environmental Research - UFZ, 04318 Leipzig, Germany

Microbial communities are prone to varying and even stochastic changes in composition and function. Natural microbial communities are important parts of ecosystems and human life, and show high complexity of microbial interactions and environmental dependencies. In contrast, using constructed artificial microbial communities might provide a chance to create communities more easily with desired functions when known strains with known functions are exploited. Designing functionally synthetic microbial communities requires two strategies, which is either a top-down design or bottom-up design. Here, we will use a design to construct microbial communities based on a looped mass transfer setup (Li et al., 2022) . As before, we will use individual-based single-cell analysis tools such as microbial community flow cytometry as a standard method: Cytometric fingerprinting will detect and evaluate variation in community structure, flow cytometry will identify the dynamics of microbial community, track labelled fluorescent strains and their abundance when grown together. Furthermore, bioinformatics tools will analyze the resulting high-throughput data and proteomics technology will be used to determine the desired function of the microbial community. We want to establish microbial ecology theory as a tool to control these types of processes such as keep proportions of organisms' constant in microbial community set ups. This study will contribute to a grand perspective of microbial ecology that meets an acute need in the health care and biotechnology industries.

Cell volume changes in confined environments on short timescales

Felix Graf

Universität Greifswald

Implementing spectral cytometry in phenotyping of human early B cell progenitors

Iga Janowska[1], Jakov Korzhenevich[1], Julian Staniek[1], Miriam Erlacher[2], Marta Rizzi[1]

[1]Department of Rheumatology and Clinical Immunology, Freiburg University Medical Center, Faculty of Medicine, University of Freiburg, Freiburg, Germany

[2]Department of Pediatrics and Adolescent Medicine, Division of Pediatric Hematology and Oncology, Medical Center, Faculty of Medicine, University of Freiburg, Freiburg, Germany

Our fundamental understanding of immunological processes originates from mouse models. However, due to significant differences between mice and humans the discoveries acquired by murine studies cannot be directly translated to humans. One of these processes is early B cell development, which has been thoroughly investigated in mice but is less well understood in humans. Therefore, we used a feeder-free in vitro system to develop human early B cells from CD34+ hematopoietic stem cells (HSC) (Kraus et al, 2014) that can reproduce all differentiation steps of early B cell lymphopoiesis. Progressive development of lymphoid progenitors and committed early B cell precursors was monitored by spectral flow cytometry. We established a 17-color panel for the spectra analyzer Cytek Aurora including 10 surface and 7 intracellular/nuclear markers, 4 of which were transcription factors. In the in vitro system cord blood-derived CD34+ HSCs cells were expanded in presence of SCF, Flt3-L, and IL-6 for 7 days. Then, the medium was supplemented with SCF, Flt3-L, and IL-7 for additional 7 days. From day 14 on, cells were cultured in medium without addition of cytokines. Between day 14 and 49 of the culture, we find all early B cell developmental stages starting with common lymphoid progenitors (CLP) expressing CD10 and CD38 on the surface and the transcription factor (TF) Ikaros. CLPs differentiate into pro-B cells by upregulating CD79a expression. At the pre-BI stage, cells start to express the TF Pax5 and on the surface CD19. Subsequently, pre-BII cells that already express the rearranged immunoglobulin heavy chain (cytoplasmic Igμ) can be divided in proliferating pre-BII large cells - expressing Ki67 and CD179a (surrogate light chain of the pre-BCR) - and pre-BII small cells, which downregulate CD179a, exit the cell cycle and start to rearrange the immunoglobulin light chain. During the last differentiation step, immature B cells induce expression of the complete IgM BCR on the surface.

By implementing spectral flow cytometry in the characterization of in vitro human early B cell development we could extend the stratification of B cell progenitors and optimize usage of scarce human samples. This powerful system enables us to study the capacity of human HSCs to differentiate into B cells in various settings, e.g. the addition of drugs, cytokines, and genetic manipulations. Finally, we can apply the in vitro system to bone marrow derived HSCs from patients to investigate the dynamics and dysfunction of human early human B lymphopoiesis in diseases.

