Cytometry.

Past, present, future

25th Annual Conference of the German Society for Cytometry

Berlin, DGfZ
October 7 – 9, 2015

DGfZ
Deutsche Gesellschaft
für Zytometrie

www.dgfz.org

Dear friends,

Welcome to the 25th meeting of the German Society for Cytometry (DGfZ e.V.) in the historic center of Berlin. It is my pleasure to host the anniversary meeting "Cytometry. Past, Present, Future" marking the 25th year since the founding of the society.

We have put together an exciting program covering innovative technologies and cutting-edge research in the fields of immunology, *in vivo* microscopy, microbiology, nanotechnology, and stem cell research.

Also, for the first time, we welcome a European partner society, the Iberian Society for Cytometry, to whom we have dedicated an entire session with the goal of fostering future collaborations between our societies.

I would like to thank our industrial sponsors for the generous financial support making this meeting possible and encourage all participants to take the opportunity to visit the industrial exhibit.

I wish you a great meeting, meeting friends and colleagues from the past, interesting interactions in the present, and lot of ideas for future research.
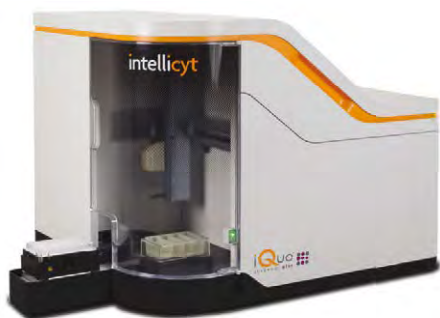
Yours,

Hyun-Dong Chang

President DGfZ

Content

Program Overview

Wednesday, October 7, 2015

9:00am - 12:00pm   **Core Managers Meeting**
DRFZ, Seminar Room 3

9:00am - 12:00pm   **Tutorials**
DRFZ, Seminar Room 1

Attila Tárnok   Publishing Cytometric Data

Andreas Grützkau   Multi-Colour Flow Cytometry

Benjamin Tiburzy   Analysis of Intracellular and Nuclear Factors

12:30pm - 12:45pm   **Welcome**
Paul-Ehrlich Lecture Hall
Hyun-Dong Chang

12:45pm - 2:15pm   **Session 1: Cells in Situ**
Paul-Ehrlich Lecture Hall
Chairs: Raluca Niesner, Attila Tárnok

Matthias Gunzer   Whole organ tomography and live cell imaging in novel animal models
to study the physiology of neutrophil granulocytes

Thomas Schüler   The regulation of CD8+ T cell responses by Interferon-γ

Willem Corver   Near-Haploidisation in Thyroid Cancer significa tly associates with the
Oncocytic Phenotype but not with Mitochondrial DNA Mutations

Randy Lindquist   Intestinal epithelial cells fragment upon isolation, and their debris is
taken up by intestinal epithelial lymphocytes or other epithelial cells

2:15pm - 2:45pm   **Coffee Break**   **Atrium Cross Over**

2:45pm - 4:15pm   **Session 2: Cells on the Move**
Paul-Ehrlich Lecture Hall
Chairs: Thomas Kroneis, Michael Nüsse

Ellen Heitzer   Insights into tumor heterogeneity and progression by the analysis of
circulating tumor cells

Anja Hauser   Dynamics of bone marrow survival niches for long-lived plasma cells

Shukun Chen   Characterization of circulating tumor cells isolated by using a functionalized *in vivo* detector: from enumeration to single cell analysis

Mairi A. McGrath   Cytometric determination of the mobility of memory CD4 lymphocytes

4:15pm - 5:30pm   **Product Slam**
Paul-Ehrlich Lecture Hall
Chairs: Frank Schildberg, Elmar Endl

All exhibitors   3-minute presentations about new products and technologies

5:30pm - 6:00pm   **Coffee Break   Atrium Cross Over**

6:00pm - 7:00pm   **Guest Lecture**
Paul-Ehrlich Lecture Hall
Chair: Jörg Hemmer

Emily Baird   Insect navigation: How miniature brains solve giant problems

7:00pm - 9:00pm   **Welcome Reception**
DRFZ Lobby

8:00pm - 10:00pm   **Core Managers Workshop**
DRFZ - Seminar Room 1
Chair: Elmar Endl

Thursday, October 8, 2015

9:00am - 10:30am   **Session 3: Cytometry of Microbial Communities**
Paul-Ehrlich Lecture Hall
Chairs: Christin Koch, Susann Müller

Josep Gasol   Usage of fl w cytometry to diagnose aquatic environment health by looking at the microbes

Susanne Günther   Assessing responsiveness towards changes in environmental factors in complex microbial communities

Susanne Dunker   Phenotypic plasticity and community dynamics of phytoplankton organisms revealed by fl w cytometry

Jakob Zimmermann   High-resolution fl w cytometry reveals dysbiosis in murine inflamm tory bowel disease

10:30am - 11:00am　Coffee Break　　Atrium Cross Over (CCO) _ _ _ _ _ _ _ _ _ _ _ _

11:00am - 12:30pm　Session 4: Nanotechnology
Paul-Ehrlich Lecture Hall
Chairs: Wolfgang Fritzsche, Ulrike Taylor

Vladimir Zharov　Recent advances in *in vivo* fl　w cytometry

Matthias Epple　Silver as antibacterial and cytotoxic agent: Nanoparticles, ions, and silver alloys

Mareike Klinger-Strobel　Blue fluo escent labeling of PLGA for particle tracking in LIVE/DEAD® stained pathogenic biofilm

Susanne Melzer　Image cytometric analysis of gold nanoparticle uptake by macrophages

Judith Krawinkel　Interaction of laser irradiation with peptide-conjugated gold nanoparticles for intracellular molecule delivery

12:30pm - 2:00pm　Lunch & Poster　Auditorium Cross Over _ _ _ _ _ _ _ _ _ _ _ _ _

1:00pm - 1:30pm　Poster Session Track A
Auditorium Cross Over
Chairs: Leoni Kunz-Schughart, Torsten Viergutz

1:30pm - 2:00pm　Poster Session Track B
Auditorium Cross Over
Chairs: Leoni Kunz-Schughart, Torsten Viergutz

2:00pm - 3:30pm　Session 5: High Content Analysis
Paul-Ehrlich Lecture Hall
Chairs: Andreas Radbruch, Cees Cornelisse

Bernd Bodenmiller　Imaging mass cytometry: A novel imaging modality to visualize dozens of biomarkers in a targeted and simultaneous manner in tumor samples.

Robert F. Murphy　Building models of cell organization, differentiation and perturbation directly from microscope images

Dieter G. Weiss　A novel functional *in vitro* phenotypic screening assay for detecting anti-Parkinson drugs using neuronal networks derived from dopaminergic human iPSCs

Kristen Feher　Comparison of fl　w cytometry samples using dimension reduction and binning

4:00pm - 5:30pm   Session 6: Iberian Society Guest Session
Paul-Ehrlich Lecture Hall
Chairs: Andrea Cossarizza, Hyun-Dong Chang

Jordi Petriz   Searching for the hematopoietic stem cells: Functional heterogeneity of CD34 positive and CD34 negative cells by co-staining with Vybrant® DyeCycleTM Violet (DCV) and an Alkaline phosphatase live stain

Julia Almeida   Flow cytometry immunophenotyping of hematological malignancies: recent advances and future challenges

Jose-Enrique O'Connor Analysis by Real-Time Cytometry of the Interaction between Nitric Oxide and Superoxide Anion in Hematic and Tumor Cells

Francisco Sala-de-Oyanguren A novel phagocytosis assay in whole blood by fl w- and imaging cytometry with GFO-Expressing bacteria

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

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

Friday, 09/Oct/2015

9:30am - 11:00am   Session 7: Immunology
Paul-Ehrlich Lecture Hall
Chairs: Gergely Toldi, Günter Valet

Andreas Thiel   Few Parameter Cytometry – How to survive in the Century of Big Data

