The many faces of Cytometry

26th Annual Conference of the German Society for Cytometry

Berlin, DGfZ
October 5 – 7, 2016

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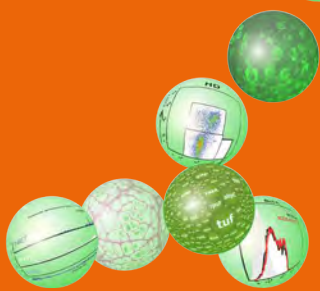

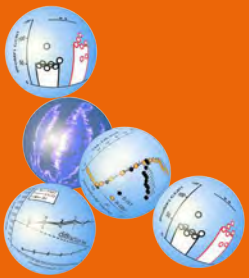

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The many faces of Cytometry

Dear friends,

It´s a great pleasure for me to welcome you again in Berlin to the 26th Meeting of the German Society for Cytometry (DGfZ). This year we are coming together to highlight "The many faces of cytometry".

I am looking forward to an inspiring program and in-depth discussion between scientists, technologists and industrial partners. Each session will cover attractive and innovative topics as nanotechnology, microbiology, microscopy and immunology.

We follow the new tradition started last year to dedicate a session to a European partner society. I am happy to announce the European Guest Session from the Czech Society for Analytical Cytometry.

Besides our scientific sessions our industrial sponsors will present their newest innovations and products within three minutes during a product slam. Last year this public event elicited extremely positive resonance. Furthermore we expect an innovative industrial exhibition that everyone is encouraged to visit. At this point I would like to thank the industrial sponsors for their generous financial support to make this meeting possible.

I wish you all an exciting meeting and a pleasant stay in the heart of Berlin.

Yours,

Hyun-Dong Chang

President DGfZ

Content

Program Overview

Wednesday, October 5, 2016

9:00am - 12:00pm **Core Managers Meeting**
Paul-Ehrlich Lecture Hall DRFZ
Chair: Elmar Endl

9:00am - 12:00pm **Tutorials: "Data Analysis"**
DRFZ, Seminar Room 3

1:00pm - 1:15pm **Welcome**
Paul-Ehrlich Lecture Hall
Hyun-Dong Chang

1:15pm - 2:45pm **Session 1: European Guest Session, Czech Society for Analytical Cytometry**
Paul-Ehrlich Lecture Hall
Chairs: Tomas Kalina, Hyun-Dong Chang

Tomas Kalina	CD Maps – Antigen Density Measurements of CD1-CD100 on Human Leukocytes
Karel Soucek	Fluorescence and cell cycle: Consequences for some flow cytometric approaches
Ondrej Pelák	Lymphocyte enrichment using CD81 targeted immunoaffinity matrix for CyTOF experiments
V. Pospíchalová	Dedicated flow cytometry: a tool for analysis of exosomes and microvesicles
Michaela Nováková	Implementing hyperspectral cytometry into immunological monitoring

2:45pm - 4:15pm **Session 2: Nanotechnology**
Paul-Ehrlich Lecture Hall
Chairs: Ulrike Taylor, Wolfgang Fritzsche

Annette Kraegeloh	Focus on Nano Cell Interactions
Dominic Docter	Impact of the nanomaterial biomolecule corona for biomedical applications

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Daniela Tiedemann Porcine gametes and embryos: screening tool for nanoparticle-cell-interaction

4:15pm - 5:00pm **Coffee Break** **Atrium Cross Over (CCO)**

5:00pm - 6:30pm **Session 3: Product Slam**
Paul-Ehrlich Lecture Hall
Chairs: Frank Schildberg, Elmar Endl

Selected industrial partners will present their newest innovative technological developments and products

6:30pm - 7:30pm **Keynote**
Paul-Ehrlich Lecture Hall
Chair: Andreas Radbruch

Tim Mosmann T Cells: From Simplicity to Complexity

7:30pm - 10:30pm **Welcome reception at the industrie area**
Atrium Cross Over (CCO)

Thursday, October 6, 2016

9:00am - 10:30am **Session 4: Technological advances for microbial single cell characterization**
Paul-Ehrlich Lecture Hall
Chairs: Christin Koch, Frank Schmidt

Wei Huang Label-free detection of single cell phenotype using Raman activated cell sorting

David Berry Single cell isotope probing and sorting via Raman microspectroscopy: A new approach for functional analyses of microbes in environmental and medical samples

Yuting Guo Heterogenic response of prokaryotes towards silver nanoparticles and ions

10:30pm - 11:30pm **Poster Session A and Coffee Break**
Atrium Cross Over (CCO)
Chairs: Torsten Viergutz, Wolfgang Beisker

11:30pm - 12:30pm **Session 5: Emerging Technologies**
Paul-Ehrlich Lecture Hall
Chairs: Wolfgang Beisker, Stephan Schmid

Presentation of novel technologies by industry and academic partners

Stefan Frischbutter Application of high throughput flow cytometry for the identification of novel immunomodulators

Christoph Herold Flow cytometry for cell mechanics

Michael Kapinsky A Web-based Software Concept To Support High Content Cytometry Antibody Panel Design

Konrad v. Volkmann Quantitative comparison study of flow cytometers using a novel ultra-stable calibration light source

12:30pm - 1:30pm Lunch Atrium Cross Over (CCO)

1:30am - 3:00pm **Session 6: Microscopy**
Paul-Ehrlich Lecture Hall
Chairs: Anja Hauser, Raluca Niesner

Ben Judkewitz Deep imaging with time-reversed light

Rainer Heintzmann Lightwedge and Lightsheet-Raman Microscopy

R. Palankar Direct Measurement of Rupture Force between Human Blood Platelets at different Degrees of Activation by Single Cell Force Spectroscopy

Daniel Schulz Dissecting the heterogeneity of murine mesenchymal bone marrow stromal cells

3:00pm - 4:00pm **Poster Session B and Coffee Break**
Atrium Cross Over
Chairs: Torsten Viergutz, Wolfgang Beisker

4:00pm - 4:30pm **Session 7: Klaus-Goerttler-Session**
Paul-Ehrlich Lecture Hall
Chair: Hyun-Dong Chang

J. L. Schmid-Burgk A FACS sorting-based genome-wide CRISPR screen identifies NEK7 as a novel genetic component of inflammasome activation

4:30pm - 5:30pm **Guest Lecture**
Paul-Ehrlich Lecture Hall
Chair: Hyun-Dong Chang
Introduction by Hyun-Dong Chang

Jens Krause Collective behaviour and collective intelligence

5:30pm - 7:00pm **Members Assembly**
Paul-Ehrlich Lecture Hall
Chairs: Wolfgang Fritzsche, Hyun-Dong Chang

8:00pm - 11:00pm **Conference Dinner - Arminius Markthalle, Berlin-Moabit**

Friday, October 7, 2016

9:30am - 11:00am **Session 8: Cutting Edge**
Paul-Ehrlich Lecture Hall
Chairs: Elmar Endl, Thomas Kroneis

Elena Levashina	Flow Cytometry Applications in Vector Biology
Randolph B. Caldwell	Flow Assisted Protein Engineering Technology
Bodo Kohring	The application of the Countstar Fluorescence (FL) in the quality monitoring of stem cells
Kristen Feher	A novel method for characterising cell properties based on pulse shapes

11:00am - 11:30am **Coffee Break** **Atrium Cross Over**

11:30am - 1:00pm **Session 9: Immunology**
Paul-Ehrlich Lecture Hall
Chairs: Gergely Toldi, Alexander Scheffold

Birgit Sawitzki	Implementation of flow cytometry in multi-center clinical trials - strategies and results from the ONE Study
Wolfgang Uckert	Designer T cells for cancer immunotherapy
Richard Addo	FACS sorting and Next Generation Sequencing of murine Bone marrow stromal cells.
Andrej Mantei	Development of a protocol for discrimination of latent and active tuberculosis infection by antigen-specific T cell activation and flow cytometry

1:00pm - 1:30pm **Farewell & Brezels** **Atrium Cross Over**

German Society for Cytometry (DGfZ)

The Society of Cytometry (Gesellschaft für Zytometrie, GZ) was founded in 1989 in Heidelberg (Germany) by the Foundation Council represented by Ceses Cornelisse, Georg Feichter, Wolfgang Goehde, Klaus Goerttler, Holger Hoehn, Andreas Radbruch, Peter Schwarzmann, and Günter Valet. An association was born dedicated to provide an interdisciplinary platform for interested scientists in the field of flow and image cytometry. Founding and current members are scientists whose personal scientific development was and is still closely interlinked with the development of cytometric technologies in Europe.

www.dgfz.org

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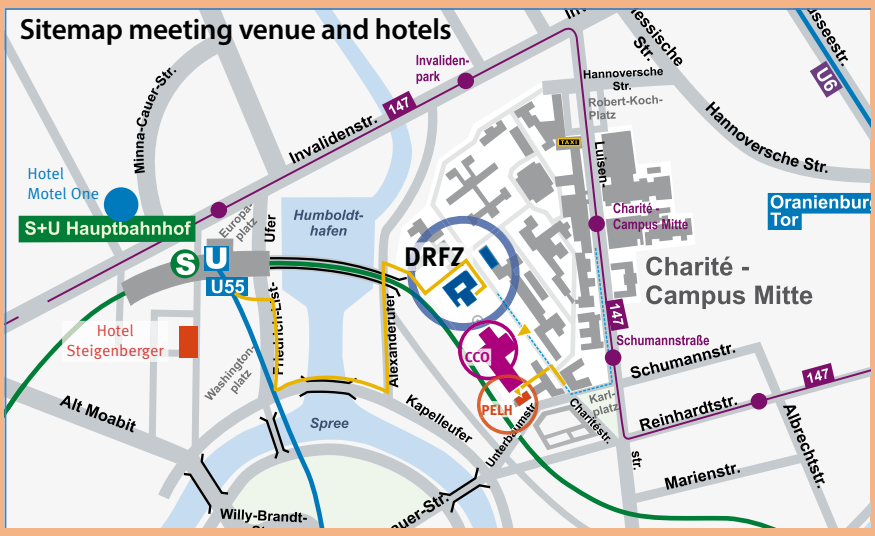
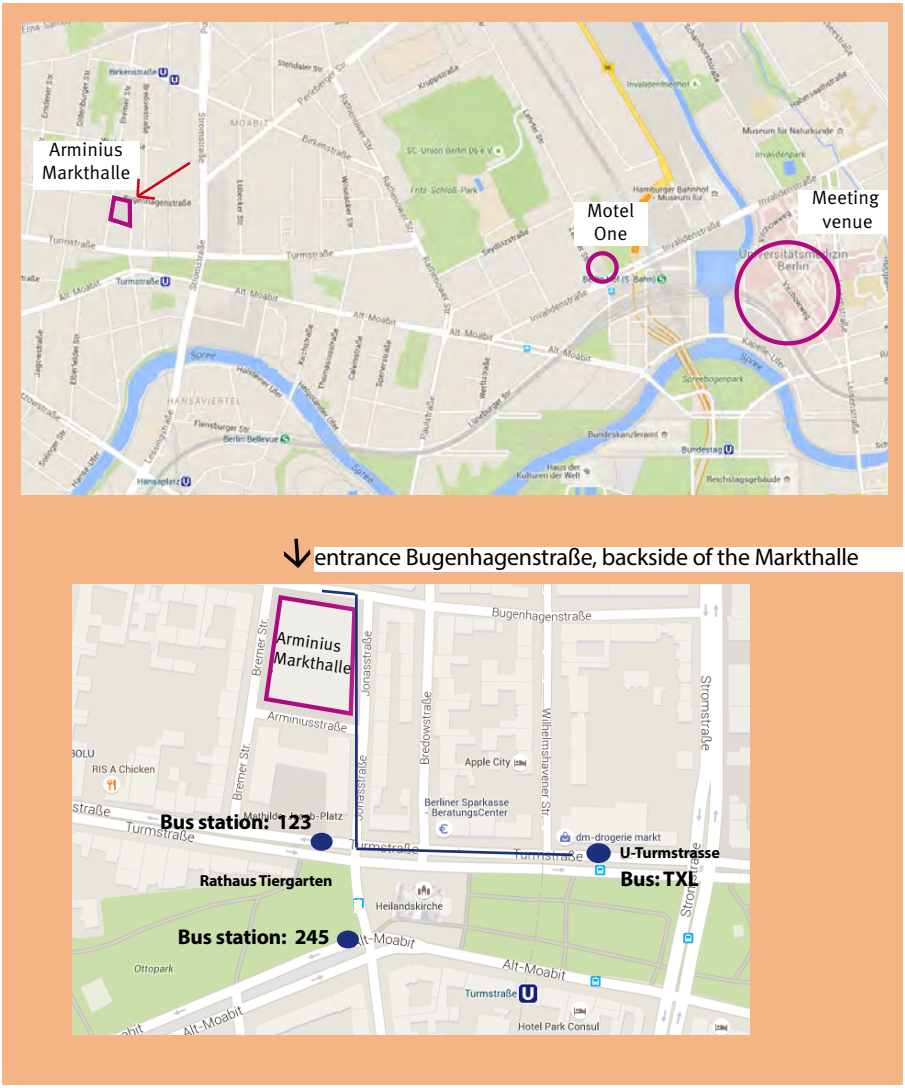


Charité Campus Mitte, Charitéplatz 1, 10117 Berlin, Germany
Campus addresses: CCO: (Charité CrossOver) Virchowweg 6
DRFZ: (Deutsches Rheuma-Forschungszentrum Berlin) Virchowweg 12
PEHL (Paul Ehrlich Lecture Hall): Virchowweg 4