A flow-through UV-C decontamination unit for sheath fluid or waste of flow cytometers

J. Kirsch[1], K. Heinrich[1], D. Kage[1], J. Glaab[2], B. Grothe[3], K. v. Volkmann[3], ***T. Kaiser***[1]

[1] German Rheumatism Research Centre Berlin (DRFZ) - Flow Cytometry Core Facility, Charitéplatz 1 (Virchowweg 12), 10117 Berlin, Germany
[2] Ferdinand-Braun-Institut gGmbH, Leibniz-Institut für Höchstfrequenztechnik, Gustav-Kirchhoff-Str. 4, 12489 Berlin, Germany
[3] APE Angewandte Physik und Elektronik GmbH, Plauener Straße 163-165 / Haus N, 13053 Berlin, Germany

Flow-cytometry and cell sorting are indispensable techniques in bio-medical research. To enable subsequent cell cultivation or to prevent misleading downstream experimental results, any contamination of the sorted cells must be avoided. However, since cell sorters typically do not operate in a sterile environment, contamination with microorganisms is not uncommon.

A chemical cleaning procedure can be applied to reduce the risk of contamination, but such a process is time-consuming, and its success not guaranteed. Furthermore, toxic residues of cleaning reagents may be critical to cell viability. Also, antibiotics are applied to the sorted cells prevent the proliferation of bacteria entering through the sheath fluid. This, however, can cause an unwanted change in gene expression and regulatory level of the cells and the widespread use of antibiotics aggravates the existing resistance issues.

As ultraviolet light is well known for inactivating microorganisms by inhibiting DNA replication, it is very interesting for applications in decontamination. Thus, decontamination of sheath fluids in flow-cytometric cell sorters by UVC irradiation is a promising alternative to chemical cleaning procedures.

Here we present a UVC unit for flow-through irradiation of sheath fluid to enable aseptic cell sorting. In a proof-of-principle study, the decontamination efficiency of the unit was tested on bacteria obtained from the laboratory's room air. A 5 log10 reduction of the contamination level was obtained.

Impact of reactive oxygen species on the mechanical properties of myeloid precursor cells

Yesaswini Komaragiri[1,2], Ricardo Hugo Pires[1,2], Stefanie Spiegler[1,2], Huy Tung Dau[1], Doreen Biedenweg[1], Clara Órtegon Salas[3], Md Faruq Hossain[1], Bob Fregin[1,2], Stefan Gross[2,3], Manuela Gellert[3], Uwe Lendeckel[3], Christopher Lillig[3], and Oliver Otto[1,2]

[1]ZIK HIKE, University of Greifswald, Greifswald, Germany
[2]DZHK, University Medicine Greifswald, Greifswald, Germany
[3]University Medicine Greifswald, Greifswald, Germany

Reactive oxygen species (ROS) are crucial in redox equilibrium by acting as secondary messengers in a variety of cellular processes such as activation, proliferation, and differentiation. Hydrogen peroxide (H2O2) is a major ROS present intracellularly at nanomolar levels that, when elevated to supraphysiological levels, promotes cell and tissue damage. While various ROS have been extensively studied at the molecular level, the precise mechanism by which they modify cell mechanics remains unknown. By employing varying H2O2 concentrations to stimulate ROS in human myeloid precursor cells (HL60), we observed a semi-quantitative relationship between ROS generation and an increase in cellular elasticity. The observed cell mechanical changes were not accompanied by changes in F-actin and microtubules levels but rather caused by lysosome disruption. Excess proton leakage into the cytosol due to lysosomal damage caused intracellular acidification, which lead to increased cell stiffness, establishing a molecular mechanism that connects redox homeostasis and cell mechanics as key mediators of cell function.

Two-photon excitation spectra of various fluorescent proteins within a broad excitation range

Ruth Leben, Randall Lindquist, Asylkhan Rakhymzhan, Raluca Niesner

Deutsches Rheuma-Forschungszentrum Berlin, ein Leibniz-Institut, Berlin, Germany

Two-photon excitation fluorescence laser-scanning microscopy is the preferred method for studying dynamic processes in living organ models or even in living organisms. Thanks to near-infrared and infrared excitation, it is possible to penetrate deep into tissue, reaching areas of interest for life sciences and biomedicine. In those imaging experiments, two-photon excitation spectra are needed to select the optimal laser wavelength to excite as many fluorophores as possible simultaneously in the sample under consideration. The more fluorophores that can be excited, the more cell populations that can be studied, and the better the access to their arrangement and interaction in complex systems such as lymphoid organs (Rakhymzhan et al. 2017). However, the two-photon excitation properties are poorly predicted by the single-photon spectra and are not yet available in literature or in databases, for many fluorophores (Ricard et al. 2018). Here we present the broad excitation range (760 nm to 1300 nm) photon flux-normalized two-photon spectra of several fluorescent proteins in their cellular environment. This includes following fluorescent proteins: mCerulean3, mTurquoise2, mT-Sapphire, Clover, mKusabiraOrange2, mOrange2, LSS-mOrange, mRuby2, mBeRFP, mCardinal, iRFP670, NirFP, iRFP720, ranging from blue to red and even infrared fluorescence.