Leo A. Hansmann   Multiple myeloma – from phenotypes to relationships

Dirk Mielenz   Reduced fluo escence versus forward scatter time-of-flig t and increased peak versus integral fluo escence ratios indicate receptor clustering in fl w cytometry

Kerstin v. Kolontaj   Automatic measurement of early T cell activation via Flow Cytometry FRET

Anna Nowak   Lack of CD154 expression discriminates stable from instable Foxp3-expressing CD137+ Treg

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

11:30am - 1:00pm   Session 8: Emerging Technologies
Paul-Ehrlich Lecture Hall
Chairs: Leoni Kunz-Schughart, Wolfgang Göhde

AYOXXA   Multiple Cytokine Detection: three dimensional assay – two dimensional read-out

Beckman Coulter   Beckman Coulter CytoFLEX Research Analyser, Exceptional Performance In A Surprisingly Small Instrument

Beckton Dickinson 20 Colors And Beyond

eBioscience   Assessment of early kinetics of sequential gag mRNA and protein expression following HIV-1 latency reversal using PrimeFlow RNA technique

Merck Millipore   Imaging Flow Cytometry Enhances the Detection of Small Particles and Rare Events Enabling Emerging Applications in Immunology and Oncology

Zellkraftwerk   High-Content Cytometry for Translational Research

1:00pm - 2:00pm   Lunch   Atrium Cross Over

2:00pm - 3:30pm   Session 9: Klaus Görttler Session
Paul-Ehrlich Lecture Hall
Chairs: Anna Rao, Alexander Grünberger

Frank Delvigne   Bet-hedging in bioprocesses deciphered by on-line fl w cytometry

Walter Schubert   The human toponome project: translating the spatial protein network code (Toponome) into effici   t therapies

3:30pm - 4:30pm   Keynote: „Meet the Expert"
Paul-Ehrlich Lecture Hall
Chair: Hyun-Dong Chang

Paul S. Frenette   Dissecting hematopoietic stem cell niches

4:30pm   Farewell

We highly appreciate the support by:

WLAN password Atrium Cross Over

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General Information

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German Society for Cytometry (DGfZ)

DGfZ
Deutsche Gesellschaft
für Zytometrie

The Society of Cytometry (Gesellschaft fuer Zytometrie, GZ) was founded in 1989 in Heidelberg (Germany) by the Foundation Council represented by Cess 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 basically in the field of fl w and image cytometry. Founding members were scientists whose personal scientific development was and is still closely interlinked with the development of cytometric technologies in Europe.

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Dr. Hyun-Dong Chang
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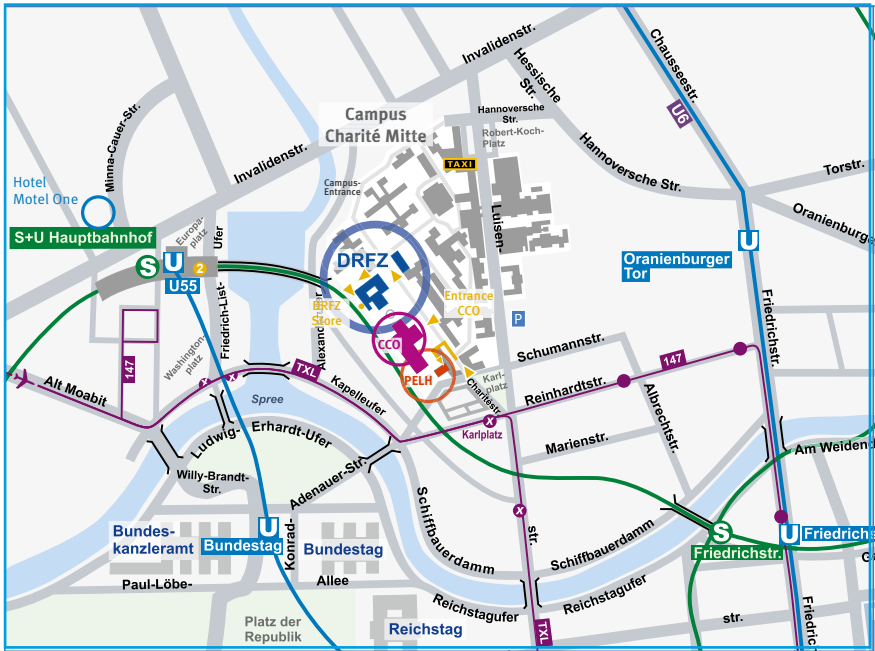Research – UFZ, Leipzig

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Dr. Frank Schmidt
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Sitemap meeting venue and hotel

Charité Campus Mitte, Charitéplatz 1, 10117 Berlin

DRFZ: Virchowweg 11+12

CCO - Ch(arité)CrossOver, Virchowweg 6

PELH - Paul-Ehrlich-Lecture Hall, Virchowweg 4

Approach with local trafic

From Airport Berlin-Schönefeld: (approx. 30 min.) Train RE7 or RB14 t o „S+U Hauptbahnhof"

From Berlin Hauptbahnhof: ❷
Walkway (approx. 8 min.) ➡➡
or Bus 147 (dir ection: „U-Bhf Markisches Museum") to "Schumanns❶aße"
or Bus TXL (direction: „Alexanderplatz") to „Karlplatz"

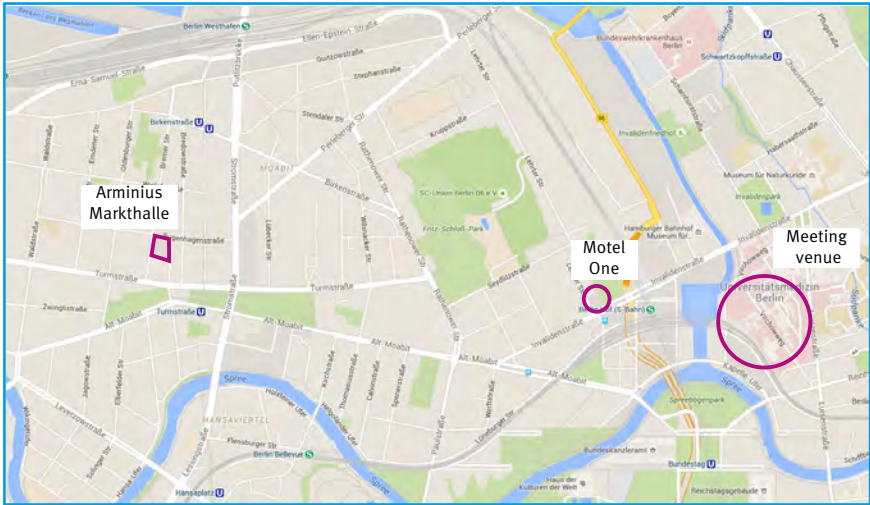**Mor information about public transfer in Berlin: www.bvg.de**
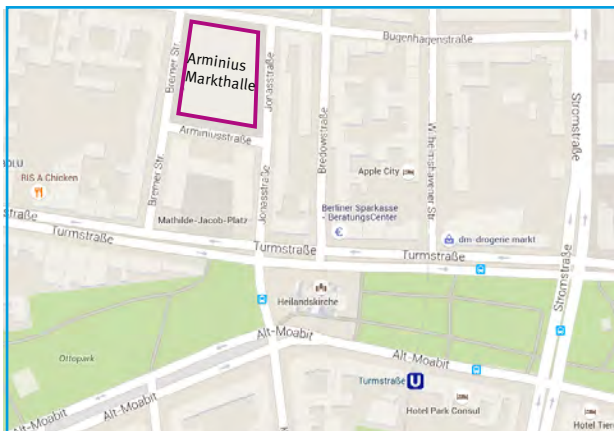**Infomation about Berlin: www.visitberlin.de**

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Conference dinner

To get to the conference dinner venue, please take the bus line "TXL" from the stop "Karlplatz" to "U-Turmstrasse" or line "123" from "Hauptbahnhof" to "Rathaus Tlergarten". From both stops it is just a short walk to the Arminius Markthalle.