Conference dinner

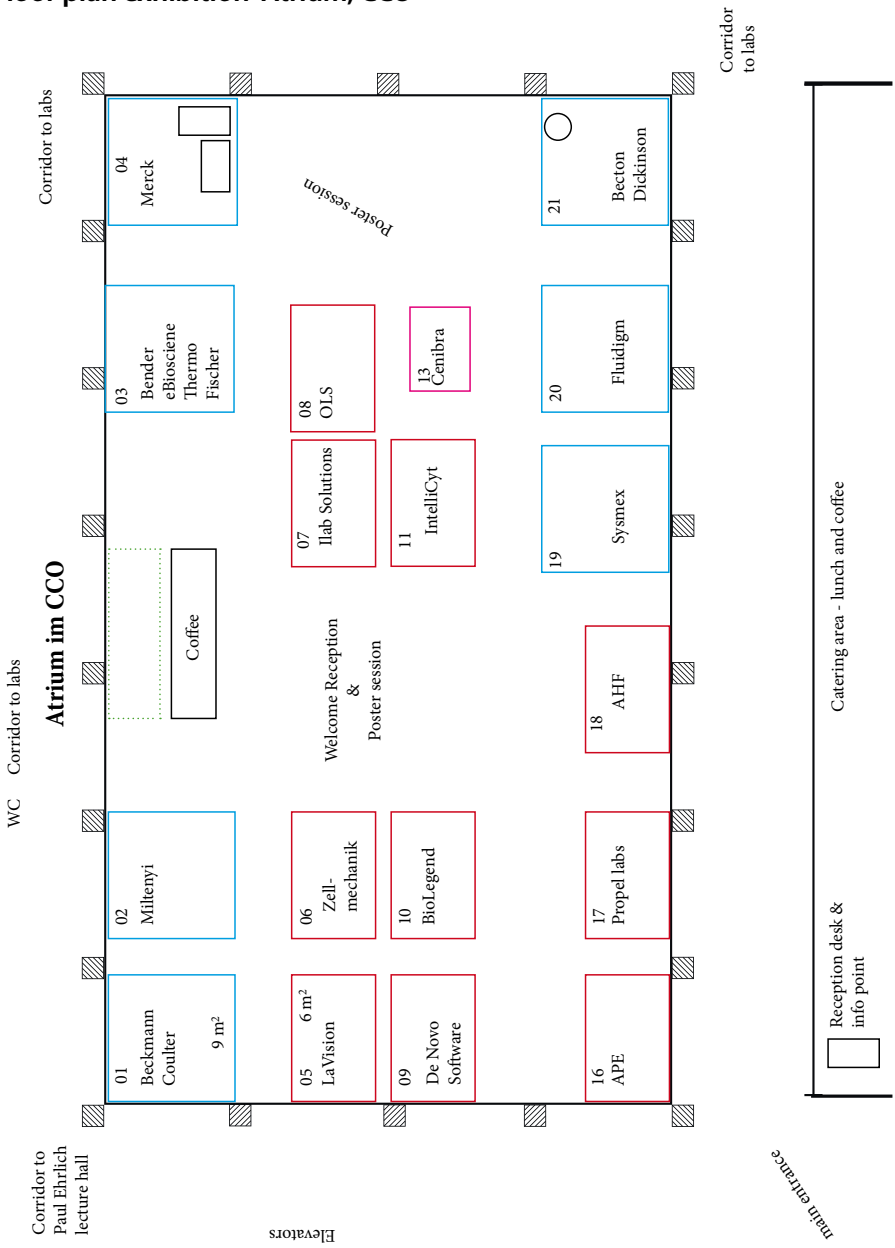
Date: on Thursday, October 6, 2016

To get to the conference dinner venue, please take the bus line "123" from "Hauptbahnhof" to "Rathaus Tiergarten" or the TXL to U-Turmstraße or the Bus 245 to Rathaus Tiergarten.
Then it is just a short walk to the Arminius Markthalle. Entry is on the back side of the Markthalle.



Arminius Markthalle, Eingang Bugenhagener Straße 18-34

Floor plan exhibition Atrium, CCO



We are most grateful to our Sponsors and Exhibitors

The exhibition is open every day

Wednesday, October 5, 2016

Core Managers Meeting

9:00am - 12:00pm Paul-Ehrlich Lecture Hall

Chair: Elmar Endl, Bonn

Keywords:
Core Facilities Survey Roundup, Network Tools and Socialising,
National Flow Cytometry Network Think Tank, Software and Management Tools.

Tutorials: Data Analysis

9:00am - 12:00pm Seminar room 3, DRFZ

Chair: Christin Koch, Leipzig

9:00	Welcome/Introduction
9:15 - 10:15	Susann Günther
10:15 - 10:30	Short break
10:30 - 11:30	Kristen Feher
11:30 - 12:00	Open for free training, discussiont

Session 1: European Guest Session, Czech Society for Analytical Cytometry

1:15pm - 2:45pm Paul-Ehrlich Lecture Hall

Chair: Tomas Kalina, Prague

Chair: Hyun-Dong Chang, Berlin

The Czech Society for Analytical Cytometry (CSAC) was established in 2001, providing a lively platform for scientific exchange during biannual meetings attended by over 200 scientists, and offering several educational workshops annually.

This session will provide a cross section of the cytometry applications within the Czech cytometry community. Overview of the CD Maps project of HCDM (Human Cell Differentiation Molecules) given by Tomas Kalina will present an effort to map the CD1 to CD100 across the range of all leukocyte subsets in the blood, thymus and tonsil. Changes in autofluorescence levels during cell cycle progression will be presented by Soucek. Alternative to ficoll separation using immunoaffinity matrix and its detailed performance will be shown by Pelak. Detailed and dedicated approach to analysis of microvesicles and exosomes will be delivered by Pospichalova. Finally, initial experience with the hyperspectral cytometry employed for immune monitoring will be introduced by Novakova.

Next conference of the CSAC will be held in Prague in September 2017, see http://www.csac.cz/en-conferences-CSACmeeting.html for more details.

CD Maps – antigen density measurements of CD1-CD100 on human lymphocytes

Tomas Kalina (CZ)

Department of Paediatric Haematology and Oncology, 2nd Faculty of Medicine, Charles University Prague , Czech Republic;
Charles University Prague, Second Faculty of Medicine, Czech Republic

Over the past 4 decades, many human leucocyte receptors have been characterized using antibodies validated by the human leucocyte typing HLDA/HCDM organization (www.hcdm.org). With rapid developments in immunology, a lot of available information is outdated or incomplete. Thus, HCDM has initiated a CD Maps project that aims at quantitative mapping expression of all CD molecules across the spectrum of leucocyte subsets.

Using the standardized approach developed by the EuroFlow consortium, we measured PE conjugated CD1-CD100 antibodies in the context of four 8-color panels on cells from three tissues (blood, thymus and tonsil). We used Quantibrite PE beads to quantify the number of PE molecules detected.

Initial analysis of 16 lymphocyte subsets in blood and 9 in thymus from 8-12 donors revealed that the top expressed molecules are CD45 (120,000 molecules in T-cells vs. 47,000 in B-cells) and CD44 (78,000 vs. 47,000). In the thymus the highest expression is that of CD99 (165 000 molecules in the DN stage).

Comparison between naive and memory T lymphocytes showed that the largest increase in expression (>20 fold) is observed in CD45RO, CD95 and CD49e in both CD4 and CD8 T-cell subsets; and CD11b and CD57 in CD8 T-cells. In contrast, CD62L, CD45RA, and CD27 were significantly decreased. Amounts of CD95, CD71, CD27, CD11b, CD54 and CD62L were significantly higher on memory than naive B cells.

The CDMaps project will generate a broad and updated online database containing the expression profiles of all CD markers on human leukocyte subsets present in blood, tonsil, and thymus. Quantitative information on receptor expression is important for mechanistic studies as well as flow cytometric panel design and design of novel biological therapeuticals. Therefore this database will serve as a useful resource to widen and advance studies into basic, translational and clinical immunology.

CD Maps project is supported with reagents by BD Biosciences, BioLegend and Exbio. TK is supported by Ministry of Health Czech Republic grant 15-26588A and LO1604.

Fluorescence and cell cycle: Consequences of gating approaches in flow cytometry

Karel Soucek (CZ)

Department of Cytokinetics, Institute of Biophysics of the CAS, v. v. i., Brno, Czech Republic;
Center of Biomolecular and Cellular Engineering, International Clinical Research Center, St. Anne's University Hospital Brno, Brno, Czech Republic;
Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic

Flow cytometry is valuable method for analysis of single cells and their separation. However, in many cases its application requires careful consideration. In particular existence of intrinsic cell background fluorescence and its dependence on cell size might affect some types of analyses and lead to the data misinterpretation. In our work we showed that distribution of the signal of both intrinsic and specific fluorescence is affected by cell cycle. We demonstrated in many experimental models and conditions that low percentile of fluorescence signal distribution is enriched mostly by the cells in G1 phase, on the other hand high percentile represents the cells in S - G2/M phase. We also show that interpretation of the data acquired based on "high vs. low" gating and subsequent sorting of this way defined fractions of cells could be misleading through significant influence of cell cycle distribution and/or cell size. Therefore researchers must be aware of the effects of cell size and/or cell cycle distribution on their flow cytometric data.

This work was supported by grant 15-11707S from Czech Science Foundation, grants 15-28628A and 15-33999A from the Grant Agency for Health Research of the Czech Republic, by project LQ1605 from the National Program of Sustainability II (MEYS CR), and by the project ICRC-ERA-Human Bridge (No. 316345) funded by the 7th Framework Programme of the European Union and by project HistoPARK – Centre for analysis and modeling of tissues and organs (Reg. no.: CZ.1.07/2.3.00/20.0185).

Lymphocyte enrichment using CD81 targeted immunoaffinity matrix for CyTOF experiments

Ondrej Pelák (CZ)

Department of Paediatric Haematology and Oncology, 2nd Faculty of Medicine, Charles University Prague , Czech Republic

Mass cytometry is a powerful new platform that enables single-cell analysis of more than 100 different parameters simultaneously. This unique feature is redeemed by its higher sample demands. In mass cytometry, the isolation of pure lymphocytes is very important in order to obtain reproducible results and to shorten the time spent on data acquisition. To prepare highly purified cell suspensions of peripheral blood lymphocytes for further analysis on mass cytometer, we used the new CD81+ immune affinity chromatography cell isolation approach, which uses low affinity reversible capture of target cells on an agarose beads matrix. Using 21 metal conjugated antibodies in a single tube we were able to identify all basic cell subsets and compare their relative abundance in final products of seven different donors obtained in parallel by density gradient (Ficoll-Paque) and immune affinity chromatography (CD81+ T-catch™) isolation approach. We show that T-catch™ isolation approach results in purer final product than Ficoll-Paque (p-value 0.0156), with less platelets bound to target cells. As a result acquisition time of 105 nucleated cells was 3.5 shorter. We then applied unsupervised high dimensional analysis viSNE algorithm to compare the two isolation protocols, which allowed us to evaluate the contribution of unsupervised analysis over supervised manual gating. ViSNE algorithm effectively characterized almost all supervised cell subsets. Moreover, viSNE uncovered previously overseen cell subsets and showed inaccuracies in Human peripheral blood phenotyping panel kit recommended gating strategy. These findings emphasize the use of unsupervised analysis tools in parallel with conventional gating strategy in order to mine the complete information from a set of samples. They also stress the importance of the impurity removal to sensitively detect rare cell populations in unsupervised analysis.

Dedicated flow cytometry: a tool for analysis of exosomes and microvesicles

Vendula Pospíchalová (CZ)

Faculty of Science, Masaryk University, Czech Republic

Exosomes and microvesicles are nanosized extracellular vesicles that were recently identified as important modes of intercellular communication as well as high potential diseases biomarkers and therapeutic targets.

Flow cytometry is a powerful method, which is widely used for high-throughput quantitative and qualitative analysis of cells. However, its straightforward applicability for extracellular vesicles, and mainly exosomes, is hampered by several challenges reflecting mostly small size of these vesicles (exosomes: ~80–200 nm, microvesicles: ~200-1,000 nm), their polydispersity and low refractive index. Dedicated flow cytometers are devices especially designed for analysis of small particles, such as bacteria, viruses or extracellular vesicles. Dedicated flow cytometry yields a physical principle, that small particles scatter more light by diffraction than reflection and refraction. Thus these cytometers build on different optical settings, powerful lasers and highly sensitive detectors.

Unlike the widely used alternative, which relies on floatation in sucrose gradient for exosome isolation and an optimized and manually adjusted version of commercial high-end cytometer, our protocol provide novel and fast approach for quantification and characterization of both microvesicles and exosomes by utilizing dedicated flow cytometer, which can be used without adjustments prior to data acquisition.

Extracellular vesicles can be fluorescently labeled with protein- (CFSE) and/or lipid- (FM) specific dyes, without the necessity to remove unbound fluorescent dye by ultracentrifugation. Additionally, double labeling with protein- and lipid- specific dyes enables to separate extracellular vesicles from common contaminants of sample preparations, such as protein aggregates or micelles formed by unbound lipophilic styryl dyes, thus not leading to overestimation of their numbers. Moreover, our protocol is compatible with antibody labeling using fluorescently conjugated primary antibodies.

Our methodology thus opens possibility for routine quantification and characterization of extracellular vesicles from various sources. Finally, it has the potential to bring desired level of control into routine experiments and non-specialized labs.

Implementing hyperspectral cytometry into immunological monitoring

Michaela Nováková (CZ)

CLIP-Dpt. Of Pediatric Hematology/Oncology, 2nd Faculty of Medicine, Charles University in Prague, Prague, Czech Republic;
Second Medical Department, University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Germany

Immunophenotyping by flow cytometry is an important method for diagnosis and monitoring of disorders of the immune system. Currently, deeper understanding of lymphocytes composition and their functional characteristics is needed in order to reveal abnormalities within immune system.

Therefore a number of analyzed markers has increased in past few years, which was primarily achieved by an increased number of stained tubes. However, this approach does not offer the complex analysis and simultaneously increases demands on quantity of material used. This is limiting the analysis in patients with immune disorders, who often have low cell count blood samples both at diagnosis and during the treatment.

Recently new methods offering complex analysis appeared: mass and spectral cytometry. Spectral cytometry has an advantage of no extra demands on reagents in comparison with conventional flow cytometry, more importantly has no increased cell loss, which has been reported in processing sample for mass cytometry measurement.

We introduced a hyperspectral flow cytometry panel for immunological monitoring in these patients. The patient group consists of patients with various immune disorders (e.g. CTLA-4 mutation) mainly treated with different immunosuppressive/immunomodulatory drugs (e.g. mTOR inhibitor). The aim was to analyze changes in lymphocyte subpopulation composition together with the analysis of proliferation and activation. After primary testing of optimal composition of the panel and conjugation antibodies with spectrally unique fluorochromes we developed a final version of 13-color panel, which was measured in 24 samples of 9 patients or controls.

We gained reproducible data in patient samples showing that spectral cytometry is mature enough to supplement conventional flow cytometry data. Nowadays we are in a testing phase of extended panel with the aim to reach 19 simultaneously stained markers.

Supported by Ministry of Health of the Czech Republic, grant nr. 15-26588A, grant nr. 15-28525A, GAUK 802214, UNCE 204012, OPPK CZ.2.16/3.1.00/21540, OPPK CZ.2.16/3.1.00/24505

Session 2: Nanotechnology

2:45pm - 4:15pm Paul-Ehrlich Lecture Hall

Chair: Ulrike Taylor, Hannover

Chair: Wolfgang Fritzsche, Jena

The interaction of nanomaterials with organisms is a field of growing interest due to the increasing use of such materials in a wide field of applications ranging from surface coatings over cosmetics to nanomedicine including both diagnostics and therapy. The session deals with this interaction on the level of cells and tissues, including some background on nanoparticles, approaches to evaluate their potential toxicity, and novel techniques with potential for nanomedicine.