Suppression of specific cellular immune response after mRNA vaccination against COVID-19 in rheumatic patients receiving SARS-CoV2 neutralizing antibody Ronapreve as pre-exposure prophylaxis.

Henrik Mei[1], Axel Ronald Schulz[1], Heike Hirseland[1], Elisa Habermann[2], Alexander ten Hagen[2], Fredrik Albach[2], Gerd-Rüdiger Burmester[2], Robert Biesen[2]

[1] Deutsches Rheumaforschungszentrum (DRFZ) Berlin,
[2] Department of Rheumatology and Clinical Immunology, Charité Universitätsmedizin, Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität, Berlin, Germany

Background: Active and passive immunizations against SARS-CoV-2 are used to protect against the development of severe COVID-19. However, the effect of passive immunization on the success of a subsequent active vaccination is unknown.

Objective: To investigate the cellular immune response to mRNA vaccination against SARS-CoV-2 in patients receiving preventive treatment with a SARS-CoV-2 neutralizing antibody cocktail, Ronapreve (Casirivimab/Imdevimab).

Methods: After poorly responding to multiple mRNA vaccinations against SARS-CoV-2, four patients with different autoimmune diseases treated with immunosuppressants including rituximab received 600 mg Ronapreve i.v. and were re-vaccinated 14-25 days later. Eleven clinically comparable patients served as controls. All participants were re-immunized with the mRNA-based Spikevax vaccine. Antigen-specific T- and B-cell responses were assessed by flow cytometry, after short-term stimulation with spike peptides and tetramer staining for spike-specific B cells before, 1 week, and 4 weeks after vaccination.

Results: As expected, most control patients showed a specific CD4 T- and B-cell response in the blood after vaccination, including induction of antigen-specific plasmablasts at week 1 and a significant increase of antigen-specific CD4 T cells producing IFNγ, IL-4, or IL-10 at week 4. In patients receiving Ronapreve, the induction of plasmablasts in the blood was effectively suppressed but not completely eliminated. In contrast to control patients, there was no consistent increase in SARS-CoV-2-specific CD4 T cells in Ronapreve-treated patients.

Our data indicate that passive immunization effectively inhibits the cellular immune response to active vaccination.

Conclusions: The timing of active and passive immunizations to homologous antigen in the same patient requires consideration. The benefit of pre-or post-exposure prophylaxis by neutralizing antibodies must be weighed against the risk of ineffective vaccination.

Hema.to

Karsten Miermans

hema.to GmbH, Munich, Germany

We’ve built hema.to to improve blood cancer diagnostics from flow cytometry data. hema.to is a CE-marked web-application that gives diagnostic recommendations in seconds from the raw .fcs or .lmd files, and can integrate into clinical workflows without SOP changes or disruptions. This is made possible by using modern machine learning technology that combines data from all laboratories. This way, smaller sites can learn from larger, more specialized hematological laboratories. hema.to grew out of a cooperation with the Münchner Leukämielabor (MLL) and has been shown to provide expert-level recommendations in a large prospective trial. Estimates indicate that the workflow can be sped up by ca. 5x, which would help clinical laboratories deal with an increasing diagnostic volume even in the current lab staff shortage crisis. In our most recent (four-center) clinical trial, we demonstrated that hema.to accelerated the diagnostic analysis by >2x and reduced variability by 15%. We’re currently commercially integrating hema.to into the first laboratories for routine use for B-cell Non-Hodgkin Lymphoma. Our final aim is twofold. First, we want to support existing flow cytometry workflows by reducing the need for subjective human input, which greatly accelerates and objectifies the analysis. Second, we want to use machine learning to extract novel insights from immunophenotyping data, for example to detect which cell populations have a strong impact on clinical endpoints. Taken together, we want to use our combined expertise in deep learning, flow cytometry and medical software development to improve patient care.