↓ entrance

Floor plan exhibition

Atrium im CCO

Corridor to labs

Corridor to labs

WC   Corridor to labs

Corridor to labs

Corridor
to labs

05 VyCap	04 Chemo-Metec	03 eBio-science

11 Ayoxxa	10 Biozol
17 APE	16 OLS

23 Beckton Dickinson
22 Merck Millipore

Coffee

⊙

09 Bio-Rad	08 Fluidigm
15 Cenibra	14 Sony

21 AHF	20 Thermo-fisher

02 Miltenyi Biotec	01 Beckmann Coulter 9 m²

07 Jackson	06 Propel-Labs
13 Biolegend	12 IntelliCyt

19 Olympus	18 Sysmex

Poster session

Catering area

Elevators

main entrance

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We are most grateful to our Sponsors and Exhibitors

Core Managers Meeting

9:00am - 12:00pm   DRFZ Seminar Room 3

Chair: Elmar Endl, Bonn

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

Tutorials

9:00am - 12:00pm   DRFZ Seminar Room 1

Attila Tárnok, Leipzig, Germany: Publishing Cytometric Data

Andreas Grützkau, Berlin: Multi-Colour Flow Cytometry

Benjamin Tiburzy, Biolegend: Analysis of Intracellular and Nuclear Factors

Session 1: Cells in Situ

9:00am - 2:00pm   Paul-Ehrlich Lecture Hall

Chair: Raluca Niesner, Berlin

Chair: Attila Tárnok, Leipzig

Modern biosciences and biomedicine currently agree that the full understanding of central physiologic and pathologic processes implies, next to the use of well established measurement techniques on cell lysates and isolated cells, the employment of technologies that allow monitoring "cells in situ", i.e. in their genuine context – the living organism. In the past two decades several approaches have been developed and successfully applied. The present session focuses on the use of intravital multi-photon microscopy and whole-body-imaging as well as on associated transgenic mouse technology as unique tools to answer key-questions in immunology.

Whole organ tomography and live cell imaging in novel animal models to study the physiology of neutrophil granulocytes

Matthias Gunzer, Duisburg-Essen

Institute for Experimental Immunology and Imaging, University Duisburg-Essen

Neutrophil granulocytes are the most frequent leukocyte subtype in the human body. Their lack or dysfunction is associated with severe health impairments. We have been studying neutrophil function under physiological and a diverse set of pathological conditions involving infectious as well as sterile inflamm tion. I will show, how neutrophils migrate in the bone marrow and in inflamed tissues and how this is explained on a molecular level. We have studied this using traditional and also newly developed animal models. From a methodological perspective we have employed advanced microscopy procedures such as intravital 2-photon and, more recently, light sheet imaging. While 2-Photon provides a direct access to cellular motility and subcellular dynamics *in vivo*, lightsheet microscopy opens the view to amazing new insights on the 3-D structure of whole organs, again in the context of normal and disease-affected conditions.

The regulation of CD8+ T cell responses by Interferon-γ

Thomas Schüler, Magdeburg

Institut für Molekulare und Klinische Immunologie, Otto Von Guericke University

In response to primary antigen contact, naïve mouse $CD8^+$ T cells (T_N) undergo clonal expansion and differentiate into effector T cells (T_E). After pathogen clearance, most T_E die and only a small number of memory T cell precursors (T_{MP}) survive to form a pool of long-lived memory T cells (T_M). Although high- and low-affin y $CD8^+$ T cell clones are recruited into the primary response, the T_M pool consists mainly of high-affin y clones. Whether the more effici t expansion of high-affin y clones and/or cell-intrinsic processes exclude low-affin y T cells from the T_M pool remains unclear. Here we show that the lack of Interferon-γ receptor (IFN-γ) signaling in $CD8^+$ T cells promotes T_M formation in response to weak, but not strong, T cell receptor (TCR) agonists. The IFN-γ-sensitive accumulation of T_M correlates with reduced mTOR activation and the accumulation of long-lived $CD62L^{hi}Bcl-2^{hi}Eomes^{hi}$ T_{MP}. Reconstitution of mTOR or IFN-γR signaling is suffici t to block this process. Hence, our data suggest that IFN-γR signaling actively blocks the formation of T_{MP} responding to weak TCR agonists thereby promoting the accumulation of high-affin y T cells finally domin ting the T_M pool.

Near-Haploidisation in Thyroid Cancer signifi antly associates with the Oncocytic Phenotype but not with Mitochondrial DNA Mutations

Willem Corver, Leiden, The Netherlands

Willem Corver van Wezel, Tom; Molenaar, Kees; Schrumpf, Melanie; van den Akker, Brendy; van Eijk, Ronald; Ruano Neto, Dina; oosting, Jan; Morreau, Hans. 1-9 Leiden University Medical Center, The Netherlands

Background

Oncocytic mitochondria-rich tumours are most frequently found in the thyroid. The accumulation of mitochondria is thought to be caused by disruptive and/or damaging mutations in the mitochondrial genome, which initiates uncoupling and subsequent mitochondrial proliferation. We demonstrated recently that oncocytic thyroid tumours frequently show near-haploidisation and endoreduplication which manifests as a near-homozygous genome (NHG). Now we extended our observations to a large series of thyroid tumours and included mitochondrial DNA (mtDNA) mutation analysis and also studied nuclear genes directly or indirectly associated with cellular metabolism.

Experimental design: In total 88 thyroid tumours of varying histology were studied. Genome-wide high-density SNP array and fl w cytometric DNA content analysis were combined to determine the chromosomal dosage (allelic state). The entire mitochondrial genome of 45 tumours (oncocytic and non-oncocytic) was studied for mutations. These tumours were also studied for IDH1/2 mutations. All tumours were also characterized for frequently observed somatic mutations in EGFR, BRAF (V600E), RAS, PIK3CA.

Results

An NHG was identified in 16/88 thyroid tumours. An oncocytic metaplasia was found in 33/88 thyroid tumours. Strikingly, all 16 tumours with a NHG were oncocytic. This correlation showed to be highly significa t (P = 0.0001, two-tailed Fisher's exact). One or more damaging or disrupting mtDNA mutations were found in 62.2% (28/45) of the tumour samples. No correlation was found between these mtDNA mutations and the oncocytic phenotype or a NHG (P = 0.3414 and P = 0.1401, respectively). The BRAF V600E mutation was found in 22 cases (20 PTC, 2 ATC). The NRAS Q61R was found in 7 cases (varying histology). IDH, PIK3CA and HRAS mutations were found sporadically and EGFR mutations were not found. None of the mutations in the nuclear encoded genes correlated with the oncocytic phenotype or a NHG.

Conclusions

A NHG correlates significa tly with oncocytic thyroid tumours. In contrast, nuclear encoded genes associated with cellular metabolism do not correlate with the oncocytic phenotype or with a NHG. This also accounted for damaging and or disrupting mtDNA mutations which were frequently found in oncocytic and non-oncocytic thyroid tumours. A combination of unknown factors likely underlies a NHG in a subset of oncocytic thyroid tumours.

Intestinal epithelial cells fragment upon isolation, and their debris is taken up by intestinal epithelial lymphocytes or other epithelial cells

Randy L. Lindquist, Berlin

Lindquist, Randy L. (1); Sarmadi, Jannike Bayat (1); Kriegel, Fabian (1); Hauser, Anja E. (1,2). Organization(s): 1: DRFZ, Berlin; 2: Charité Universitätsmedizin, Berlin

Gastrointestinal tissue has a complex milieu of cells, with a relatively high proportion of "unconventional" immune cells. Intraepithelial mononuclear phagocytes and intraepithelial lymphocytes (IELs) normally maintain tolerance to food and commensal bacteria-derived antigens, while protecting against pathogens. However, in response to inflamm tory signals, IELs can also induce epithelial damage directly, and by recruiting and stimulating inflamm tory leukocytes.