Focus on Nano Cell Interactions

Annette Kraegeloh, Saarbrücken

INM - Leibniz-Institute for New Materials

Nanomaterials and nanoparticles exhibit a high application potential in technical fields but especially in biomedicine. The dimensions of nanoobjects together with other properties are the basis for their intended or even undesired effects on living organisms and cells. The latter are promoted by a) specific entry pathways and distribution within the body and tissues, b) uptake into single cells and specific intracellular distribution, and c) a high chemical reactivity, influencing dissolution, molecule adsorption or agglomeration. To take this even further, the composition and morphology of nanoobjects, their surface properties as well as the composition of the surrounding media influence the interactions of nanoobjects with molecules and living entities. The detection and localization of nanoobjects in the cellular environment or even within cells is one prerequisite to understand their interaction mechanisms and effects.

Fluorescence microscopy is a versatile tool for the detection of nanoparticles as well as cellular structures. It can be applied for analysis of nanoparticle internalization pathways, intracellular distribution and localization as well as intracellular transport of nanoparticles, especially fluorescently labeled ones. Due to the small size of nanoscale objects, STED microscopy -as superresolution technique- has the potential to contribute to a detailed analysis of nanoparticle cell interactions, in addition to other microscopy techniques.

Several examples are given, addressing applications of fluorescence and STED microscopy for analyses of nanoparticle cell interactions. Fluorescently-labeled silica nanoparticles in a size range of 15-130 nm were prepared and applied for most of the studies.

Using live-cell microscopy, the association of nanoparticles with various types of vesicles within A549 cells was shown. STED microscopy was used to quantify the internalization of silica nanoparticles into A549 cells, allowing for a comparison between extracellular and intracellular nanoparticle numbers.

Using Caco-2 cells, the intracellular distribution and time-dependent migration of internalised nanoparticles towards the nuclei were analysed. STED microscopy revealed that 32 nm nanoparticles entered the nuclei and formed agglomerates within this compartment. In contrast, larger, 83 nm nanoparticles were not detected within the nuclei. Recent investigations showed that differentiated Caco-2 cells did not internalise quantum dots. In addition, the results indicated that these nanoparticles do not seem to translocate across the intact cell layer.

Current investigations target the internalisation of nanoparticles by hepatocytes, applying a three-dimensional cell-culture model.

Porcine gametes and embryos: screening tool for nanoparticle-cell-interaction

Daniela Tiedemann, Hannover

Unit of Reproductive Medicine, University of Veterinary Medicine Hannover, Germany;
Institute of Farm Animal Genetics, Friedrich-Loeffler-Institut, Mariensee, Germany

The increasing use of nanoparticles (NPs) in consumer products and their application as imaging agents or vectors is due to their unique and tunable properties. These properties are the result of their small size and comparatively high surface area. NPs can be characterized by material composition, size, surface coating, functionalization, and concentration. Once introduced to biological systems, these characteristics determine the interaction of cells and NPs. The effects vary from inertness to highly toxic behavior. Further, NPs have the ability to cross biological barriers (testis, brain) and accumulate in certain tissues. Predicting and influencing this behavior is still fairly difficult, because only little is known about the mechanisms of nanoparticle-cell-interaction.

The goal of the presented study was to gain reliable and comparable information about the effects of NPs on reproductive cells and functions. The use of porcine gametes and embryos as a screening tool makes it possible to observe the influence of NPs on very sensitive cell functions of primary cells. Additionally, reproductive function and embryonic development are very important parameters for toxicity assessment, because negative influence can effect following generations.

To achieve this goal, NPs of different material (gold: AuNP, gold-silver-alloy: AuAgNP, silver: AgNP, nickel-titanium-alloy: NiTiNP, chromium-nickel-iron-alloy: CrNiFeNP; all coated with bovine serum albumin (BSA)) were introduced to oocytes during *in vitro* maturation (46h, 38.5°C) and to ejaculated sperm (2h, 37°C). None of the tested NPs had an influence on sperm vitality (motility, membrane integrity, morphology). The maturation of the oocytes was significantly reduces by AuAgNPs with a high silver content and AgNPs (only 12% mature oocytes compared to 77% in the control group). Interestingly, this negative effect could be reduced by using a different conjugation method of NPs and BSA. Confocal microscopy revealed the uptake of large amounts of AuNPs into the cytoplasm of the oocyte during maturation, while NPs with silver content accumulated in the surrounding cumulus cells. In vitro fertilization in the presence of AuNPs did not result in different fertilization rates (pronuclear formation) or cleavage rates (2-cell-stage). Oocytes which had AuNP contact during maturation were also fertilized *in vitro* and cultured to the blastocyst stage. The blastocyst rate was not affected. Transfer of some of these embryos to recipient sows in a small study lead to the birth of live piglets. They showed no impairment of health or vitality and developed normally until weaning.

The conducted experiments have shown that reproductive functions can be impaired by NPs. Especially *in vitro* oocyte maturation has proven to be a viable tool for reprotoxic screening and observing nanoparticle-cell-interaction.

Impact of the nanomaterial biomolecule corona for biomedical applications

Dominic Docter, Mainz

University Medical Center, Germany

Besides the wide use of engineered nanomaterials (NM) in technical products, their applications are not only increasing in biotechnology and biomedicine, but also in the environment. Whereas the physico-chemical properties and behaviour of NM can be characterized accurately under idealized conditions, this is no longer the case in complex physiological or natural environments. Here, proteins and other biomolecules rapidly bind to NM, forming the protein/biomolecule corona critically affecting the NM' (patho)biological and technical identity (Fig. 1) [1-6]. The biomolecules-coated nanomaterials can be considered as new materials compared to the pristine nanomaterials during their manufacturing. As most biological systems are (highly) dynamic, a time-resolved knowledge of particle-specific protein fingerprints is required to understand the corona evolution, enabling predictions, prevention or rational enforcement of nanoparticle-induced (patho)physiological effects, including nanotoxicology. As the corona impacts *in vitro* and/or *in vivo* NM applications in humans and ecosystems, a mechanistic understanding of its relevance and the biophysical forces regulating corona formation is mandatory.

Based on recent insights, we here critically present an updated concept of corona formation and evolution. We comment on how corona signatures may be linked to effects at the nano-bio interface in physiological and environmental systems. In order to comprehensively analyse corona profiles and to mechanistically understand the coronas' biological/ecological impact, we present a tiered multidisciplinary approach. To stimulate progress in the field and we introduce the impact of the corona for NM-microbiome-(human)host interactions. We conclude by discussing relevant challenges, which need to be resolved by the field.

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[4] Docter D. et al. (2014), Nature Protocols, Sep;9(9):2030-44.

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[6] Tenzer S. and Docter D. et al. (2011). ACS Nano, 5, 7155-7167.

Session 3: Product Slam

5:00pm - 6:30pm, Paul-Ehrlich Lecture Hall

Chair: Frank Schildberg, Boston

Chair: Elmar Endl, Bonn

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Keynote: "Meet the Expert"

6:30pm - 7:30pm Paul-Ehrlich Lecture Hall

Chair: Andreas Radbruch, Berlin

Speaker: Tim Mosmann, Rochester/USA

Every immunologist knows his discovery together with Dr. Robert Coffman: the TH1 and TH2 subsets of T lymphocytes. This was a crucial event to understand the regulation of immune responses and disease pathogenesis. Dr. Tim Mosmann is one of the 0.5% of all publishing researchers in science that are called "highly cited".
Currently Tim Mosmann and his colleagues use multicolor flow cytometry and Spot assays to define the precise T cell phenotypes induced by vaccination, infection and allergy in infants, adults and the elderly. He defined an uncommitted CD4 T cell population that rapidly produces IL-2, but does not differentiate into either TH1 or TH2 cells. Recently, the challenges of interpreting high-dimensional flow data have led him to collaborations on algorithmic analysis of this data, resulting in a suite of programs for pre-processing, cluster identification and facilitated exploration of flow data. In turn, the computational work has led to a heightened appreciation of diversity within cell populations, and suggested alternative ways to look at cell differentiation.
Tim Mosmann is Director of the Human Immunology Center (HIC), and Director and Michael and Angela Pichichero Director's Endowed Chair of the David H. Smith Center for Vaccine Biology and Immunology.
He studied Chemistry and Physiology, and Microbiology, at the University of Natal and Rhodes University in South Africa. He obtained his Ph.D. in Microbiology at the University of British Columbia/Canada. After research fellowships at the University of Toronto and University of Glasgow and assistant professorship at the University of Alberta he spent eight years in industry, as a research scientist at DNAX Research Institute in Palo Alto, California. Afterwards he went back to University of Alberta. In 1998, he was recruited to the University of Rochester.

Tim Mosmann was honored with the William B. Coley Award, Avery-Landsteiner-Prize,Paul-Ehrlich-and-Ludwig-Darmstaedter-Prize and the Novartis Prize for Basic Immunology.

T cells: From Simplicity to Complexity

Tim Mosmann

University of Rochester Medical Center, Rochester, USA

The development of high-throughput bioassays, allied with the identification of cytokines by molecular cloning, led to the discovery of functional T cell subsets expressing separate cassettes of cytokines. Initial results identified just two effector types, and even this division was considered to be 'too much complexity' by some researchers. These two types were the main recognized subsets for some years, partly because there was a good 'fit' between Th1 cells and delayed hypersensitivity or 'cellular' reactions, and between Th2 cells and antibody responses (although the Th2 response was actually a better fit with allergic responses). Several years later, several labs showed that CD4 T cells were much more complex, by discovering several additional states expressing relatively stable effector functions, matched with important biological responses. Even the idea of defined T cell subsets with specific effector function cassettes was then thrown into doubt, with the discovery in several labs that most or all 'defined' T cell subsets had the ability to further differentiate into other states.

Meanwhile the ability to resolve different cell subsets had been improving markedly, particularly by multi-parameter flow cytometry, measuring up to 35 markers or more with several modern technologies. This in turn created a strong need for advanced algorithmic methods for analyzing this highly complex data. We developed a flow cytometry cell population clustering tool, SWIFT, that separates sub-populations with high resolution, and provides a detailed map of the cell populations, thus beginning to exploit the rich data in flow cytometry files.

Because the complexity of this clustered data is still high, we have also produced visualization and exploration tools that facilitate the interpretation of the quantitative clustering results, and allow comparisons and inferences to be made between groups of samples.

The 'clouds' of cells representing each of these clusters in multi-dimensional space focused attention on an issue that has been perplexing for some time – populations of cells behave predictably, yet individual members of a sub-population can vary by about ten-fold in the expression of each of many markers. To reconcile this cellular diversity with predictable differentiation behavior, we developed a visualization tool that represents cells as balls rolling across a landscape under the influence of differentiative forces, as well as variable amounts of stochastic 'noise'. These models suggest explanations for some biological behaviors, such as the importance of fringe populations that are better positioned (with a higher probability) to explore new states. Thus to predict biological effects, the spread of values around the population median may be more important than the median itself. Thus our models of CD4 T cell effector diversity have moved from a uniform 'helper' function, through the Th1/Th2 dichotomy, the addition of other subsets, and the plasticity of each subset, to a model that includes these states as nodes on a complex landscape in which probability influences the behavior of individual cells, while regulatory networks exert homeostatic forces that define population trends rather than linear, inevitable pathways.

Thursday, October 6, 2016

Session 4: Technological advances for microbial single cell characterization

9:00am - 10:30am Paul-Ehrlich Lecture Hall

Chair: Christin Koch, Leipzig

Chair: Frank Schmidt, Greifswald

Technological advances have significantly enhanced the application range of high resolution single cell analysis techniques for microbiological questions. The session will introduce current technologies like single cell isotope probing, single cell based Raman microspectroscopy or the combination of those techniques with modern OMICs technologies and cover examples from biotechnological applications as well as functional characterization of complex natural microbial communities. Future trends and limitations will be in the focus of discussion.

Single cell isotope probing and sorting via Raman microspectroscopy: A new approach for functional analyses of microbes in environmental and medical samples

David Berry, Vienna, Austria

University of Vienna, Austria

Using Raman microspectroscopy, microbiologists can within seconds obtain an „optical fingerprint" of a single living microbial cell that contains an astonishing amount of information regarding its chemical composition. When combined with the use of stable isotope labeled substrates or deuterated water, this technique offers a direct means to identify microbial cells performing a defined physiological activity within complex samples, which can subsequently be sorted for cultivation or single cell genomics. Using examples from environmental and medical microbiology, the potential of single cell stable isotope probing by Raman microspectroscopy as well as Raman-activated cell sorting in capillaries and microfluidic devices will be demonstrated.

Label-free detection of single cell phenotype using Raman activated cell sorting

Wei Huang, Oxford, UK

University of Oxford, United Kingdom

Single cell Raman spectra (SCRS) are phenotypic fingerprints of cells which can be used to characterise cell type, physiological state and functionalities. We have developed Raman activated cell sorting (RACS) and Raman activated cell ejection (RACE) techniques, which

sort cells according to their Raman spectra. Raman-based cell sorting will be useful to study single cells in their native states and pave the way for single cell '-omics' including genomics, transcriptomics, proteomics and metabolomics.

Reference:
Song, Y., Yin, H. and Huang,W.E.* (2016) Raman activated cell sorting. Current opinion in Chemical Biology. 33: 1-8.

Wang, Y., Huang, W.E.,* Li, C. and Wagner, M. (2016) Single cell stable isotope probing in microbiology using Raman microspectroscopy. Current opinion in Biotechnology 41:34-42.

McIlvenna, D., Huang, W.E., Davison, P.A., Glidle, A., Cooper, J.M. and Yin, H. (2016) Continuous cell sorting in a flow based on single cell resonance Raman spectra. Lab on a Chip 16:1420-1429.

Berry et al. (2015) Tracking heavy water (D2O) incorporation for identifying and sorting active microbial cells. Proc Natl Acad Sci U S A 112:E194-203.

Zhang et al. (2015) Raman-activated cell sorting based on dielectrophoretic single-cell trap and release. Anal Chem 87:2282-2289.

Huang et al. (2004) Raman microscopic analysis of single microbial cells. Anal Chem 76: 4452-4458.