Multiparametric mass cytometry data analysis in the search for the chronic lymphocytic leukemia cell of origin

Mikulová, Antonia[1], Vondálová Blanářová, O.[1], Plešingerová, H.[1,2], Stančíková, J.[3], Stuchlý, J.[3], Kalina, T.[3], Kotašková, J.[4], Arpáš, T.[2], Bryja, V.[1] and Janovská, P.[1]

[1]Laboratory of Cell Communication, Department of Experimental Biology, Masaryk University, Brno, Czech Republic
[2]Centre of Molecular Biology and Genetics, IHOK, University Hospital Brno, Brno, Czech Republic
[3]Childhood Leukaemia Investigation Prague (CLIP), Department of Pediatric Hematology and Oncology, 2nd Medical School of Charles University, Prague, Czech Republic
[4]Medical Genomics, Central European Institute of Technology (CEITEC), Brno, Czech Republic

Chronic lymphocytic leukemia (CLL) is a lymphoproliferative disease characterized by clonal expansion of CD5+ B cells which can be found in peripheral blood, lymph nodes and bone marrow. It is the most common leukemia in the western countries and despite the effort dedicated to the research of CLL, only little is still known about the origin of the disease. CLL cells almost uniformly express surface receptor tyrosine kinase-like orphan receptor (ROR1). It is also true for clonal B cell subsets in monoclonal B cell lymphocytosis (MBL), stage preceding CLL, where cells are also ROR1+. In contrast, only 1-5% of B cells in peripheral blood of healthy young individuals is ROR1-positive.

Our aim is to characterize ROR1+ subsets of B cells in peripheral blood of healthy donors and define their connection to MBL and CLL cells. To achieve that, we have used flow cytometric and mass cytometric approaches to measure expression of selected markers on B cells from peripheral blood of healthy donors (including multiple samples enriched for ROR1+ B cells), patients with MBL and CLL. Subsequent analysis of B cell subpopulations allowed us to characterize ROR1+ subsets and their similarities with MBL and CLL cells. Mass cytometric data using 30 markers were employed to reconstruct the CLL progression in pseudotime using topological data analysis tools available in tviblindi package, with the main aim to elucidate whether any ROR1+ population from healthy individuals B cells precedes acquisition of MBL and CLL cell phenotype.

High dimensional spectral flow cytometric analysis of human blood and tissue correlates

Michael Müller

Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie, Hans-Knöll-Institut, Jena, Germany

We use a 5L Cytek Aurora Analyzer and Aurora CS with a 40-colour surface marker panel to characterize the immune system in patient samples. Our focus lies on tissue resident T cell populations within different tissues and different diseases. The setup and application of this panel on several tissues is challenging because the different isolations techniques have an impact on marker expression. With the combination of spectral flow cytometry and single cell transcriptomics we want to identify mechanisms of tissue specific immune homeostasis and immune abrogation.

Viscoelastic characterization of biological cells in hyperbolic microfluidic channels

Felix Reichel

Max Planck Institute for the Science of Light, Erlangen, Germany

Research over the last decades revealed that single-cell mechanical properties can serve as label-free markers of cell state and function and that mechanical changes are a sign of alterations in the cell's molecular composition. This led to the development of a number of microfluidics tools to rapidly measure the deformability and also the viscoelastic properties of cells. The quantification of the stresses, that cause the deformation of the cells in these channels, is often challenging and with that the derivation of a stress-strain relation for such a system becomes complex. Here, we used hyperbolic channels to create an extensional flow field where the acting stresses can be measured using calibration particles and yield a simple relationship between acting stress and resulting cell strain. We then used the setup to measure the Young's modulus and bulk viscosity of HL60 cells over a wide range of time scales. Our simple setup offers a straightforward measurement of the viscoelastic properties of cells, soft particles and possible also liquid-liquid phase separated droplets.