In standard GI tissue preparations, a significa t proportion of IELs are positive for EpCAM by FACS, but EpCAM expression does not correspond to any identifiable subpopulation. Further, many CD45+ cells in the lamina propria, which would not be expected to interact with IELs, are also EpCAM-positive by FACS. Peyer's patch leukocytes, however, are only rarely EpCAM+. Immunostaining of frozen sections revealed that EpCAM was expressed brightly throughout the epithelial layer, but was absent from the LP. By imaging cytometry, EpCAM on IEL T cells has a punctate appearance at the cell membrane, often colocalizing with a bleb in bright fiel or scatter images. These EpCAM+ blebs are not themselves positive for Annexin V or for cell-impermeable live/dead stains, but are often found on cells positive for Annexin V or a live/dead stain. We suspect that this EpCAM is in fact derived from epithelial cell-derived blebs, not from the IELs themselves.

When we examined the cytotoxicity of various IEL subsets, in some experiments we unexpectedly found high frequencies of dead cells in the absence of stimulation. By imaging cytometry, over 80% of the cells positive for live/dead staining in some samples were in fact conjugates of a live target cell with a dead bleb. Doublet discrimination in conventional fl w cytometry using time-based parameters fails to distinguish these conjugates from actual dead cells. However, imaging cytometry is able to unambiguously resolve dead cells from conjugates of live cells and dead cells. These results indicates that fl w cytometric assays of cytotoxicity should be interpreted with caution, especially when several different populations are being compared simultaneously, and underline the importance of imaging cytometry for these assays.

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Session 2: Cells on the Move

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

Chair: Thomas Kroneis, Graz, Austria

Chair: Michael Nüsse, Frankfurt

Migration is a key feature of a subset of human cells and fundamental to a number of processes. In this session we will have two key talks with migration playing an important role: One is based on migration as a malignant key aspect of cancer cells escaping primary tumors giving rise to circulating tumor cells (CTCs) and, subsequently, metastasis. The main aspect discussed will be tumor heterogeneity and how the analysis of cells on the move (CTCs) may contribute to trace cancer.

Second, both immobility and high mobility plays a key role in memory plasma cells: Responsible for providing stable antibody titers, memory plasma cells home to special niches in the bone marrow. Maintaining their environment is associated with cells that stay within this microenvironment and cells that need to be recruited. Hence, this talk will focus on the dynamics of the bone marrow with regard to its survival niches for plasma cells.

Furthermore, the session will provide room for additional contributions related to cells on the move.

Insights into tumor heterogeneity and progression by the analysis of circulating tumor cells

Ellen Heitzer, Graz, Austria

Institute of Human Genetics, Medical University of Graz, Graz, Austria

Circulating tumor cells (CTC) are shed into the bloodstream from primary and metastatic tumor deposits and refle t the current status of tumor genotype. Therefore, clinical applications of CTC analysis include the identific tion of prognostic, predictive, and pharmacokinetic biomarkers. Due to the fact the tumors are known to be genetically heterogeneous and to constantly evolve during progression or under the selective pressure of a given therapy, the analysis of CTCs can provide new biological insights into the tumor heterogeneity and tumor progression.

However, a major hurdle in CTC analysis is that CTCs constitute as few as 1 cell per 1 × 109 normal blood cells in patients with metastatic cancer, and therefore it is difficu to identify and isolate them. Furthermore, sequencing of single-cell amplific tion products can be prone to experimental and technical errors. Therefore there is need for unbiased amplific tion methods for the analysis of single cells. This talk will briefl cover our experiences with different single cell sequencing kits and new strategies for CTC detection and isolation.

Furthermore, results of a recent study of our group will be presented, in which we were able to reconstruct complex tumor genomes from the peripheral blood of patients with colorectal cancer. We showed that we can elucidate relevant changes in the tumor genome refle ted CTCs that had either not been present or not been observed at the time of initial diagnosis. In addition, we observed a tremendous heterogeneity in CTCs of patients and many mutations were only traced back to the tumor sample after ultra-deep sequencing. These results may pave the way to use CTCs as a liquid biopsy in patients with cancer, providing more effective options to monitor tumor genomes that are prone to change during progression, treatment, and relapse.

Dynamics of bone marrow survival niches for long-lived plasma cells

Anja Hauser, Berlin, Germany

Deutsches Rheuma-Forschungszentrum Berlin, ein Institut der Leibniz-Gemeinschaft

Long-lived memory plasma cells contribute essentially to protective immunity by securing the maintenance of stable antibody titers over extended time periods. However, they can also be harmful in the case of certain antibody-mediated autoimmune conditions. Plasma cells are able to survive for months to years in a special microenvironment, the survival niche in the bone marrow. Several cell types have been described to contribute to plasma cell maintenance within these niches: plasma cells have been reported to contact bone marrow reticular stromal cells secreting CXCL12, a chemokine which has not only been shown to mediate the migration of plasmablasts to the bone marrow, but also prolongs plasma cell survival *in vitro*. Whether CXCL12 is actually needed to retain plasma cells in their niches is unclear. Additionally, several hemato¬poietic cell types have been described to contribute to the survival niche *in vivo*. Among them are eosinophils, which provide the soluble survival factor APRIL. Most cell types of hematopoietic origin show a high turnover, thereby raising the question of how a stable supply of plasma cell survival factors by these accessory cells is guaranteed. We studied the interactions of plasma cells with reticular stromal cells and hematopoietic accessory cells and the turnover of the different cell types in the microanatomical context of the plasma cell niche.

To this end, we combined a mouse model for the visualization of stromal cells, confocal microscopy and developed an automated image analysis approach to quantify the interactions of bone marrow plasma cells with reticular stromal cells and various hematopoietic cell types in situ.

To test whether the observed co-localization counts were the result of a random tissue distribution of these cells, we developed a tool simulating random bone marrow cell positioning. Plasma cells were predominantly contacting reticular stromal cells in a non-random fashion, while eosinophils were not contacting, but neighboring one third of plasma cells. By intravital microscopy, we show for the first time *in vivo* that bone marrow plasma cells are sessile and form stable contacts with stromal cells. In vivo proliferation analysis of reticular stromal cells of the survival niches revealed that they were non-proliferative and represent a stable niche component. In contrast, accessory eosinophils displayed a high turnover and proliferated vigorously. If accessory cells were depleted by irradiation, newly generated eosinophils localized in the vicinity of surviving plasma cells and stromal cells, supporting the idea of a stable microenvironment being maintained through attraction of accessory cells, probably organized by static reticular stromal cells.

Characterization of circulating tumor cells isolated by using a functionalized *in vivo* detector: from enumeration to single cell analysis

Shukun Chen, Graz, Austria

Chen, Shukun1); El-Heliebi, Amin (1); Wendt, Gabi (2); Krusekopf, Solveigh (2); Lücke, Klaus (2); Ståhlberg, Anders (3); Kroneis, Thomas (1,3); Sedlmayr, Peter (1). 1: Institute of Cell Biology, Histology and Embryology, Medical University of Graz, Austria; 2: GILUPI GmbH, Potsdam, Germany; 3: Institute of Biomedicine, Sahlgrenska Cancer Center, University of Gothenburg, Sweden

Background

An increasing number of studies suggest that circulating tumor cells (CTCs) may constitute the most potential biomarker for prognostic and therapeutic effect evaluation in cancer. In recent years new techniques for CTC isolation are constantly emerging. The GILUPI CellCollector, type CANCER01® (DC01, GILUPI GmbH, Potsdam, Germany), a medical wire functionalized with anti-EpCAM antibodies, was first described in 2012 and has been CE certified as medical device by the notifie body for applications in Europe. Unlike other methods detecting CTCs from volume-limited sample, the Detektor allows *in vivo* isolation and enumeration of CTCs directly from the blood stream. In our ongoing clinical trial, the DC01 is applied on high-risk prostate cancer patients for CTC enumeration for the purpose of evaluating its performance compared to other techniques (EPISPOT assay, CellSearch). However, when it comes to personalized medicine, analyses exclusively based on enumerating CTCs are rather insuffici t. Hence, in a second approach we aim at retrieving single CTCs for downstream analysis using an improved device ("catch and release", C&R). This new device, which currently awaits CE certific tion, was evaluated *in vitro* with respect to molecular characterization of single cells recovered from the detector.