Heterogenic response of prokaryotes towards silver nanoparticles and ions

Yuting Guo

Yuting Guo, Hans J. Stärk, Hauke Harms, Lukas Y. Wick, Susann Mülle, UFZ, Germany

Using Raman microspectroscopy, microbiologists can within seconds obtain an „optical fingerprint" of a single living microbial cell that contains an astonishing amount of information regarding its chemical composition. When combined with the use of stable isotope labeled substrates or deuterated water, this technique offers a direct means to identify microbial cells performing a defined physiological activity within complex samples, which can subsequently be sorted for cultivation or single cell genomics. Using examples from environmental and medical microbiology, the potential of single cell stable isotope probing by Raman microspectroscopy as well as Raman-activated cell sorting in capillaries and microfluidic devices will be demonstrated.

Postersession Track A

Auditorium Cross Over

Chair: Torsten Viergutz, Dummerstorf
Chair: Wolfgang Beisker, München

10:30am - 11:30am Poster Session A

A01 Longitudinal Imaging of Germinal Centers

Randy L. Lindquist1, Carolin Ulbricht1, Anja E. Hauser2

1DRFZ, Berlin, Germany; 2Charite Universitätsmedizin, Berlin, Germany

Intravital imaging, the only way to study the dynamics and interactions between immune cells in situ, has revealed the cellular dynamics of developing immune reactions and of steady-state immunosurveillance in both lymphoid and non-lymphoid tissues. However, the preparations commonly used are too invasive to be survival procedures, which limits them to one imaging session per animal. A less invasive preparation that permitted longitudinal imaging of the same region would allow us to follow the evolution of immune responses over time, greatly enhancing *in vivo* studies.

The plasticity of the immune system can be used to generate such a preparation: lymph nodes transplanted to an orthotopic site induce lymphangiogenesis, to integrate the transplanted lymph node into the lymphatic system of the host. Once engrafted, the normal lymphoid architecture is maintained, and is capable of supporting immune reactions. Transplantation of a LN to a superficial site in the skin permits multiphoton imaging through the skin, without the surgical exposure necessary for typical intravital imaging. This transforms imaging of LNs into a survival procedure and permits repeated imaging of the same lymph node, without surgical preparation that causes inflammation and alters the process we wish to image.

We are adapting this system to longitudinally image various signalling processes along with the development of clonality over the course of the GC reaction. Specifically, we are investigating how the dynamics of calcium signalling in GC B cells relates to affinity maturation and selection. Another question we are focusing on is the dynamics of antibody diversification within GCs, using a Brainbow-based lineage-tracing model in which individual B cells are marked by a unique combination of fluorescent proteins. This enables us to follow the progression to monoclonality in individual GCs within the same LN over time.

A02 Longitudinal Intravital Imaging of the Retina for Early Diagnosis in Chronic Inflammation and Neurodegenerative Diseases

Daniel Bremer[1], Florence Pache[2], Alexander Brandt[2], Friedemann Paul[2], Anja E. Hauser[3], Helena Radbruch[4], Raluca A. Niesner[1]

[1]German Rheumatism Research Center Berlin, Germany; [2]NeuroCure Clinical Research Center, Charité; [3]Intravital microscopy and immunedynamics, Charité; [4]Neuropathology, Charité

A hallmark of autoimmune retinal inflammation is the infiltration of the retina with cells of the innate and adaptive immune system. In worst cases, the inflammatory processes can lead to detachment of the retinal layers and to retinal tissue injury, e.g. loss of the retinal photoreceptor layer. These local autoimmune processes can be investigated using non-invasive longitudinal imaging of the eye, which, as a window to the central nervous system (CNS), also reflects

inflammatory processes within the CNS. Histological studies in murine neuroinflammatory models, such as experimental autoimmune uveoretinitis (EAU) and experimental autoimmune encephalomyelitis (EAE) indicate that immune infiltration is initialized by effector CD4+ T cells, with the innate compartment (neutrophils, macrophages and monocytes) contributing crucially to the tissue degeneration that occurs in later phases of the disease.

However, how an immune attack is orchestrated by various immune cell subsets and how the latter interact with the target tissue during the course of disease *in vivo* is poorly understood. Our study addressed this gap with a novel setup for intravital two-photon microscopy of the retina, used to repeatedly visualize and track CD4+ T cells and LysM phagocytes in the entire retina over the course of EAU. The repeated retina imaging allowed us to monitor the dynamic modifications of the microglial network induced by the inflammatory processes. Thanks to the new technology, non-invasive correlation of clinical scores of CNS-related pathologies with longitudinal immune infiltrate behavior, as well as tissue dysfunction on a cellular basis, is now possible for the first time, paving the way for deeper insights into the pathology of neuroinflammatory processes over the entire disease course.

A03 Platelets bridge between the innate and adaptive immune system in bacterial host defense via platelet factor 4 and FcγRIIA

Raghavendra Palankar1, Thomas P. Kohler[2], Nikolay Medvedev[1], Sven Hammerschmidt[2], Andreas Greinacher[1]

[1]Department of Transfusion Medicine, University Medicine Greifswald, Germany; [2]Department Genetics of Microorganisms, Interfaculty Institute for Genetics and Functional Genomics, University of Greifswald, Greifswald, Germany

Introduction:Platelets contribute to innate immunity. They interact directly and indirectly via with bacteria. Here we addressed the role of the chemokine platelet factor 4 (PF4) and human platelet FcγRIIA in bacterial recognition and killing using biomimetic and live bacterial micropatterned arrays

Methods:Platelet activation on 2D planar and biomimetic bacteria-like micropatterns or E. coli wild-type (WT) BW30270 and mutants KPM53 (ΔwaaC)/KPM121 (ΔwaaA) expressing truncated LPS on lithographically micropatterned arrays were coated with IgG, PF4, or PF4/polyanion (P)-IgG complexes. These E.coli show enhanced PF4-binding activity (WT<ΔwaaC<ΔwaaA) (1). Coating was quantified by live imaging in the presence or absence of platelet receptor or cytoskeletal inhibitors. FcγRIIA - and antibody-dependent bacterial killing by platelets was assessed by quantiative fluorescence microscopy and co-culture experiments.

Results:Platelets adhered and spread on planar micropatterned arrays functionalized with IgG [% micropatterned area covered 60.23% ±13.1 mean (SD)] and aggregated IgG [83.41%±11.36]. FcγRIIA blocking mab IV.3 reduced ($P < 0.0001$) platelet spreading on IgG [3.39% ±3.35] and aggregated IgG [11.41%±3.96]. Similar, blocking of αIIbβ3 or the use of cytochalasin D and blebbistatin reduced platelet spreading. Biomimetic bacteria-like microbead arrays functionalized with IgG, aggregated IgG, PF4/P or anti-PF4/PIgG complexes inhibited platelet spreading as well. In the presence of anti-PF4/P-IgG, platelets were able to kill E.coli strains

within two hours, directly dependent on the bacterial PF4 binding capacity (up to 65.4 ±6.3% killing rate). This killing was inhibited by mab IV.3 blocking FcγRIIA.

Conclusions:The chemokine PF4 binds to polyanions on bacteria thereby enabling opsonization by anti-PF4/P IgG, which in turn mediates killing of E.coli via FcγRIIA. Because PF4 binds to Gram-negative and but also to Gram-positive like staphylococci, preformed anti-PF4/P IgG rapidly recognizes even bacteria which the host has never recognized before. Thus, this IgG-mediated innate immune defense mechanism may also relevant to control bacterial infections. Furthermore, a combination of lithography, quantitative fluorescence microscopy and live bacterial micropatterned arrays in combination can be used to understand the host-pathogen interactions and antimicrobial mechanisms of cells of the immune system.

A04 Silver nanoparticles for the specific detection of cellular antigens in mass cytometry

Axel Ronald Schulz, Silke Stanislawiak, Sabine Baumgart, Andreas Grützkau, Henrik E. Mei

Deutsches Rheumaforschungszentrum Berlin, Germany

The development of mass cytometry (CyTOF technology) has pioneered a new era of multiparametric single-cell analysis. Cells and cellular networks can now be studied at an unprecedented depth and complexity. Current instrumentation allows for the theoretical detection of up to 135 parameters, but the availability of metal tags suitable for labeling specific probes limits analyses to currently ~40 parameters, curtailing exploitation of the full analytical capacity of mass cytometers.

We explored the application of elemental silver nanoparticles (AgNP) for reporting specific cellular targets on human lymphocytes in a mass cytometry assay. We demonstrate the successful use of streptavidin-coated AgNP of different sizes for the specific detection of human cell surface antigens such as CD4, CD8, CD45RO, CD16 and CD244 via biotinylated primary antibodies. Signal intensities elicited by cell-bound AgNP were higher than conventional lanthanide-based probes and improved substantially detection of the low abundant antigen CD25. Further, AgNP were compatible with standard mass cytometry staining protocols for PBMC and lyzed whole blood and showed low background signals in control assays. The detection of AgNP, containing both 107Ag and 109Ag at natural abundance, did not overlap or interfere with the detection of any other metal isotopes and further development may lead to the application of isotopically purified AgNP.

Collectively, usage of AgNP extends the analytical capacity of mass cytometry staining panels by one, prospectively two, additional parameters, well suitable for cellular targets of low abundance.

A05 Mastering the challenges of fluorescence intensity calibration

Thomas Thiele[1], Heike Borcherding[1], Peter Carl[2], Uwe Schedler[1]

[1]PolyAn GmbH, Germany; [2]Bundesanstalt für Materialforschung und –prüfung (BAM), Germany

The calibration of fluorescence signals in assays as well as in biological systems is a key requirement. Especially for quantitative studies of living cells, e.g. expression of biomolecules the generation of concentration-proportional analytical statements obtained by the fluorescence intensity plays a significant role. This also applies for cell assays like immunofluorescence assays. Also, the determination of local concentrations of target proteins within cells or within their environment is a major challenge in modern biology.

Proper calibration of the fluorescence imaging systems is the prerequisite to ensure that test results from different instruments and different test environments are comparable. The existing calibration solutions for fluorescence imaging systems, however, mostly depend on simple instrument calibration without reference to absolute physical standards or with insufficient stability for multiple measurements. In order to obtain the necessary quantitative information, it is required to calibrate both instruments and the corresponding assays, ideally with one calibrating system.

We present several new calibration tools for the most commonly used platforms for cytometry, i.e. fluorescence microscope based systems and flow cytometry based systems. They are comprised of ultra-stable fluorophores that are encapsulated in bio- and cell-compatible polymer matrices. Each tool is tailored to the specific requirements of the different read-out platforms. Both re-usable single-color calibration slides for fluorescence microscopes as well as new multi-color calibration beads are presented.

The traceability of the calibration tools to international standards and the wide calibration range of the fluorescence intensity are illustrated. They are thus suitable for calibration of the signal over concentration and quantum yield to that of the targets.

A06 Denitrifying electroactive microbiomes – understanding functions and process improvement

Christin Koch[1], Narcís Pous[2], Sebastià Puig[2], Susann Müller[1], Falk Harnisch[1]

[1]Helmholtz Centre for Environmental Research - UFZ, Germany; [2]LEQUiA, University of Girona, Spain

Denitrifying bioelectrochemical systems are a promising technology for nitrate removal from wastewaters and polluted groundwaters. Continuous microbial community monitoring is required for a better understanding and improvement of the process. In this study, flow cytometry was applied to monitor and determine the structure–function relationship of the microbiome of a denitrifying biocathode under different experimental conditions.

A 1L-biocathode denitrifying bioelectrochemical reactor was built. The biocathode was filled with granular graphite and poised to a potential of -320mV (vs. Ag/AgCl). It was fed with 200 ppmN-NO3- at 0.5 L·day-1 and removal efficiency (NO3-, NO2- and N2O), electrochemical performance as well as microbial dynamics were monitored regularly. The applied stress-tests shifted the reactor microbiome and the denitrification performance and revealed functional relevant subcommunities. Combined with cell sorting and molecular analysis a key role for Thiobacillus sp. in the bioelectrochemical reduction of nitrate was suggested.

Bioelectrochemical enrichment studies of Thiobacillus sp. revealed further denitrification mechanisms. Based on cyclic voltammetry, it was demonstrated that nitrate and nitrite reduction proceed at –0.30 V and –0.70 V (vs. Ag/AgCl) by surface associated extracellular electron transfer sites.

In summary, this study provides an excellent example how intensive monitoring of microbial community structure and bioelectrochemical activity combined with mechanistic understanding can be used to improve bioreactor performance in general and especially denitrification in bioelectrochemical systems.

Pous, N., Koch, C., Vilà-Rovira, A., Balaguer, M. D., Colprim, J., Mühlenberg, J., Müller, S., Harnisch, F., Puig, S., Monitoring and engineering reactor microbiomes of denitrifying bioelectrochemical systems, 2015, RSC Adv. 5, 68326-68333.

Pous, N., Koch, C., Colprim, J., Puig, S., Harnisch, F., Extracellular electron transfer of biocathodes: revealing the potentials for nitrate and nitrite reduction of denitrifying microbiomes dominated by Thiobacillus sp., 2014, Electrochem. Commun. 49 , 93 - 97.

A07 Evaluating the stability of microbial community with flow cytometry

Zishu Liu, Nicolas Cichocki, Fabian Bonk, Johannes Lambrecht, Susanne Günther, Hauke Harms, Florian Centler, Susann Müller

Helmholtz-Zentrum für Umweltforschung GmbH - UFZ

Quantitative cytometrically based information on natural or engineered microbial community dynamics are used to describe, interpret and predict community behavior. Core concepts from macro-ecology that determine diversity and stability indices of populations are adapted to microbiology. Mainly next generation sequencing data (NGS) are used for such approaches. However, PCR based technologies are limited in this regard due to the usual small sampling frequency, complex preparation procedures and intrinsic features such as susceptibility to misconducted data processing. Especially in a real biotechnological process, in nature, or in case of human microbiomes the time for a complete assessment (weeks to months) would not mirror microbial community variations which occur within minutes or hours, depending on the cells generation time. As a consequence, there is a growing interest for high-resolution methods, which cost less and which are fast in sample assessment. Microbial flow cytometry can be used for this purpose.

Here, we propose a workflow for the evaluation of cytometric community fingerprinting data which allows by calculation, comparison and selection, to evaluate community stability. The definition of the term stability comprises definitions and formula for the terms resistance, persistence, and resilience. The results of stability assessment strongly suggest that dissimilarities in structures of sub- communities caused by perturbations could be used to predict the further community behavior.

We present a fast and robust analysis method for quantifying the stability of natural or engineered microbial communities. Our approach may allow nearly on-line monitoring of microbial community stability in future and thus contribute to fast evaluation pipelines and decision making.