Functional CyTOF profiling of SARS-CoV-2-specific T cells induced during B-cell depletion

Axel Ronald Schulz[1*], Addi J. Romero-Olmedo[2*], Svenja Hochstätter[3], Sebastian Ferrara[1], Dennis Das Gupta[2], Iiris Virta[1], 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. Mei1[#]

[1] German Rheumatism Research Center, Berlin, Germany
[2] Institute of Medical Microbiology and Hospital Hygiene, Philipps-University Marburg, Marburg, Germany
[3] Institute of Virology, Philipps-University, Marburg, Germany
[*, #] equal contribution of first and senior authors

T-helper cells are known regulators of B-cell responses, but whether and how B cells influence T cells is less clear. Here we longitudinally investigated spike-specific CD4 T cells in a B-cell-depleted unvaccinated survivor of severe COVID-19, using single-cell flow and 48-plex mass cytometry, to determine the quantity, durability, and quality of the CD4 T-cell response under conditions of induced B-cell deficiency. Specific CD4 T cells were identified as CD40L+ CD137+ T cells after overnight stimulation with a SARS-CoV-2 spike peptide pool. Four age-, gender- and infection-severity-matched controls and one age-matched vaccinated control were analyzed for comparison.

Our results show remarkably high frequencies of approximately 1% of spike-specific CD4 T cells in the B-cell-depleted COVID-19 survivor, which were exceptionally stable over the 5-month observation period. B cells returned seven months after recovery, and the patient was vaccinated with BNT162b2, which further increased specific T-cell frequencies. Mass cytometry of spike-specific CD4 T cells revealed combinatorial expression of 14 cytokines. Boolean analysis in recovered patients identified a predominant Th1 response characterized by combinations of TNF, IFNγ, GM-CSF and/or IL-21. Notably, the BNT162b2 vaccination induced additional Th2 cells and hybrid Th1/2 cells, expressing combinations of IL-4, IL-5, and IL-13, along with a combination of Th1 cytokines, respectively. This indicates a multifaceted T-cell response with a broadened effector range after BNT162b2 vaccination in a previously recovered individual. While the T-cell-cytokine combinome revealed rather minor differences between the B-cell depleted and the control subjects, the high frequency and persistence of peripheral spike-specific CD4 T cells was unmatched versus controls and published cohorts.

Within the limitations of a single-patient analysis, our results indicate that relatively normal spike-specific CD4 T cells develop when B cells are depleted in a primary infection, although the absence of B cells appears to be compensated by a massive expansion of the spike-specific CD4 T-cell compartment. In summary, we here combined sensitive antigen-specific T-cell detection with high dimensional mass cytometry to provide insight in the quantitative and qualitative features of antigen specific T-cell responses, serving a blueprint for functional T cell studies in infection, vaccination and autoimmunity.

IgA-Seq enhances discrimination between Crohn's Disease patients and healthy controls, but IgA1- and IgA2-bound microbiota do not differ significantly

Toni Sempert[1,2], Lisa Budzinski[1], René Maier[1], Victoria von Goetze[1], Amro Abbas[1], Gi-Ung Kang[1], Leonie Lietz[1,2], Robin Kempkens[1], Janine Büttner[3], Bettina Bochow[3], Anja Schirbel[3], Benjamin Moser3, Hyun-Dong Chang1,2

[1]Deutsches Rheuma-Forschungszentrum Berlin – A Leibniz Institute, Berlin, Germany
[2]Department for Cytometry, Institute of Biotechnology, Technische Universität Berlin, Berlin
[3]Medizinische Klinik mit Schwerpunkt Hepatologie und Gastroenterologie, Charité-Universitätsmedizin Berlin, Berlin, Germany

Crohn's Disease (CD) shows alterations in immune response and composition of intestinal microbiota. Previous studies reported different effector functions of IgA1 and IgA2 on immune tolerance or inflammation (Steffen et al. 2020, Michaud et al. 2022). Here, we apply multi-parameter microbiota flow cytometry (mMFC) to identify and isolate bacterial populations coated by host IgA1 and IgA2 on single-cell level comparing Crohn's Disease patients to healthy controls. We can show that the majority of bacteria coated with IgA are coated by both IgA1 and IgA2. CD patients have a higher frequency of IgA1- and IgA2-bound bacteria compared to healthy controls, while the amount of IgA per cell is not significantly different. Full-length 16S rRNA gene sequencing shows that bacterial composition differs between CD patients and controls (Bray-Curtis beta diversity). The distinction between CD patients and controls is enhanced when analyzing IgA-coated bacterial only. Our data indicate that (1) there is little difference between the specificity of intestinal IgA1 and IgA2 regardless of disease state and that (2) CD patients show a pattern of IgA-coated bacteria which differs significantly from healthy controls. Thus, multi-parameter microbiota flow cytometer combined with 16S rRNA gene sequencing is as a powerful tool to obtain disease-specific signatures and to better describe microbial composition regarding host-microbiota-crosstalk.