Methods

1) In the clinical study, the DC01 was exposed to patient's peripheral blood for 30 min. Cells captured by DC01 were stained directly on-Detektor by immunofluo escence (IF).

Cells characterized as cytokeratin positive, CD45 negative, and nucleus positive. were counted as CTCs.

2) In the *in vitro* approach cultured HT-29 cells were used to test the C&R Detektor. Captured cells were visually checked and released from the new device using a special release buffer. Recovered cells were collected onto chamber slides, picked by micromanipulation and subjected to RT-qPCR or single-cell whole genome amplific tion (WGA). WGA products were forwarded to single-cell array comparative genome hybridization (array-CGH) to characterize the recovered cells on the genomic level.

Results

1) So far, 18 of 30 (60%) patient samples were positive for at least one CTC (range 1-6 CTCs) representing a higher enrichment efficie y as compared with other techniques used in parallel.

2) Cells captured by the C&R device could be released with an efficie y ranging from 50% to 96%. Detached cells could be recovered at rates of 12% to 50% (recovering efficie y) for downstream analysis. Array-CGH profiles of recovered single cells shared similar gains and losses compared to both bulk as well as non-treated control cell genomic HT-29 DNA. This indicates that DNA quality of detached cells was not altered by catch & release procedure. RT-qPCR of recovered single cells or cell pools yielded data in the range expected for single cells and cell pools analysis indicating that we have no

issues with RNA quality which in turn allows for downstream gene expression analysis.

Conclusions

The GILUPI CellCollector CANCER01® represents a promising tool for detection of CTCs. Our new approach using the C&R detector proved to be valuable for both genomic and transcriptomic single-cell analysis. Further *in vitro* studies will be focused on array-CGH and RT-qPCR analysis using prostate cancer cell lines where we aim at developing prostate cancer specific assays for the purpose of personalized medicine.

Cytometric determination of the mobility of memory CD4 lymphocytes

Mairi A. McGrath, Berlin

Mairi A. McGrath1, Francesco Siracusa1, Katrin Lehmann1, Markus Bardua1, Anja E. Hauser1,2, Koji Tokoyoda1, Hyun-Dong Chang1, Andreas Radbruch1. 1: DRFZ, Germany; 2: Charité Universitätsmedizin, Germany

We have developed the concept that long-term immunological memory is maintained by resting memory cells in dedicated survival niches of the bone marrow (BM). While we have found that BM memory CD4 T cells are quiescent in terms of proliferation and gene expression in their resting state, it has remained unclear as to whether these cells are long-term residents of the bone marrow, or if they maintain the ability to re-circulate between the blood and tissue. Therefore, we have developed a cytometric approach to obtain a "snap-shot" of cellular mobility of BM memory CD4 T cells in their resting state and following re-activation.

Using such an approach, we addressed 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, confi ming that they were unlikely to leave the BM in their resting state. Furthermore, we found that the BM niche is critical in maintaining BM memory CD4 T cells as they survived extremely poorly in ex vivo survival assays without the support of stromal cells.

Following re-activation, we found that BM memory CD4 T cells rapidly proliferated, as assessed by up-regulation of the proliferation marker Ki67. Moreover, we found that a number of mobility related molecules were altered in BM memory CD4 T cells following re-activation, including up-regulation of F-actin, which is essential for the ability of T cells to migrate within tissue. Despite this apparent mobilization of BM memory CD4 T cells after re-activation, we did not see a decrease in the number of BM memory CD4 T cells, suggesting that not all activated T cells left the BM following re-activation.

Overall, using this cytometric determination of mobility we believe we can confi m the novel concept that immunological short-term memory is maintained by memory cells circulating between blood and secondary lymphoid organs, while immunological long-term memory is maintained by immobile cells, resting in dedicated survival niches of the bone marrow, and most likely other organs, as tissue-resident memory cells. This new understanding of immunological memory impacts tremendously on the development of vaccination strategies, and on the development of therapies against immune-mediated diseases, like chronic inflamm tion.

Notes

Product Slam

4:15pm - 5:30pm, Paul-Ehrlich Lecture Hall

Chair: Frank Schildberg, Boston, USA

Chair: Elmar Endl, Bonn, Germany

All exhibitors are invited to give a 3-minute presentation about new products and technologies

Guest Lecture: Emily Baird

6:00pm - 7:00pm   Paul-Ehrlich Lecture Hall

Chair: Jörg Hemmer, Nersingen

It is a popular misconception that evolution leads to perfection. One reason is the burden of ancestry. Take the evolution of eyes. Vertebrates and arthropods went different ways, by that constraining adaptation to the modification of inherited structures. Both the lensed eyes of vertebrates (and molluscs) and the compound eyes of arthropods have functional advantages and flaws. The winner for low light conditions, however, is the lensed eye. But how do dung beetles navigate through pitch-black night, and how may certain nocturnal bees breeze through the densest undergrowth without risking injury, and which role does the central nervous processing capacity play in overcoming poor optical instrumentation?

At least some answers are given by our guest speaker, Professor Emily Baird. Born and raised in Australia, she started her academic career at Australian National University, Canberra, where she obtained her Ph.D. in 2007 with a study on how honeybees visually control their flight. After a six-months postdoctoral fellowship at Bielefeld University, Germany, she moved to Lund University in Sweden, where she joined the Lund Vision Group, one of the world leading research clusters in comparative visual science. Emily Baird will introduce us into the fascinating visual world of insects, which is so fundamentally different from ours. However, extracting information at the edge of visibility: that is where entomology meets cytometry.

Insect navigation: How miniature brains solve giant problems

Emily Baird, Lund, Sweden

Lund Vision Group, Department of Biology, Lund University

The brains of most insects are smaller than a grain of rice, yet they are capable of using these tiny processors to achieve the most astounding feats of navigation. South African dung beetles, for example, use the Milky Way to orient along straight lines when rolling their dung balls across the Savannah on moonless nights: they are currently the only animals known to use this celestial cue as a compass. On the other side of the world, the orchid bees of Central America can navigate over tens of kilometers through the densely cluttered tropical rainforest in the search for scent from spatially rare orchid fl wers. No man-made systems are capable of performing these or similar feats with such limited sensors and processing power, so how do the beetles and bees, with their tiny brains and sensory systems do it?

The ultimate goal of my research is to answer this question by describing – through anatomical, physiological and behavioural analyses, as well as computer modelling, simulation and robotics – the visual specialisations and behavioural strategies that enable insects to orient and navigate in the natural world. In this talk, I will present some of my research group's latest finding that help us to understand how miniature brains cope with giant problems.

Core Managers Workshop

8:00pm - 10:00pm   DRFZ, Seminar Room 1

Chair: Elmar Endl, Bonn

The workshop is aimed at managers and staff of research core facilities to share knowledge and experience among people that run or work within a core facility.
Moreover we will discuss better ways to establish collaborations among the core managers by modern means of networking. Managers, core staff, and industrial partners are all invited to participate.

Session 3: Cytometry of Microbial Communities
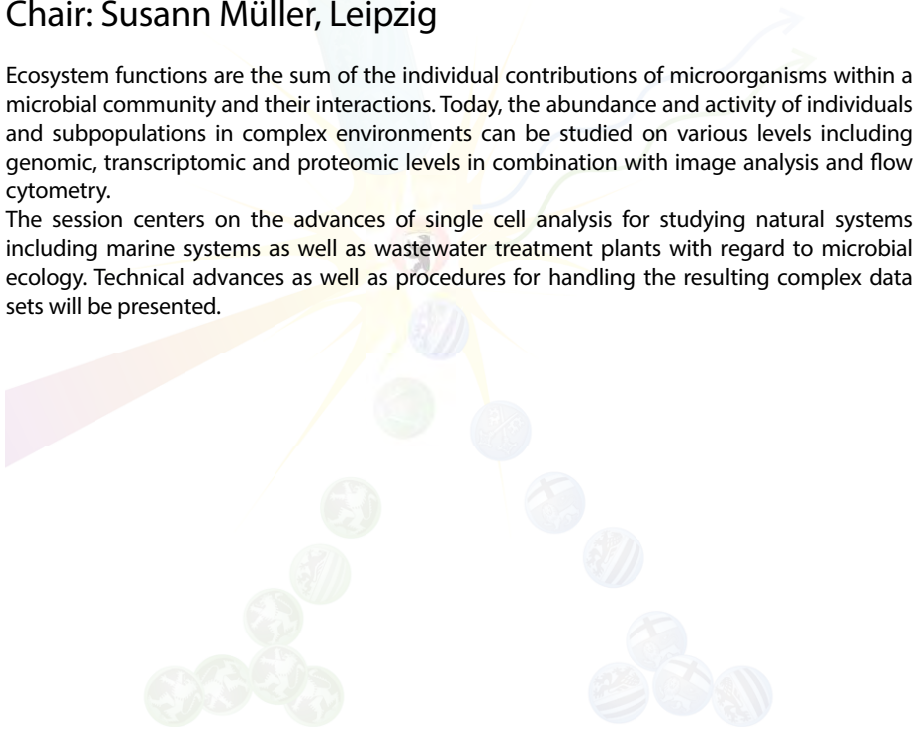
9:00am - 10:30am   Paul-Ehrlich Lecture Hall