A08 Decreased percentage of natural-Tregulatory cells in typte 2 diabetes patients

Faheem shahzad, Nadeem Afzal, Khursheed Javaid, Waqas Latif, Foqia Naz, Abu Zafar

University of Health Sciences Lahore pakistan, Pakistan

Type-2 diabetes mellitus (T2DM) is not an autoimmune disease but various complications during the course of disease includes production of autoantibodies. These autoantibodies indicate dysregulation of immune mechanisms. T regulatory cells (Tregs) are key players in the maintenance of immune regulation, therefore a study was designed to determine the level of these cells in T2DM patients. This study included 84 subjects. Subjects without diabetes were labelled as group-I (n=28), group-II (n=28) T2DM patients who had diabetes of -5 years duration, and group-III (n=28) T2DM patients with more than 5 years of diabetes. Tregs were enumerated by FACScalibur flow Cytometer (BD) by using anti-CD4, anti-CD25, anti-CD45 and anti-FOXP3 antibodies. Data was analysed using SPSS-20.0. Highest mean ± SD of percentage of nTregs was observed in group-I (5.6±2.8), followed by group-III (.7±.5) and group–II (.4±.7).

On comparison, a statistically significant difference was observed among the three groups (p=0.000), between group-I and group-II (p= 0.000) and between group I and group-III (p= 0.000). There was no significant difference between group-II and group-III. No correlation was observed between the percentage of nTregs and age of the subjects and it was concluded that the "Percentage of nTreg was decreased in patients of T2DM as compared to healthy controls".

A09 Characterization of bovine neutrophil oxidative burst and phagocytic activity during transition period – Effects of parity and Vitamin E and/or CLA supplementation

Jana Frahm, Stephanie Schäfers, Ulrich Meyer, Susanne Kersten, Sven Dänicke

Friedrich-Loeffler-Institute, Germany

OBJECTIVE The main objective of this study was to evaluate the impact of parity and supplementation of exogenous antioxidants on peripartal polymorph nuclear cell (PMN) function. The transition period defines critical weeks around parturition, is described by negative energy balance, caused mainly by the onset of lactation, and a state of immunosuppression characterized by an impairment of neutrophil phagocytosis and killing capacity. PMNs are important cells at the first line of innate immune reactions and generate large quantities of oxygen radicals when activated, which is required for the efficient destruction of pathogens and signaling cascades mediating immune responses. Previous studies have been hypothesized that higher incidences of peripartum health disorders in dairy cows are associated with oxidative stress during the transition period, which is possibly leading to dysfunctional immune cell reactions.

EXPERIMENTAL DESIGN AND METHODS This study investigates the oxidative burst and phagocytic activity of bovine PMN during periparturient period with or without supplementation of 2,327 IU vitamin E/d (VitE, BASF Lutavit® E 50) or 8.4g trans-10, cis-12 and 8.4 cis-9, trans-11 conjugated linoleic acid/d (CLA, BASF Lutrell®) or both. Six weeks prior to calving, fifty-eight healthy multiparous German Holstein cows were divided into four dietary groups with VitE and/or CLA or without supplementation. Furthermore, for each feeding group cows were allocated on the basis of parity: 2nd calving (n=34) and more than 2nd calving (n=24). Cell functional assays were carried out at fifteen times around calving and early lactation. The production of reactive oxygen species (ROS) in unstimulated or phorbol myristate acetate (PMA)-stimulated PMN were analyzed in whole blood samples using DHR-assay and flow cytometry. Additionally, phagocytic activity was tested with fluorescein isothiocyanate (FITC)-labelled opsonized bacteria E. coli and the percentage and phagocytic activity of PMN which have ingested bacteria was measured by flow cytometry.

RESULTS Our results show alterations in the steady-state production of oxygen radicals in untreated PMNs of all feeding groups during transition period. The basal ROS generation differs according to parity and supplementation. Untreated neutrophils of cows of the 2nd parity are significantly affected by VitE around calving contrary to cells of cows' higher parity. In cows of higher parity, the VitE supplementation caused a higher efficiency of oxidative burst activity in neutrophils treated with PMA around calving, whereas the proportion of activated PMNs remains constant. Determining the phagocytic activity, a time-dependent progression was shown in all groups with calving being a distinct event.

CONCLUSION The multifactorial nature of almost all metabolic disorders in dairy cows around

calving makes it necessary to discuss this situation from different perspectives. Our results suggest that this complexity is mainly affected by age-depending cellular immune functions. There are also indications that the cellular responsiveness and adaptation to physiologic processes is differently influenced by nutritional supplementation and age.

A10 Addressing the Analysis Bottleneck of Large Multiparameter Data: ForeCyt Software Combines Rapid Acquisition Times with Plate Level Analytics, Reducing the Time to Actionable Results

Miguel Jimenez

IntelliCyt Corporation, United States of America

Traditional flow cytometry, while powerful, has seen limited adoption in cell-based screening laboratories. Limitations in speed of data acquisition, and large sample volume requirements, coupled with lack of software visualization and analysis tools for mining large amounts of high content data poses a significant challenge for large, combinatorial flow cytometry experiments. IntelliCyt's iQue Screener PLUS platform addresses these limitations and provides rapid, high content per-cell analyses of suspension cells in 96 well- (in < 5 min), 384 well- (in <20 min), and 1536 well- (in < 60 min) formats. ForeCyt software provides visualization capabilities such as multiplexed hit finding based on user-defined criteria using profile map, dose response curves, and heat maps in addition to the standard flow cytometry per-cell analysis tools for gating and statistical analysis. To demonstrate the rapid, versatile workflow of the iQue Screener platform, we highlight a 9-color PBMC-immunophenotyping screen to quantify regulatory T-cell and T-helper cell induction in 96-well plate format. Staphylococcal enterotoxin B (SEB) was utilized as a positive assay control to induce expression of the CD127low, CD25+ Treg cells and HLA-DR+ T-helper cell populations. Additional treatments on the plate were comprised of randomly spiked wells with various combinations of SEB and/or phytohemagglutinin (PHA) at various concentrations to simulate compound "hits" of different intensities. Cells were directly plated into 96 well plates and treated with compounds for 24 hours at 37°C, 5% CO2. After incubation, an antibody cocktail containing CD3, CD4, CD8, CD45RA, CD45RO, CD62L, CD127, CD25, and HLA-DR was directly added to each well of the 96 well plate. After staining was complete, data was acquired on the iQue Screener PLUS. The total run protocol to acquire all 96 samples was 6 minutes, with an average of ~6000 cells per well (~3000 CD3+ T-cells for this particular donor). Individual plots demonstrated strong staining and excellent resolution of positive and negative signals for all the tested markers. In addition, ForeCyt software enabled the use of templated analyses, which facilitated the generation of heat maps and dose response curves (for the SEB positive control). Plate level analytics are seen via the profile maps, which quickly identify wells that meet pre-defined criteria and help to aggregate information across the multi-parameter data set. For this analysis, the profile map was used to identify positive hits as those wells with (1) high Treg cell (%), (2) high HLA-DR+ T helper cells (%), and (3) low cell toxicity. These results demonstrate that the iQue Screener platform not only overcomes the limitations of traditional flow cytometers for throughput, but also reduces the analysis bottleneck and quickly provides actionable results from high-content, multiplex assay screens.

Session 5: Emerging Technologies

11:30am - 12:30am Paul-Ehrlich Lecture Hall

Chair: Wolfgang Beisker, München

Chair: Stephan Schmid, Regensburg

Since its first development in the 60ties, cytometry was always driven either by the technology of the instrument and/or by the development of new drugs and compounds to measure new and more parameters of cells and particles at the same time. From light scatter to immunofluorescence, from analyzing the physics of light emission to high speed cell sorting up to cytometric mass spectroscopy, the technical development droves the science and vice versa.

An increasing number of parameters in combination with higher speed of analysis and/or cell sorting has brought the attention again to the need for a precise quantification of light in the cytometer. New instruments come up on the commercial market every year. The determination of its sensitivity and quality poses a permanent challenge. An even more, the necessary day to day calibration of instruments in the cytometric laboratory needs clear quantification techniques.

The development of software to facilitate the insight in the large universe of data has been of increasing importance in the last years. Only with the appropriate software, the user is able to extract the information he wants from his experiment. The ease of handling the data and the clear graphic representation of results is essential. Software can help to design panels of antibodies for high content cytometry, to allow its design even for less experienced users.

The number of parameters in cytometry, such as light scatter, fluorescence or physical properties of cells has been increased over the last 20 years. Today, up to 28 colors can be measured with specially designed instruments. However, their application strongly depends on the capability of the user to find its way through the jungle of wavelengths and dyes.

The treatment of autoimmune diseases requires the identification of immunomodulatory agents by multiparameter cell analysis combined with a high throughput regime in cytometry. Thousands of samples per day for testing immune cells against libraries of small molecules have to be handled and inspected. Instrumentation as well as software are designed to help the development of new therapeutic approaches.

Since the "old days" of cytometry, data about the physical structure of single cells have been collected. The approach to measure morphological changes of cells in a label free situation could supply some "extra" information, if labeling is not possible for various reasons. May be, because the number of available emission and excitation wavelengths is already exhausted or because biological reasons prevent the application of fluorescence markers. The deformability of cells can be measured in real time, allowing a label-free characterization of blood subpopulations. Different cell cycle stages show variations in these mechanical parameters, which are different from what has been done up to today.

For all all these approaches the combination of basic science and industrial applications give a new insight in a field, which may be the basis of cytometry in the near and farer future. The session will provide an insight in exciting new fields, which are on the brink to be used in every day scientific instrumentation for cytometry.

Application of high throughput flow cytometry for the identification of novel immunomodulators

Intellicyt, Stefan Frischbutter

Treatments of autoimmune diseases like Rheumatoid Arthritis, Multiple Sclerosis, or Type I Diabetes

mainly target cells or inflammatory processes involved in disease progression but in most cases a lifelong treatment is required to prevent disease relapse. However, constant immunosuppression increases the risk of severe side effects like infections or cancer. Therefore, elaborated immunmodulatory therapies are needed that very specifically target mechanisms responsible for disease progression or yet more important strengthen endogenous regulatory elements of the immune system like regulatory T cells (Tregs) to re-establish immune balance.

To follow the latter approach, a high throughput screening assay was developed to monitor compound depended increase of Treg frequencies. Primary immune cells from mice containing green fluorescent protein (GFP) reporter for Foxp3 (master transcription factor of Tregs) were cultured in the presence of compounds from a library consisting of 40.000 small molecules (FMP Berlin). Reporter expression in CD4+ T cells and compound toxicity was analyzed using a high throughput flow cytometry platform capable of handling 384 well plates allowing a daily throughput of more than 3000 samples. Samples were analyzed by specialized flow cytometric analysis software allowing quick batch analysis of whole plates for overall assay performance, Z-Factor, and Hit threshold calculation as well as zooming into individual wells for detailed analysis and hit selection.

Setting a threshold of 3σ according to untreated controls the screen resulted in 846 hits from which 32 compounds were selected for further validation. Dose response analysis finally revealed one compound reproducibly increasing Foxp3+ T cell frequencies. Additionally, subsequent studies with primary human PBMC´s showed downregulation of the proinflammatory cytokines IL-4, IL-6, IL-17, IFN-y, and TNFa in the presence of the hit compound.

The combination of multiparameter cellular analysis and high throughput in a straightforward measurement and data analysis workflow allows for design of various screening assays enabling the identification of immunomodulatory agents useful to study immunological processes and might help the development of novel therapeutic approaches.

FLOW CYTOMETRY FOR CELL MECHANICS

Zellmechanik Dresden, Christoph Herold

The cytoskeleton is the fundamental, dynamic, filamentous network in every biological cell. It constitutes a link between the morphological phenotype of the cell and its biological (dys-) function. For example, cells become softer during certain malignant transformations, change stiffness during differentiation or rapidly alter their shape while migrating. Properties like the mechanical behavior, shape or size are intrinsic parameters of cells, thus representing a label-free biomarker for cell physiology. Detection of those distinct morphological alterations of cells requires a convenient, robust, high-throughput technique to be utilized for basic sciences and clinical research.

The research group of Prof. Jochen Guck at the Biotechnology Center (TU-Dresden) and ZELLMECHANIK DRESDEN, a university spin-off, have recently developed the innovative method, real-time deformability cytometry (RT-DC). Designed as a modular and high-throughput microscope extension it allows for the first time measuring cell morphological properties at rates of more than 100 cells per second in real-time (Nat. Methods 12, 199–202 2015).

We demonstrated the capability of RT-DC for biological and medical applications, e.g. by mechanical phenotyping of human hematopoietic stem cells showing source specific differences. By analyzing whole blood, we have established the mechanical hemogram – the first label-free and functional characterization of blood cell sub-populations including lymphocytes and myeloid cells. Additionally, we showed that the mechanical fingerprint of different cell cycle stages allows distinguishing cells in G2 and M phase. This is currently not possible using standard flow cytometry approaches. Collaborating scientists and first external owners of the device are now starting to exploit this highly innovative flow-cytometric method in their respective research areas.

We believe that the annual meeting of the DGfZ provides the perfect platform to present this emerging technology to the experts.

A Web-based Software Concept To Support High Content Cytometry Antibody Panel Design

Beckman Coulter, Michael Kapinsky

The menu of detectors, excitation wavelengths and fluorochromes is continuously expanding making high content cytometry an increasingly powerful research tool. More channel, more lasers and more fluorochromes means more possibilities but also imposes the challenge of a combinatorial growth of possible antibody panels whose complex performance characteristics hardly can be managed by paper and pencil. Often experiences made already with one or more specific antibody panels cannot be transferred to modified or entirely new panels and testing of multiple panels without rigorous and meaningful ranking of expected panel sensitivities seems cost-prohibitive.

In this session we will present an expert software system that enable less-experienced cytometry users to design high content panels based on embedded instrument configurations, fluorochrome properties and cell biology. Advanced users are provided a tool that allows them to create their own individual experimental environment first before having their panels designed with a software tool that takes all their technical and biological input into consideration.

In this session you will:

• Learn on the determinants of sensitivity in high content cytometry as related to panel design, instrument characteristics and antigen co-expression patterns

• See a way to translate the seemingly complex and fuzzy problem of high content panel design into a systematic, easy-to-use software approach

• Learn how a web-based software solution may help you to design sensitive high content antibody panels

Quantitative comparison study of flow cytometers using a novel ultra-stable calibration light source

APE, Konrad von Volkmann

Characterization, calibration and monitoring a flow cytometer are essential for high quality data acquisition. Especially for long term studies or large comparison studies involving various clinics or flow cytometry core facilities distributed over several places a high degree of coherence within the data and the results derived from it is absolutely essential. Over the past years a lot of work has been put into this endeavor [1,2]. The proposed standard operation procedures are basically divided in measuring of various kinds of calibration particles and analyzing of light emitted by a well defined light source. In most labs especially the first method is used for quality control. This leads to good results where the variation resulting from hardware differences or from other sources is negligible when compared to biological differences between cell types. Nevertheless the effort for this procedure is quite substantial and still does not allow a calibration such that absolute statements about MFI and measured quantities are possible.

In our talk we will discuss ways quantify flow cytometers in terms of sensitivity and resolution. As shown by others [3,4] the coefficient of variation (CV) of a stable light source can be used for scale calibration in numbers of detected photoelectrons. This calibration allows the quantitative comparison of flow cytometers in terms of light detection efficiency. We will present a comparison study of various cytometers available in the Core Facility of the DRFZ employing a novel calibration light source, called quantiFlash. This device is commercially available and implements ideas from Steen and Wood [5], who was actively involved in the development and the design of the quantiFlash. Furthermore we will show how the quantiFlash can be used for finding an optimal fluorochrome-marker combination with regard to cytometer sensitivity.

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Session 6: Microscopy

1:30pm - 3:00pm Paul-Ehrlich Lecture Hall

Chair: Anja Hauser, Berlin

Chair: Raluca Niesner, Berlin

Fluorescence microscopy has a long tradition in the investigation of cells in health and disease. We experienced in the last decades developments such as super-resolution imaging or optogenetics that revolutionized our view on the cell as an orchestration of millions of molecules. The access to the cell within its own environment gained unpreceded relevance and surprising answers were delivered by using other phenomena than fluorescence in microscopy. The present session gives insight into newest developments, pushing the edge of our cytometric understanding in tissue context.

Deep imaging with time-reversed light

Ben Judkewitz, Berlin

Bioimaging and Neurophotonics, Exzellenzcluster NeuroCure, Charité Berlin

Recent advances in resolution, speed, labelling and the advent of optogenetics have greatly extended the use of optical techniques and enabled many biomedical breakthroughs. Yet, when light propagates through thick biological tissues, refractive index inhomogeneities cause diffuse scattering that increases with depth. This poses a major challenge to optical techniques, limiting their biomedical usefulness *in vivo* to superficial layers of tissue (in rodents) or to larval stages (in zebrafish). In this talk I will describe several strategies to address this key challenge using techniques based on wavefront engineering and optical time reversal, in order to enable optical imaging at unprecedented depths in biological tissues.

Lightwedge and Lightsheet-Raman Microscopy

Rainer Heintzmann, Jena

Leibniz Institute of Photonic Technology, Albert-Einstein Str. 9, 07745 Jena, Germany; Institute of Physical Chemistry and Abbe Center of Photonics, Friedrich-Schiller-University Jena

Two recently developed modes of lightsheet imaging will be presented. Lightwedge microscopy aims at mesoscopic imaging of fixed and optically cleared samples at 1 µm isotropic resolution without the need for sample rotation. The key-idea is to focus a lightsheet at an unusually high NA (thus the name "lightwedge") and still obtain a large field of view due to refocusing of the lightwedge and stitching the multiple small regions of thin illumination back together. This has been simplified by electrical tunable lens technology which has become available recently.

The second mode is hyperspectral Raman imaging in a lightsheet illumination configuration. To recover the spectral information a full-field Fourier-spectroscopic approach has been chosen. The difficulty here is that in a Michelson approach, it would be technically very hard to maintain the angular stability and common path approaches usually tolerate a relatively low product of étendue and maximal optical path difference. We thus developed an optically stable Mach-Zehnder like scheme based on the use of retro-reflecting corner cubes, which is inherently stable. This enabled us to obtain full spectrally-resolved Raman images consisting of over four million spectra in about 10 minutes. Advantages over the conventional Raman imaging are the reduced maximum power on the sample and out of focus heating, the lightsheet-inherent good suppression of crosstalk from the illumination side and the avoidance of glass close to the sample mounting.

Light sheet illumination for Raman imaging at few specific wavelengths was previously reported [2,3].

With a total laser power of 2 W at an illumination wavelength of 577 nm, we obtained images (2048 × 2048 pixels) of polystyrene beads (fig. 1b), zebrafish and a root cap of a snowdrop at a spectral resolution of 4.4 cm-1 with only few minutes of exposure. The olefinic and aliphatic C-H stretching modes, as well as the fingerprint region are clearly visible along with the broad water peak of the embedding medium (fig. 1a).

Spectrally resolved spontaneous Raman microscopy therefore promises high-throughput imaging for biomedical research and on-the-fly clinical diagnostics

Direct Measurement of Rupture Force between Human Blood Platelets at different Degrees of Activation by Single Cell Force Spectroscopy

Raghavendra Palankar

Raghavendra Palankar, Thi-Huong Nguyen, Van-Chien Bui, Nikolay Medvedev, Andreas Greinacher, Mihaela Delcea
University Medicine Greifswald, Germany

The measurement of biophysical and biomechanical forces during platelet activation and aggregation is of major biological and biomedical importance since they eventually contribute to the strength of the blood thrombus (e.g. clot stability and platelet plug retraction).So far, no previous study has measured directly the adhesion forces at single platelet-platelet interfaces during interaction at different platelet activation states. Here, we filled this void by using single-cell force spectroscopy (SCFS) experiments. SCFS quantitatively measures interaction forces and binding kinetics at single cell regimes and reveals the contribution of single or a few molecules to such interaction. In this study we describe using SCFS, a strategy to achieve strong adhesion of a single platelet to an atomic force microscopy cantilever (AFM-cantilever) with no-/or minimal platelet activation, which allows for measurements of rupture forces among single platelets. We first monitored single platelet activation levels on substrates passivated with extracellular matrix proteins (e.g. different types of collagen and fibronectin) and determined the most suitable material to modify the AFM-cantilever for immobilization of a single platelet. We then applied our setup to measure interaction forces of single platelets with these substrates and rupture forces between two single platelets at different platelet activation states. We used pharmacological blockers of platelet receptors to investigate how this influences platelet-platelet interaction. We found the rupture force increases proportionally to the degree of platelet activation, and upon blocking of specific platelet receptors significantly reduced. Quantification of single platelet-platelet interaction provides major perspectives for testing and improving biocompatibility of new materials for vascular tissue engineering, quantifying the effect of drugs on platelet function and assessing the biophysical and biomechanical characteristics of platelet related defects.

Dissecting the heterogeneity of murine mesenchymal bone marrow stromal cells

Daniel Schulz

Daniel Schulz[1], Richard Addo[1], Joachim Grün[1], Pawel Durek[1], Sandra Zehentmeier[2], Koji Tokoyoda[1], Anja Hauser[1], Koichi Ikuta[3], Hyun-Dong Chang[1], Özen Sercan-Alp[1], Andreas Radbruch[1]
[1]Deutsches Rheumaforschungszentrum, Berlin, Germany; [2]Yale University, New Haven, USA; [3]Institute for Virus Research, Kyoto University, Japan

Introduction

Bone marrow stromal cells gain increasing amounts of attention lately due to their ability to support the long-term survival of memory lymphocytes and long-lived plasma cells as well as hematopoietic stem cells in stromal niches.

While the architecture of the niches is similar as seen e.g. by uniform expression of the cell adhesion molecule VCAM-1 the survival factors and cues provided by the stromal cells are different. CD4+ T Helper memory cells are supported by Interleukin-7 (IL-7) expressing stroma whereas plasma cells depend on stroma-derived CXCL12 among others to survive.

Objectives

Traditionally, stromal cells are studied *in vitro* due to their strong adherence to plastic surfaces. Assessment of stromal cells ex vivo is desireable as it better reflects surface marker expression but difficult due to low numbers and yield in efficiency.

Thus, an efficient protocol and subsequent ex vivo characterization of stromal cells of the murine bone marrow should be carried out to dissect stromal heterogeneity and further elucidate their functions in supporting the long-term maintenance of immunological memory in the bone marrow.

Materials & Methods

Hindlegs from C57Bl/6 or IL-7 reporter mice were dissected to obtain the bones. For histology, bones were formaldehyde-fixed, frozen and subsequently cut and stained for confocal imaging. For flow-cytometrical analysis, bone marrow strings were flushed as intact as possible from the bones. Both empty bones and marrow were then digested in RPMI containing Collagenase IV, DNase I and Dispase II. Additionally, cytoskeleton inhibitors Latrunculin B and Cytochalasin D were added. Cells were stained for stromal markers (LegendScreen, BioLegend) and analyzed on a MACSQuant (Miltenyi) or further sorted at BD Influx. Sorted stromal and endothelial fractions were lysed to obtain RNA for gene expression analysis.

Results

Addition of cytoskeleton inhibitors vastly improved the stromal yield. Furthermore, sorting cells in sufficient numbers for gene expression analysis was feasible. Expression data from total VCAM-1+ stromal cells or an IL-7+ subset confirm the overall heterogeneity of bone marrow stromal cells. This could be confirmed by an unbiased surface marker screen as well as histological stainings.

Conclusion

Ultimately, we developed a protocol to investigate the stromal heterogeneity in the bone marrow ex vivo by using an enzymatic digestion followed by flow-cytometrical analysis and sorting to gain insights into the role of stromal cells in the generation and maintenance of immunological memory. In addition, data from histology and surface marker screenings implicate a diverse stromal population lacking functional translation, yet.

Postersession Track B

Auditorium Cross Over

Chair: Torsten Viergutz, Dummerstorf
Chair: Wolfgang Beisker, München

3:00pm - 4:00pm Poster Session B

B01 L-10 induction in human naïve and memory T cell subsets by the Notch signaling pathway

Jonas Ahlers[1,2], Andrej Mantei[1], Petra Bacher[1], Thordis Hohnstein[1], Nadine Mockel-Tenbrinck[3], Laura Lozza[4], Manuela Staeber[4], Alexander Scheffold[1,2]

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IL-10 is a major immunosuppressive cytokine, which can be produced by many cell types including CD4 T helper cells. However, the molecular signals supporting selective IL-10 induction in human T cells are currently not defined. Furthermore whether IL-10 is restricted to certain memory T cell subsets with regulatory function or whether it can principally be induced in all T cell subsets is not clear. We have previously shown that IL-10 production from murine Th1 cells was strictly dependent on Blimp-1 but was further enhanced by the synergistic function of c-Maf, a transcriptional regulator of IL-10 induced by multiple factors, such as the Notch pathway, activated by its ligand Delta-like 4 (DLL4). Now we demonstrate that recombinant DLL4 together with the cytokines IFN-alpha, IL-6 and IL-21 potently induce the expression of IL-10 also in human T cells. In contrast to murine T cells the expression of IL-10 itself, as well as the transcriptional regulation, is not restricted to a certain T helper subset: DLL4 together with this cytokine cocktail induces IL-10 via Blimp-1 and c-Maf in all major memory T cell subsets (Th1, Th2, Th17, Th1/17), indicating that IL-10 production and it's regulation is in fact a universal feature of human memory/effector cells, which can be triggered by DLL4 and probably plays a role for self-limitation of inflammatory T cell responses.

B02 Synthesis of the physiological bone marrow memory plasma cell niche

Stefanie Schmidt[1], Adriano Taddeo[2], Bimba Franziska Hoyer[1,3], Pawel Durek[1], Falk Hiepe[1,3], Hyun-Dong Chang[1], Andreas Radbruch[1]

[1]German Rheumatism Research Centre, Berlin; [2]University of Bern, Department of Clinical Research; [3]Charité - Universitätsmedizin Berlin

In the bone marrow, long-lived memory plasma cells (mPC) are maintained in dedicated survival niches, provided by mesenchymal stromal cells and accessory cells. We are currently synthesizing this niche from its hypothetical elements, for a molecular understanding of the maintenance of mPC. Since mPC die very fast, when removed from their niche, we developed a method to purify viable mPC. As stromal cells we used murine ST2 cell line, which express VCAM and CXCL12, but do proliferate *in vitro*, unlike their physiological counterparts *in vivo*.

When isolated mPC are co-cultured with ST2 cells in the presence of APRIL, as a ligand of the mPC receptor BCMA (B-cell-maturation-antigen), at 4.2% oxygen, 60 - 80% of the mPC survive for up to six days of culture and continue to secrete antibodies. Beyond day 6, the continued proliferation of ST2 cells makes the cultures unreadable. The survival of mPC was impaired when APRIL and therefore signaling via BCMA was missing or when cell-cell contact

was inhibited. Thus, survival and function of mPC seems to be dependent on two synergistic signals.

We hypothesize, that BCMA signaling induces high expression of anti-apoptotic proteins like mcl-1 and ST2 contact activates PI3K/AKT pathways for inhibition of pro-apoptotic proteins.

Here, we present a system, which enables the study of how long-lived plasma cell survival is supported by the niche components on molecular level.

B03 Analysis of reactivation of BM memory CD4 T cells

Mairi McGrath, Francesco Siracusa, Markus Bardua, Koji Tokoyoda, Hyun-Dong Chang, Andreas Radbruch

DRFZ, Germany

We have provided evidence that long-term immunological memory is maintained by memory cells in dedicated survival niches such as in the bone marrow (BM). We have shown that BM memory CD4 T cells are quiescent in terms of proliferation and gene expression in the memory phase of an immune response, but it has remained enigmatic what is the fate of these cells following reactivation. We have addressed this question by analyzing the reactivation response of memory CD4 T cells in mice following restimulation with antigen *in vivo*.

We have assessed the retention versus circulation potential of BM memory CD4 T cells via the expression of CD69, KLF2, S1pr1, CD62L and CCR7, which are key indicators of the ability of cells to translocate from bone marrow into blood and secondary lymphoid organs. We found that many memory CD4 T cells of the bone marrow expressed the retention marker CD69, whilst expressing little or no CD62L, CCR7, KLF2 and S1pr1, confirming that they were unlikely to leave the BM in their resting state.

Following rechallenge with antigen in the memory phase of the immune response we found that memory CD4 T cells rapidly proliferated, leading to a large accumulation of memory CD4 T cells in the BM. Moreover, the reactivation response in the BM could occur independently of both circulating cells and of secondary lymphoid organs. The majority of the newly generated cells appeared to remain in the BM. We are currently investigating what role these newly generated BM effector memory T cells play in secondary immune responses.