Chair: Christin Koch, Leipzig

Chair: Susann Müller, Leipzig

Ecosystem functions are the sum of the individual contributions of microorganisms within a microbial community and their interactions. Today, the abundance and activity of individuals and subpopulations in complex environments can be studied on various levels including genomic, transcriptomic and proteomic levels in combination with image analysis and flow cytometry.

The session centers on the advances of single cell analysis for studying natural systems including marine systems as well as wastewater treatment plants with regard to microbial ecology. Technical advances as well as procedures for handling the resulting complex data sets will be presented.

Usage of fl w cytometry to diagnose aquatic environment health by looking at the microbes

Josep M Gasol, Barcelona, Spain

Departament de Biologia Marina i Oceanografia, nstitut de Ciències del Mar-CMIMA

Flow cytometry has the potential to enumerate almost simultaneously all the microbes (algae, protozoa, viruses, prokaryotes) inhabiting aquatic environments, as well as the interaction of these microbes with inorganic particles. In addition, some derived parameters within each microbe category (such as the high-nucleic acid content vs. low-nucleic acid content bacteria) obtained with the use of physiological probes, inform us about the relative activity and physiological state of some of the populations. All these methods allow for various strategies that can inform us about the ecological state of a given body of water without the need for cultivation.

I will present examples based on i) the presence or absence of some specifi populations; ii) the number and position of the different populations, including different types of fl w cytometric diversity measurements, and iii) various population abundance ratios. These various indices of ecological function can be used to test for the health of aquatic environments. The easy of use, affordability and transportability (including the existence of in situ machines) of fl w cytometers make these techniques serious and robust candidates for the standard monitoring of aquatic environments.

Assessing responsiveness towards changes in environmental factors in complex microbial communities

Susanne Günther, Leipzig

Helmholtz Centre for Environmental Research, Environmental Microbiology, Leipzig

Circulating tumor cells (CTC) are shed into the bloodstream from primary and metastatic tumor deposits and refle t the current status of tumor genotype. Therefore, clinical applications of CTC analysis include the identific tion of prognostic, predictive, and pharmacokinetic biomarkers. Due to the fact the tumors are known to be genetically heterogeneous and to constantly evolve during progression or under the selective pressure of a given therapy, the analysis of CTCs can provide new biological insights into the tumor heterogeneity and tumor progression.

However, a major hurdle in CTC analysis is that CTCs constitute as few as 1 cell per 1 × 109 normal blood cells in patients with metastatic cancer, and therefore it is difficu to identify and isolate them. Furthermore, sequencing of single-cell amplific tion products can be prone to experimental and technical errors.

Therefore there is need for unbiased amplific tion methods for the analysis of single cells. This talk will briefly cover our experiences with different single cell sequencing kits and new strategies for CTC detection and isolation. Furthermore, results of a recent study of our group will be presented, in which we were able to reconstruct complex tumor genomes from the peripheral blood of patients with colorectal cancer. We showed that we can elucidate relevant changes in the tumor genome refle ted CTCs that had either not been present or not been observed at the time of initial diagnosis. In addition, we observed a tremendous heterogeneity in CTCs of patients and many mutations were only traced back to the tumor sample after ultra-deep sequencing. These results may pave the way to use CTCs as a liquid biopsy in patients with cancer, providing more effective options to monitor tumor genomes that are prone to change during progression, treatment, and relapse.

Phenotypic plasticity and community dynamics of phytoplankton organisms revealed by fl w cytometry

Susanne Dunker, Leipzig

iDiv/UFZ Leipzig

Phytoplankton organisms are the main primary producers in aquatic systems and include microalgae as well as cyanobacteria. Toxic cyanobacteria are a common problem of strongly nutrient polluted waters and therefore their impact on phytoplankton dynamics and food chain is important to know.

The physiology and the resulting growth of phytoplankton organisms are strongly dependent on cellular properties, especially on cell size. Different cell sizes mean a trade-off between competitiveness and grazing resistance, because in contrast to large species, small species have a high efficie y of light absorption, good surface/volume-ratio for accumulation of nutrients, but a low grazing resistance and nutrient storage capacity. The size of the organisms can be adapted on species-, but also on community-level. Several cyanobacteria are very small, but they can form aggregates and also many filame tous organisms are known. In which range species can vary cellular properties, depends on their phenotypic plasticity and indicates the adaptability to varying environmental factors.

With variation of different biotic and abiotic factors we tested the phenotypic plasticity and impact on community dynamics under controlled laboratory conditions in contrast to field conditions by the help of fl w cytometry. Therefore three representative organisms of green algae, diatoms and cyanobacteria were cultivated as monoculture and in mixed culture.

It could be shown that the species with highest phenotypic plasticity (*Desmodesmus armatus* - a green algae) was strongly dominant in mixed culture over the cyanobacterium *Microcystis aeruginosa*, whereas another green algae (*Chloroidium saccharophilum*) with a homogenous population and low phenotypic plasticity was overgrown by the cyanobacterium *Synechocystis sp.*. This picture changed during grazing pressure, where *Synechocystis sp.* was mostly outcompeted, but *M. aeruginosa* was still present in mixture, due to toxicity. The data give an insight into the complex regulation of population dynamics under natural conditions and hopefully allow to develop management strategies of nutrient polluted waters in future.

High-resolution fl w cytometry reveals dysbiosis in murine inflamm tory bowel disease

Jakob Zimmermann, Berlin

Zimmermann*, Jakob (1); Hübschmann*, Thomas (2); Schattenberg, Florian (2); Schumann, Joachim (2); Durek, Pawel (1); Radbruch, Andreas (1); Chang°, Hyun-Dong (1); Müller°, Susann (2). 1: DRFZ Berlin, Germany; 2: UFZ Leipzig, Germany

The intestinal microbiota is a complex and heterogeneous consortium of 10e14 total bacteria from several hundred different bacterial species. In mice, the microbiota was shown to be crucial for the control of immunity and immune-mediated diseases such as inflamm tory bowel disease (IBD), a chronic inflamm tory disorder of the gastro-intestinal tract. In humans, the situation is less clear, largely due to the lack of high-throughput analytical and preparative methods to dissect the heterogeneity of the microbiota dynamically and in large cohorts. Currently, most studies of the microbiota rely on qPCR- or next generation-sequencing-based analysis of the bacterial metagenome. This is not only expensive, population-based, and restricted to the genome and transcriptome but does also not allow for the isolation of defined bacteria for further analysis.

Here we present a cytometric approach to unravel the complexity of the intestinal microbiota. High-resolution detection of bacterial forward and side scatter, together with quantific tion of DNA content, allowed us to distinguish up to 80 different bacterial subpopulations from formaldehyde-fi ed, Tween-permeabilized murine stool samples.