Overall, we have shown that in steady state conditions, the BM consists of resting and resident memory CD4 T cells. Following re-encounter with antigen, these cells can be rapidly reactivated, resulting in an enormous amplification of effector memory CD4 T cells in the BM. This new understanding of immunological memory impacts tremendously on the development of vaccination strategies, and on the development of therapies against immune-mediated diseases.

B04 Lipids grease the way: Pollen-derived (glyco)-lipids at the onset of type 2 responses

Nestor Gonzalez Roldan, Regina Engel, Dominik Schwudke, Katarzyna Anna Duda

Research Center Borstel, Germany

Grass pollens are an important source of environmental antigens that cause allergic responses. Most studies on pollen have focused on the role of protein antigens as allergens. However, proteins are not the sole bioactive agent present in grass pollen. Other structural components of pollen grains such as glycolipids have been recently recognized as potential initiators and regulators of allergic inflammation through the activation of innate-like and lipid-reactive populations of lymphocytes.

We aimed to isolate and characterize all lipid species present in Timothy grass (Phleum pratense) pollen that are involved in development of type 2 immune responses related to allergic inflammation.

Diverse lipid-containing fractions were obtained from Timothy grass pollen by extraction with Chloroform/Methanol/Water. After silica gel separation three compound classes, namely phytocerebrosides, cerebrosides and glycerolipids eluting with CHCl3/MeOH 93/7, v/v and 90/10, v/v were identified. These fractions were then analyzed for their biological activity. In primary cultures of splenocytes and liver lymphocytes from C57BL/6 mice, the lipid compounds induced the release of cytokines detectable in culture supernatants, and selectively induced the proliferation of invariant Natural Killer T cells (iNKT). In vivo, intranasal administration of these lipid compounds induced a type 2 response involving iNKT cells, evidenced by eosinophilic infiltration into the airways in a CD1d-restricted manner. In human peripheral blood mononuclear cells, pollen glycolipids activated iNKT and a subpopulation of delta/alpha/beta T cells as evidenced by CD69 upregulation. Further HPLC purification of the fraction CHCl3/MeOH 90/10, v/v allowed for the isolation of its main glycerolipid, which structure was resolved applying various analytical methods (gas chromatography, mass spectrometry and NMR analyses).

Our results demonstrate that pollen-derived lipids target cells and pathways that are critically involved in type 2 responses in mice. However, the specific target of theses glycolipids for the initiation and regulation of allergic inflammation in humans needs further investigations.

B05 Dual fluorescence/mass-tag antibody conjugates as a new tool for comparative flow and mass cytometry analyses and pre-enrichment strategies

Sabine Baumgart

DRFZ, Germany

Background: Mass cytometry allows high-dimensional profiling of immune cells in a clinical setting. Although many cell types can be analyzed in parallel, rare cell types, such as dendritic cells or plasma cells, will be not acquired in sufficient numbers due to the limited acquisition speed of the instrument. Pre-enrichment strategies would be helpful to overcome this limitation. In this respect we have developed antibody conjugates with two labels: a fluorochrome necessary for a pre-enrichment by fluorescence activated cell sorting and a heavy metal isotope necessary for the detection by mass cytometry. These dual conjugates would be also helpful for comparative analyses of both technologies.

Method: Dual conjugates were generated from carrier protein-free fluorescent Ab conjugates subjected to routine metal isotope labeling via the Ab's reduced disulfide bridges. Exemplarily, different CD4 antibody conjugates were prepared using disulfide-free fluorochromes, such as FITC, VioBlue, VioGreen and Vio667 dyes, which were subsequently labeled with 141Pr. From all fluorescence and dual conjugates the molar ratio dye/Ab was determined and emission spectra were collected while the number of metal isotopes per Ab was calculated for metal and dual conjugates.

Results: CD4 dual conjugates retained their fluorescent emission spectra after metal labeling. In addition we have confirmed that dual conjugates revealed their target specificity in both, flow and mass cytometry, and showed similar titration curves on both platforms. In five different PBMC samples assayed, mass and flow cytometry data on CD4+ cell frequencies and signal intensities strongly correlated when either CD4-VioGreen-141Pr or CD4-Vio667-141Pr were used.

Finally, these conjugates were used for isolation of CD4+ T cells from PBMC using a fluorescence-based high-speed cell sorter. Subsequent mass cytometric analysis of the sorted cells revealed that non-CD4 PBMC were efficiently depleted and that the phenotypical composition of CD4+ T cells was maintained after sorting. CD4-141Pr staining was not impaired after sorting, so that the CD4 parameter was also available for mass cytometric data analysis.

Conclusion: Dual fluorescence/mass-tag Ab conjugates enable a direct comparison between flow and mass cytometry assays and provide a versatile applicable tool for the pre-enrichment of target cells for mass cytometry by fluorescence-based high-speed cell sorting. Our approach provides access to mass cytometry of rare cell subsets present in complex mixtures while keeping sample acquisition times at a minimum.

B06 Analysis of mast cell degranulation by multiparametric flow cytometry - looking for stimulus-specific degranulation signature.

Philipp Hagemann, Frauke Koops, Zane Orinska

Forschunszentrum Borstel, Germany

Mast cell (MC) degranulation is a complex and precisely regulated process. Upon stimulation within a few minutes mast cells are releasing their granules containing biogenic amines, proteases, proteoglycans, lysosomal enzymes, pre-stored cytokines, and other biologically active compounds, leading to local and systemic, vascular and inflammatory responses.

The exocytosis of granules is orchestrated by membrane receptor complexes responding to the environmental signals, intracellular signalling modules transducing the incoming signals, cytoskeletal proteins and granule membrane protein complexes controlling exocytosis. IgE-mediated Fc epsilon RI-dependent mast cell degranulation is the main effector reaction in allergies. IgE-independent G-protein coupled receptor-mediated mast cell degranulation in response to polycationic compounds (e.g. compound 48/80 or antibiotics of fluoroquinolone family) is the second type of hyper-responsiveness described for MCs. Furthermore MC degranulation could be induced by compounds of different venoms and pharmacological agents e.g. calcium ionophores. Cytometric analysis of mast cell degranulation mainly is based on analysis of CD107a (LAMP-1) translocation from the granular to the plasma membrane.

Our starting hypothesis is that different MC stimuli acting through different receptor complexes will generate multiparametric stimulus specific signature. To investigate the stimulus-specific MC response, degranulation of peritoneal MCs were analysed with multicolor flow cytometry. For the purpose of MC identification, the T1/ST2 (IL33R) and CD117 (c-kit) double staining was used. Upon degranulation changes in the forward scatter (FSC) and side scatter (SSC) as well as translocation of granular membrane proteins to the plasma membrane were measured. Our first results indicate that MCs degranulate in a stimulus-dependent manner and multiparametric analysis is generating stimulus-specific pattern of MC response. The used parameters let us to discriminate between completely degranulated and partially degranulated MCs. Thus, the multiparametric analysis of stimulus-specific receptor expression/translocation will provide additional information about MC degranulation requirements. This will help us to identify environmental signals required for successful MC tolerization.

B07 Different flow cytometry approaches to analyze the regulation of the interferon-gamma expression in human T cells

Stefanie Gryzik, Timo Lischke, Ria Baumgrass

German Rheumatism Research Center, Germany

Excess of interferon-gamma (IFNg) expression turned out to be involved in immune pathologies such as systemic lupus erythematosus, multiple sclerosis and insulin-dependent diabetes mellitus. Thus, IFNg expression needs to be tightly controlled to counteract those

diseases. We need to better understand how IFNg expression magnitude in individual cells is regulated in order to interfere with pathophysiological processes.

Using flow cytometry and imaging flow cytometry we studied stimulation-induced IFNg expression on single cell level concerning its variability and robustness within the human memory T cell population, the correlation with the amount and translocation of transcription factors, and how intermediate inhibition (low dose) by the drug cyclosporin A (CsA) acts.

Our results illustrate the utility of flow cytometry and imaging flow cytometry for facilitated studies of regulatory processes on single-cell level.

B08 Optimization of protocol for isolating human skin T cells

Weijie Du[1], Carla Cendon[1], Axel Schulz[1], Erping Zhang[2], Juliane Bodo[3], Hyun-Dong Chang[1], Andreas Radbruch[1], Jun Dong[1]

[1]Deutsches Rheuma-Forschungszentrum (DRFZ), Germany; [2]Sankt Gertrauden Krankenhaus, Berlin, Germany; [3]Plastische und Ästhetische Chirurgie, Berlin, Germany

Background:
Memory T cells are heterogeneous, consisting of not only circulating subpopulations (i.e. central memory T cells and effect memory T cells) but also non-circulating, tissue-resident memory T (TRM) cells, such as in the skin. In mice, skin TRM cells have been shown to provide rapid local protection during secondary infections [1]. To further dissect human skin TRM cells, various isolation approaches have been applied, such as EDTA isolation [2], collagenase or collagenase P digestion [2] [3] [4], and skin explants [2]. However, these protocols either suffer from low yield or require long *in vitro* culture periods. To solve these problems, we established a modified collagenase digestion protocol for rapid isolating high yield viable T cells while preserving intact epitopes of interest.

Methods:
Skin samples were obtained from healthy donors under plastic surgeries with informed consent and following the ethical committee approval. Subcutaneous fat and hair were removed and the remaining tissue was minced into small fragments. Around 1 cm2 skin fragments were incubated in 3 ml digestion medium at 37°C for 6 or 12 hours. Subsequently, the digested skin fragments were dissociated using a gentleMACS dissociator and the resulting cell suspension was filtered and stained with various surface markers of interest. Viable cells were counted using a MACSQuant; the expressions of CD45 and resident memory T cell markers were further analyzed by a multicolor flow cytometer. In parallel, this protocol was compared with other protocols such as whole skin dissociation and collagenase P digestion.

Results and conclusion:
In terms of yield and cell viability, the modified collagenase digestion protocol resulted in at least 1.5 times more T cells per cm2 with relatively higher viability than other protocols. Moreover, this modified protocol could well preserve critical surface marker expressions (such

as CD4, CD8 and CD69), as opposed to other protocols. Therefore, the modified collagenase digestion protocol is suitable for further functional assays which acquire relative high amount of viable skin T cells, providing an opportunity for better understanding of human skin TRM cells.

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B 09 FACS sorting and Next Generation Sequencing of murine bone marrow stromal cells.

Richard Addo, Daniel Schulz, Toralf Kaiser, Pawel Durek, Mir-Farzin Mashreghi, Hyun-Dong Chang, Andreas Radbruch

DRFZ, Germany

Despite the crucial role bone marrow stromal cells play in the organization of niches for hematopoiesis and subsequent role in immune system, the challenging task of isolating these rare cells has hampered efforts in understanding their identity, nature and potential functions. Answers to questions in the field of bone marrow cells will pave way for their envisioned therapeutic use.

To tackle the technical difficulty, we developed an optimised enzymatic and mechanical isolation of bone marrow stromal cell and FACS protocol that makes it possible to sort this rare population of cells to very high purity with decent recovery. Using positive (VCAM-1) and negative markers (CD45,CD31) surface marker we sorted two population of bone marrow stromal cells and endothelial cells. Taking advantage of modified kits to isolate RNA from low cell numbers ,we performed a thorough-put Next Generation Sequencing(NGS) to generate transcriptomes of genes expression.

The transcriptomic signature gives us clues into the nature and possible function of stromal and endothelial cells. Finally to get a global overview of bone marrow stromal and lymphocytes interaction, we sequenced and compared the transcriptomes from different strains of mice.

In conclusion, in overcoming the technical hurdle in handling of bone marrow stromal cells , we will further seek to understand the unique identity and functions of stromal cells in relation to other immune cells with special focus on memory lymphocytes.

B10 VOCAL Verification and Optimization of Cellcounting after Automated Lysis

Monica Killig, Esther Schiminsky, Martin Mengel, Martin Büscher

Miltenyi Biotec GmbH, Germany

In clinical or diagnostic flow cytometry, lysis protocols are a standard way of processing whole blood samples. However, since lysis often involves utilization of osmotic pressure to selectively disintegrate Red blood cells, it is always questionable if other cells or cell subsets are selectively affected as well. Often the validation is limited by the effect that lysed cells are becoming "invisible" in normally triggered flow cytometric event detection.

In order to improve the ease of validation efforts and generally to improve interpretability and accuracy, we have worked out a special procedure that enables the tracking of lysed cells and non lysed cells before and after lysis. Since this method is purely dependent on fluorescence, it is a very robust and bias free method not suffering from the scatter limitations.

In this work we demonstrate the application of the above described method to optimize a fully automated lysis protocol on a MACSQuant® flow cytometer. The results are showing that even after lysis more than 90% of all original cells can be tracked throughout the process independently of the fact that they are lysed or not. And we could show that there are different lysis effects on CD14+ monocytes and CD19+ B cells relative to all other investigated subsets. This could be a clinically relevant finding.

Session 7: Klaus-Goerttler-Session

4:00pm - 4:30pm Paul-Ehrlich Lecture Hall

Chair: Hyun-Dong Chang, Berlin

Klaus-Goerttler-Prize

Since 1996 the DGfZ awards a younger scientist with the so called Klaus-Goerttler-Prize, which goes along with prize money of 1000 €.
The award is named after Prof. Klaus Goerttler, he was a pathologist and trend-setting member of the foundation board of our society.

The Prize is dedicated to award a younger scientist for a scientific work out of the wide field of Cytometry which appears outstanding both in scientific quality and innovation as well as presentation and layout. The scientific work reflects a (almost) finished scientific graduation (Diploma, Bachelor, Ph.D. or an equivalent work) and was selected out of several submissions by the council board of the society.

A FACS sorting-based genome-wide CRISPR screen identifies NEK7 as a novel genetic component of inflammasome activation

Jonathan Schmid-Burgk

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The NLRP3 inflammasome is a high-molecular weight protein complex assembled upon pathogen encounter in myeloid cells, leading to both IL-1b maturation and cell death. Recent studies have implicated the NLRP3 inflammasome as a critical driver of inflammation in a number of common human diseases such as atherosclerosis, Alzheimer's disease, and gout.

While it has been shown that known NLRP3 stimuli converge on potassium ion efflux upstream of NLRP3 activation, the exact molecular mechanism of NLRP3 activation remains elusive.

We established a genome-wide CRISPR/Cas9 screen in immortalized mouse macrophages aiming at the unbiased identification of gene products involved in NLRP3 inflammasome activation. We employed a FACS sorting-based screen for NLRP3-dependent cell death that we could optimize to an on-target enrichment rate of >50-fold. Using a genome-spanning library consisting of >70,000 gRNAs, we identified five gRNAs leading to a specific survival phenotype in macrophages stimulated with Nigericin, a ionophoric compound that induces potassium efflux. While two of these gRNAs targeted NLRP3 itself, the other three gRNAs targeted genes that had not been implicated in innate immunity before. Of those, the kinase NEK7 was validated to be critically required for NLRP3 activation, while the AIM2 inflammasome was not dependent on the presence of NEK7. We next strive to identify the phosphorylation targets of NEK7 in order to further understand the molecular mechanism of NEK7-dependent NLRP3 activation.