Using this method in combination with 16s ribosomal RNA sequencing, we demonstrate for the first time that murine T cell –dependent IBD reveals a strikingly similar dysbiosis pattern compared to the human disease. In murine IBD, overall microbial complexity was reduced and pathobionts like *Enterobacteriaceae* replaced physiological symbionts such as *Bacteroidaceae*, *Lachnospiraceae*, *Rikenellaceae*, and *Ruminococcaceae*. Flow cytometry further revealed that the changes in the fecal microbiota timely coincided with IBD symptoms like weight loss and diarrhea suggesting a causative link between dysbiosis and IBD pathogenesis.

Flow cytometry thus provides unique oportunities for the analysis of the microbiota and its role in disease, at the level of proteome, phenotype and single cells, and with the option to isolate defined bacteria, for functional and molecular analysis, but also for therapeutic corrections of the microbiota in dysbiosis.

*/°equal contribution

Notes

Session 4: Nanotechnology

11:00am - 12:30pm   Paul-Ehrlich Lecture Hall

Chair: Wolfgang Fritzsche, Jena

Chair: Ulrike Taylor, Neustadt

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.
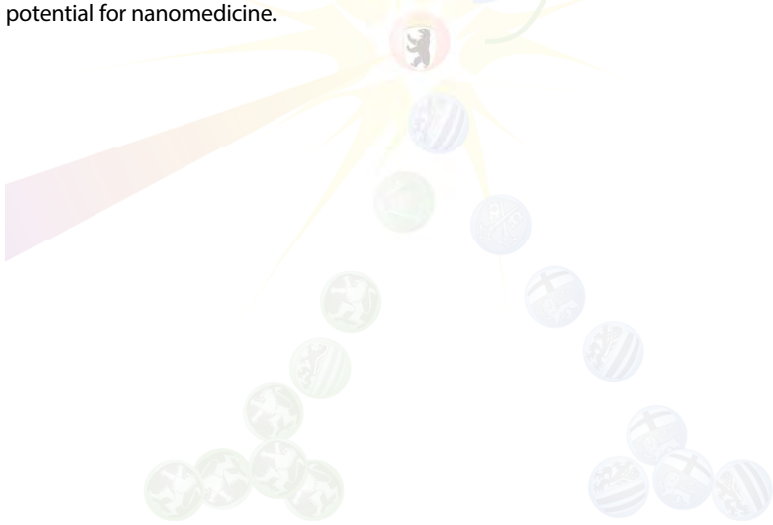
Recent advances in *in vivo* fl w cytometry

Vladimir P. Zharov, Arkansas, USA

Arkansas Nanomedicine Center, Winthrop P. Rockefeller Cancer Institute, University of Arkansas for Medical Sciences, Little Rock, AR

The sensitivity of conventional fl w cytometry is limited by the small volume of blood collected, in which no less than one disease-specific markers can be detected. It leads to missing many thousands of abnormal cells in the whole blood volume (~5 L in adults), which can be suffici t for disease progression to barely treatable or incurable stage. For example, despite enormous efforts to detect circulating tumor cells (CTCs) responsible for 90% of all cancer deaths as a result of the development of deadly metastases, the mortality rates for metastatic cancer have still been almost fl t. To solve this problem we introduced *in vivo* fl w cytometry for detection of rare circulating biomarkers directly in the bloodstream using blood vessels as natural tubes with native cell fl w. In this technology, laser irradiates vessels of interests followed by the detection of photoacoustic (PA) waves from circulating objects using ultrasound transducer attached to the skin. The PA waves are generated either using intrinsic contrast agents (e.g., melanin) or absorbing low toxic plasmonic nanoprobes functionalized to specific markers. In this report we summarize recent advances of new generation of *in vivo* multicolor fl w cytometry platform using multispectral lasers, fibe -based transducers, label-free and/or multiplex molecular targeting, plasmonic probes with ultrasharp PA spectral resonances, *in vivo* magnetic capturing of CTCs, and combination of PA diagnosis with photothermal (PT) elimination of abnormal cells.

The capacity of this new technology was first demonstrated in preclinical models for real-time detection of bulk and stem CTCs, S. aureus, and sickle cells. Then, the clinical prototype of *in vivo* PA fl w cytometry (PAFC) provided the assessment of ~1 L of patient blood during 30-60 minutes. The first clinical studies focused on detection of CTCs and cancer-associated clots in melanoma patients. The PAFC data *in vivo* were validated *in vitro* with conventional fl w cytometry, immunohistochemical staining, and magnetic kits. The obtained results demonstrate that PAFC is more sensitivity (≥100-fold), accurate and rapid (≤ 1 hour) that conventional technique. Unlike typical blood sampling involving extraction of a volume of blood ranging from 10 μL (drop) to a few mL (CTC assays), *in vivo* examination involves nearly the entire volume of blood passing through 1–2-mm-diameter peripheral vessels over 0.5–1 h and thus will enable a dramatic increase in diagnostic sensitivity, ultimately up to 10^3–10^5 times, refle ting the ratio of the volume of blood sampled *in vivo* to that *in vitro*. According to pilot clinical results, the portable personal fl w cytometer can provide a breakthrough in early diagnosis of cancer, infection and cardiovascular disorders with a potential to inhibit, if not prevent, metastasis, sepsis, and strokes or heart attack through the use of well-timed personalized therapy.

Silver as antibacterial and cytotoxic agent: Nanoparticles, ions, and silver alloys

Matthias Epple, Duisburg-Essen

Inorganic Chemistry and Center for Nanointegration Duisburg-Essen (CeNIDE), University of Duisburg-Essen, 45117 Essen, Germany

Silver has a well-known bactericidal effect. This effect has been exploited by mankind since several thousand years. In the last decades, silver nanoparticles have gained increasing importance, e.g. to coat medical implants and consumer commodities. In these cases, they act as depot for a slow release of silver ions. However, silver is also toxic against eukaryotic cells.

The mode of action of silver ions and silver nanoparticles towards bacteria and cells is illustrated and compared. Based on an extensive literature survey and own experiments it is demonstrated that the therapeutic window of silver (critical concentrations for bacteria and cells) is more narrow than typically assumed. Furthermore, there is no evidence of an inherent "nano-effect" of silver nanoparticles. Rather, the dissolution of silver nanoparticles leads to the release of silver ions which constitute the toxic species.

Finally, the tunable biological effect of alloyed silver-gold nanoparticles will be shown.

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Blue fluo escent labeling of PLGA for particle tracking in LIVE/ DEAD® stained pathogenic biofilm

Mareike Klinger-Strobel, Jena

Klinger-Strobel, Mareike (1,2); Ernst, Julia (3); Lautenschlaeger, Christian (4); Pletz, Mathias W. (1,2); Fischer, Dagmar (3); Makarewicz, Oliwia (1,2). 1: Center for Infectious Diseases and Infection's Control, Jena University Hospital, Jena, Germany; 2: Center for Sepsis Control and Care, Jena University Hospital, Jena, Germany; 3: Department of Pharmaceutical Technology, Friedrich-Schiller University Jena, Jena, Germany; 4: Clinic of Internal Medicine IV, Jena University Hospital, Jena, Germany

Bacterial communities are predominantly organized in complex matrix-enclosed sessile structures known as biofilms that are formed on nearly all surfaces. Due to high number of implanted devices (catheters, pacers, artificial joints, etc.) in clinical practice, and immunologically compromised patients in intensive care units, this form of bacterial affection is of high therapeutic relevance. Accrued biofilms are almost impossible to eradicate by common drug treatments since microbes embedded in a biofilm benefi from the protective biofilm environment. In consequence, the only practical measure to achieve focus control in patients with life-threatening biofilm associated infections is to surgically remove the implant or infested body part (e.g. heart valves) – imposing the risk of complications for the individual patient and increasing health care costs. Therefore, alternative therapeutic strategies with controlled biofilm eradication would offer great benefits or clinical treatment.

Biodegradable polymers, e.g. polyester poly lactic–co-glycolic acid (PLGA), offer interested particular carrier systems for drugs into bacterial biofilm . To investigate the penetration and effic y in biofilm , the nano-carriers has to be labelled in suitable manner that has to be different to the most commonly used green or red fluo escent dyes for biofilm staining. Therefore, we investigated an alternative blue fluo escent labelling of organic nano-carriers by a coumarin derivative. This could be covalently and stabile coupled to the carboxyl groups of the PLGA and allowed the visualization of the nano-carriers inside the biofilms that stained with SYTO9 (green, vital cells) and propidium iodide (red, dead cells).