Guest Lecture: Jens Krause

4:30pm - 5:30pm Paul-Ehrlich Lecture Hall

Chair: Hyun-Dong Chang, Berlin

Prof. Dr. Jens Krause´s research interests focus on mechanisms and functions of animal social networks and their evolutionary and ecological consequences. His findings contribute to a better understanding of swarm intelligence of animals and human beings as well. Most of all his conclusions from studies about dynamics of collective human behavior have a high practical impact and are most interesting for industry and economy.

Jens Krause is head of the department of "Biology and Ecology of Fishes" at the Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB). The IGB was founded in 1992 and covers a wide range of disciplines from hydraulics to socioeconomics, from molecular biology to catchment modelling, and from microbial ecology to fish behavior (www.igb-berlin.de).

Jens Krause studied Biology at the Free University in Berlin and moved to Cambridge/UK. He got his PhD in 1993 and went as post doc to Canada, Princeton/USA and Leeds/UK. From 1996 he became lecturer, reader and finally professor for behavioral ecology at the University of Leeds/UK. Since 2009 Jens Krause is professor for fish ecology at the Humboldt University Berlin.

Collective behaviour and collective intelligence

Jens Krause

Leibniz-Institut für Gewässerökologie und Binnenfischerei, Berlin

Many group-living species exhibit complex and coordinated behaviours from the motion of fish schools to bird flocks, ungulate herds and human crowds. The common property of these biological phenomena is that of inter-individual interaction, by which individuals can influence the behaviour of others. Individual-based models provide predictions regarding collective processes which we tested in a set of experiments that explore the behaviour of fish schools and human crowd dynamics. From this work it appears that in some cases individuals in groups are capable of solving cognitive problems that singletons cannot solve. In this context I will discuss the topic of swarm intelligence (or collective intelligence) and present a number of case studies from the human domain to show commonalities between animal and human problem solving strategies.

Members Assembly

5:30pm - 7:00pm Paul-Ehrlich Lecture Hall

Chair: Wolfgang Fritzsche, Jena

Chair: Hyun-Dong Chang, Berlin

Conference Dinner

8:00pm Arminius Markthalle, Eingang Bugenhagener Straße 18-34

Friday, October 7, 2016

Session 8: Cutting Edge

9:30am - 11:00am Paul-Ehrlich Lecture Hall

Chair: Elmar Endl, Bonn

Chair: Thomas Kroneis, Billdal

Cutting-edge research evolves from newly invented and optimized technologies and strategies or arises from known technologies being applied to new fields of research tackling questions from an unexpected angle. Thus, this session was launched to create space for this aspect bouncing between a proof-of-principle and translational level of research. The keynotes of this session will allow insight into flow cytometry-assisted protein engineering and vector biology and beyond.

Flow Cytometry Applications in Vector Biology

Elena A. Levashina, Berlin

Vector Biology Unit, Max Planck Institute for Infection Biology, Germany

Vector-borne infectious diseases persist as a major scourge for humanity. Malaria alone, caused by Plasmodium parasites transmitted by the bite of infected female Anopheles mosquitoes, annually infects 250 million people worldwide and kills half a million, mostly children in sub-Saharan Africa (WHO, 2010). On the other hand, the Aedes mosquito species transmit a series of viral diseases including zika and dengue to name a few. The field of vector biology has been recently revolutionized by development of efficient transgenic approaches that offer unprecedented opportunities for better understanding of mosquito – pathogen interactions but also new tools to learn more about mosquito biology in general. In my talk I will discuss examples of flow cytometry applications at the organismal and cellular levels, that can be used for high-throughput screening of large numbers of mosquito larvae or for analyses of mosquito blood cell populations.

Flow Assisted Protein Engineering Technology

Randolph Caldwell, München

Helmholtz Zentrum München - Deutsches Forschungszentrum für Gesundheit und Umwelt, Germany

We have developed a technology platform for the evolution of novel or improved commercially relevant proteins. The approach is based on the in cell evolution of target genes and the flow cytometry-assisted selection of the desired protein variants. Our platform accomplishes protein diversification utilizing unique features of the avian B cell line DT40. We harness the same "diversification machinery" that creates target specific antibodies possessing extremely high binding affinities. Enormously diverse protein libraries of a gene of interest can be quickly generated in DT40 by utilizing random templating of genetic domains (gene conversion) and/or by the introduction of non-templated genetic information (somatic hypermutation). This ability distinguishes itself from all other currently used technologies of protein evolution because the genetic diversification of the gene is target specific, highly efficient and achieved within living eukaryotic cells.

The application of the Countstar Fluorescence (FL) in the quality monitoring of stem cells

Bodo Kohring

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Mesenchymal stem cells are a subset of pluripotent stem cells which can be isolated from the mesoderm. With their self-replication renewal and multi-direction differentiation characteristics they possess a high potential for various therapies in medicine. Mesenchymal stem cells have a unique immune phenotype and immune regulation ability. Therefore, mesenchymal stem cells are already widely used in stem cell transplantations, tissue engineering and organ transplantation. Beyond these applications, they are used as an ideal tool in tissue engineering as seeder cells in a series of basic and clinical research experiments. Up to now, there is not a widely accepted method and standard for the quality control of mesenchymal stem cells. The Countstar FL can monitor the concentration, viability and phenotypical characteristics (and their changes) during the production and differentiation of these stem cells. The Countstar FL has also the advantage in obtaining additional morphological information, provided by the permanent brightfield and fluorescence based image recordings during the whole process of cell quality monitoring. The Countstar FL offers a fast, sophisticated and reliable method for the quality control of stem cells.

A novel method for characterising cell properties based on pulse shapes

Kristen Feher

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In standard flow cytometry, cells are characterised by using an estimate of scatter and fluorescence intensities. These estimates are derived from an electronic pulse corresponding to the physical response of a detector (PMT or Photo diode), which in turn corresponds to the characteristics of emitted and scattered light from a cell. The estimates are based on pulse area, height and width. A very simple method of using pulse shape is to compare the relationships of area, height and width to each other, e.g. the pulse height and width are typically used to distinguish between single cells and doublets.
Based on this standard practice, we suppose that a more complete analysis of the shape could yield novel information about cell type without having to stain extra markers. Using custom hardware, we capture these pulses and analyse their shapes using a discrete wavelet transform. We confirm the stability of this method with the quantiFlashTM device as well as with microspheres.

We are able to efficiently filter out cell doublets and non-specific pulses (due to electronic artefacts) to increase data quality. Furthermore, we are able to identify erythrocytes which we confirm with a specific erythrocyte staining. This method shows great promise in identifying a greater range of cell types, as well the potential to be implemented in a sorter yielding high purity sorts. Furthermore, this approach could be an interesting tool for the measurement of small particles, e.g. bacteria.

Session 9: Immunology

11:30am - 1:00pm Paul-Ehrlich Lecture Hall

Chair: Gergely Toldi, Birmingham

Chair: Alexander Scheffold, Berlin

This year's immunology session will focus on two clinical aspects of cytometry dealing with diagnostic and therapeutic applications. Besides individualized diagnostics, flow cytometry plays a key role in clinical trials. In this respect, standardization and the reduction of inter-site variability in measurements is an important factor for a successful trial. Novel approaches in the immunotherapy of cancer brought T cells in the focus of attention. Modification strategies to enhance target recognition and response of T lymphocytes against cancer cells are already making their way from bench to bedside for the benefit of patients.

Implementation of flow cytometry in multi-center clinical trials - strategies and results from the ONE Study

Birigit Sawitzki, Berlin

Institute of Medical Immunology, Charité University Medicine Berlin, Germany

Flow cytometry is now accepted as an ideal technology to reveal changes in immune cell composition and function. However, it is also an error-prone and variable technology, which makes it difficult to reproduce findings across laboratories. It is especially apparent for functional assays but also evident when "only" performing phenotypical analysis of leukocyte subsets.

Within the EU-funded project the "ONE Study" the safety and also partially efficacy of different leukocyte subsets with regulatory potential such as polyclonal Tregs or tolerogenic macrophages in kidney transplant recipients are investigated. In particular, the consortium is interested in whether and how cell therapies induce changes in leukocyte composition and function. This is done in a multi-clinical approach involving 8 different clinical sites.

Analysis of leukocyte subset composition is done by whole blood (EDTA) staining, which allows simultaneous acquisition of proportional and absolute leukocyte numbers. Each centre is performing the staining and cell acquisition.

In order to minimize variability in such a multi-centre approach we had decided to perform an extensive standardization involving flow panel set-up, staining procedure and sample acquisition prior to study initiation. This resulted in an acceptable variability of all (>70) leukocyte subsets being reported when tested in a world-wide interlab comparison (Streitz 2013).

Using the standardised approach, we could recently define age- and gender-dependent differences in leukocyte subset composition.

During the talk I will shortly summarize the standardization strategy and reported on the identified age- and gender-dependent differences in leukocyte subset composition. Furthermore, I will present identified changes in leukocyte subset composition according to clinical events such as acute rejection and cell therapy.

Designer T cells for cancer immunotherapy

Wolfgang Uckert, Berlin

Humboldt University Berlin-Max Delbrück Center for Molecular Medicine, Germany

Genetically engineered T cells carrying T cell receptor (TCR) genes are effective in cancer immunotherapy (TCR gene therapy). However, the modification of T cells requires ex vivo gene transfer, which most often is performed using retrovirus vectors (RV). This process is laborious, time-consuming, expensive and not suitable for TCR gene therapy targeting mutated, tumor-specific antigens. New technologies have to be developed to fulfill the requirements of personalized immunotherapy and to characterize gene-modified cells used in TCR gene therapy.

We modified the Sleeping Beauty (SB) transposon system to achieve efficient gene transfer into human T cells. Since the transfection of large amounts of DNA by electroporation results in T cell damage, we replaced the transposase-encoding DNA plasmid vector by *in vitro*-transcribed RNA and the TCR-encoding transposon plasmid vector by a minicircle DNA vector. Due to these modifications, T cell viability increased from 5 to 50 %. Moreover, stable transfection of about 50 % of human T cells was achieved, which is similar to RV transduction. TCR-engineered T cells were functional and can be generated within a very short time. Thus, SB transposon-based gene transfer can be used to produce engineered T cells for TCR gene therapy.

We also developed targeted RV capable of transducing subsets of T cells. We used the measles virus (MV) envelope glycoproteins hemagglutinin (H) and fusion and modified the H-protein by adding sequences encoding single-chain antibody fragments, derived from CD8α or CD4 hybridoma. The generated CD8α (MVm8) and CD4 (MVm4) targeted RV exclusively transduced CD8+ and CD4+ murine T cells, respectively. By i.v. injection of the CD8 targeted RV, encoding an ovalbumin-(OVA) reactive TCR, we demonstrated that MVm8 RV mediated specific *in vivo*-transduction of CD8+ T cells. Upon *in vivo* CD8 T cell engineering by MVm8, mice were challenged with OVA-expressing Listeria monocytogenes in an infection model and developed protective immunity.

We established an *in vivo*-mouse model which allows the prediction whether or not TCR-engineered T cells and the selected target antigen are suitable for TCR gene therapy and allow the eradication of tumors or select escape variants.

Development of a protocol for discrimination of latent and active tuberculosis infection by antigen-specific T cell activation and flow cytometry

Andrej Mantei

Tim Meyer[1], Andrej Mantei[1], Petra Bacher[2], Christian Meisel[1], Alexander Scheffold[2]
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Approximately one third of the world's population is infected with M. tuberculosis (TB). However, most of infected persons will stay latently infected and less than 10% will progress to an active TB disease. A major problem in TB diagnosis is the fact that the standard tests (Tuberculin skin test, Interferon release assay, PCR) fail to discriminate between an active TB (ATB) and latent infections (LTBi) as well as a cured TB. A definitive diagnosis still relies on the cultivation of M. tuberculosis from patient material, which provides reliable results only after several weeks. Therefore, the decision for the start of an anti-TB therapy usually relies on the evaluation of clinical symptoms.

Based on a recently developed assay for the identification of antigen-specific T cells, we have established a flow cytometry-based protocol that allows characterization of M. tuberculosis–specific CD4 T cells from peripheral blood. Through single cell analysis of activation and proliferation markers like CD38 and Ki67 on TB-specific CD154+ (CD40L) CD4 T cells our protocol allowed discrimination of latently infected patients from patients with an active TB with a sensitivity and specificity of 100 % in our patient cohort (n=20). This protocol provides valuable information on the state of a TB infection within 24 hours and complements the current standard methods in TB diagnostics. Most importantly, it will facilitate clinicians' early decisions for or against anti-TB therapy.

Silver nanoparticles for the specific detection of cellular antigens in mass cytometry

Axel Ronald Schulz

Axel Ronald Schulz, Silke Stanislawiak, Sabine Baumgart, Andreas Grützkau, Henrik E. Mei
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The development of mass cytometry (CyTOF technology) has pioneered a new era of multiparametric single-cell analysis. Cells and cellular networks can now be studied at an unprecedented depth and complexity. Current instrumentation allows for the theoretical detection of up to 135 parameters, but the availability of metal tags suitable for labeling specific probes limits analyses to currently ~40 parameters, curtailing exploitation of the full analytical capacity of mass cytometers.

We explored the application of elemental silver nanoparticles (AgNP) for reporting specific cellular targets on human lymphocytes in a mass cytometry assay. We demonstrate the successful use of streptavidin-coated AgNP of different sizes for the specific detection of human cell surface antigens such as CD4, CD8, CD45RO, CD16 and CD244 via biotinylated primary antibodies. Signal intensities elicited by cell-bound AgNP were higher than conventional lanthanide-based probes and improved substantially detection of the low abundant antigen CD25. Further, AgNP were compatible with standard mass cytometry staining protocols for PBMC and lyzed whole blood and showed low background signals in control assays. The detection of AgNP, containing both 107Ag and 109Ag at natural abundance, did not overlap or interfere with the detection of any other metal isotopes and further development may lead to the application of isotopically purified AgNP.

Collectively, usage of AgNP extends the analytical capacity of mass cytometry staining panels by one, prospectively two, additional parameters, well suitable for cellular targets of low abundance.

Farewell & Brezels

Auditorium Cross Over

Address book

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