Image cytometric analysis of gold nanoparticle uptake by macrophages

Susanne Melzer, Leipzig

Melzer, Susanne (1); Ankri, Rinat (2); Fixler, Dror (2); Tárnok, Attila (1,3). 1: Department of Pediatric Cardiology, Heart Center Leipzig GmbH, Universität Leipzig, Germany; 2: Faculty of Engineering and Institute of Nanotechnology and Advanced Materials, Bar-Ilan University, Israel; 3: Translational Center for Regenerative Medicine, Universität Leipzig, Germany

Introduction: In atherosclerosis stable and vulnerable atherosclerotic plaque types are distinguished that behave differently concerning rupture, thrombosis and clinical events. The stable are rich in M2 macrophages (MF), whereas the unstable are rich in inflamm tory M1 subtype and are highly susceptible to rupture, setting patients at risk for thrombotic events when they undergo invasive diagnosis such as coronary angiography. Therefore, novel approaches for non-invasive detection and classific tion of vulnerable plaques *in vivo* are needed. Whereas classical approaches fail to differentiate between both plaque types, a new biophotonic method (combination of the diffusion refle tion method with fl w and image cytometry (FCM and IC)) to analyze gold nanoparticle (GNP) loading of MF-rich plaques could overcome this limitation. Within here we present the results of the IC analysis.

Methods: Two types of GNP were used in this study: three types of gold nanorods GNR with 40x18nm, 65x25nm and 52x13nm in size; and gold nanospheres GNS with an average diameter of 18.5 nm. The GNS had an absorption peak at 520 nm and the GNR at 630 nm. Monocytes were isolated from human buffy coat samples by Ficoll-density gradient centrifugation, differentiated into MF within 6 days and labelled with GNR and GNS for 24 h.

GNS and GNR loading was determined by FCM and IC.

Results: After GNR labelling of MF the FCM light scatter values increased up to 3.7 fold. IC showed additionally that all particle variants were taken up by the cells in a heterogeneous fashion.

Conclusion and outlook: The combination of FCM and DR measurements provides a potential novel, highly sensitive and non-invasive method for the identific tion of atherosclerotic vulnerable plaques, aimed to develop a potential tool for *in vivo* tracking. Further experiments will show, if differently polarized MF (M1 or M2) take up the particles differently and may thereby serve to distinguish stable from vulnerable plaques.

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Interaction of laser irradiation with peptide-conjugated gold nanoparticles for intracellular molecule delivery

Judith Krawinkel

Judith Krawinkel1, Undine Richter1, Maria Leilani Torres-Mapa2, Bayaraa Tumursukh1, Martin Westermann4, Lisa Gamrad5, Christoph Rehbock5, Stephan Barcikowski5, Alexander Heisterkamp2,3,. 1: Institute of Applied Optics, Friedrich-Schiller-University Jena; 2: Institute of Quantum Optics, Leibniz University Hannover; 3: Excellence Cluster REBIRTH, Hannover; 4: Electron Microscopy Centre, Friedrich-Schiller-University Jena; 5: Technical Chemistry I, University of Duisburg-Essen and Center for NanoIntegration Duisburg-Essen CENIDE

Different approaches for new delivery methods using cell-penetrating peptides or utilizing the interaction of gold nanoparticles and lasers for cell manipulation are currently being developed. We combined the benefit of both approaches and explored the use of peptide-conjugated gold nanoparticles (CPP-AuNPs) fabricated by laser ablation to manipulate cells on a subcellular level applying pulsed laser radiation (532 nm, 1 ns). The CPP-AuNPs are taken up via endocytosis followed by laser-triggered release of the endosomal content while maintaining cell viability.

The interaction of the incident laser light with the CPP-AuNPs leads to a field enhancement and heat deposition in the CPP-AuNPs which locally ruptures the enclosing endosomes and delivers the target molecule into the cytoplasm. Further, we observed desagglomeration of the CPP-AuNPs which is advantageous for a better biocompatibility as smaller nanoparticles exocytose faster.

Session 5: High Content Analysis

2:00pm - 3:30pm   Paul-Ehrlich Lecture Hall

Chair: Andreas Radbruch, Berlin

Chair: Cees Cornelisse, Middelburg, The Netherlands

Today technologies allow for the analysis of more and more parameters. This enables the phenotyping of cells and tissues in unprecedented depth but also generates highly complex data sets. This session is dedicated to the application of novel technologies for the analysis of complex tissues but also to bioinformatic tools and modeling which unravel the complexity and multidimensionality of the data.

Imaging mass cytometry: A novel imaging modality to visualize dozens of biomarkers in a targeted and simultaneous manner in tumor samples.

Bernd Bodenmiller, Zurich, Switzerland

Institute of Molecular Life Sciences, University of Zurich

Each year approximately 1.1 million new cases of breast cancer are diagnosed and about 0.3 million women worldwide die from this disease. Tumor metastases, relapse, and resistance to therapy are the main causes of death in breast cancer patients. Communication between heterogeneous cancer cells and normal cells in the so-called tumor microenvironments (TME) drives cancer development, metastasis formation, and drug resistance. To understand the TME and its relationship to clinical data, comprehensive investigation f the components of the microenvironment and their relationships is necessary. We recently invented a novel imaging modality based on mass cytometry, called imaging mass cytometry (IMC) that enables this type of study. In IMC, tissues are labelled with antibodies that carry pure metal sotopes as reporters. Theoretically 135 antibodies can be visualized simultaneously; in practice, we routinely quantify 44 markers. The antibody abundance is determined using laser ablation coupled to inductively coupled plasma mass spectrometry. Here we show the results of the analysis of hundreds of breast cancer samples by IMC. To extract biological meaningful data and potential biomarkers from this dataset, we developed a novel computational pipeline for the interactive and automated analysis of large-scale, highly multiplexed tissue image datasets.

Our analysis revealed a surprising level of inter- and intra-tumor heterogeneity and diversity within known human breast cancer subtypes as well as the stromal cell types in the TME.

Furthermore, we identified cell-cell interaction motifs in the tumor microenvironment that correlated with clinical outcomes. In summary, our results show that IMC provides targeted, high-dimensional, subcellular resolved images of tissue samples. The identified spatial relationships among complex cellular assemblies have potential as biomarkers. We envision that IMC will enable a systems biology approach to diagnosis of disease and will ultimately guide treatment decisions.

Building models of cell organization, differentiation and perturbation directly from microscope images

Robert F. Murphy, Freiburg

Ray and Stephanie Lane Professor of Computational Biology and Professor of Biological Sciences, Biomedical Engineering and Machine Learning, Carnegie Mellon University, Pittsburgh, USA

Honorary Professor of Biology and Senior External Fellow, Albert Ludwig University of Freiburg, Germany

Given the complexity of biological systems, machine learning methods are critically needed for building systems models of cell and tissue behavior and for studying their perturbations. Such models require accurate information about the subcellular distributions of proteins, RNAs and other macromolecules in order to be able to capture and simulate their spatiotemporal dynamics. Microscope images provide the best source of this information, and we have developed tools to build generative models of cell organization directly from such images. Generative models are capable of producing new instances of a pattern that are expected to be drawn from the same underlying distribution as those it was trained with. Our open source system, CellOrganizer (http://CellOrganizer.org), currently contains components that can build probabilistic generative models of cell, nuclear and organelle shape, organelle position, and microtubule distribution. These models capture heterogeneity within cell populations, and can be dependent upon each other and can be combined to create new higher level models.

The parameters of these models can be used as a highly interpretable basis for analyzing perturbations (e.g., induced by drug addition), and generative models of cell organization can be used as a basis for cell simulations to identify mechanisms underlying cell behavior.

A novel functional *in vitro* phenotypic screening assay for detecting anti-Parkinson drugs using neuronal networks derived from dopaminergic human iPSCs

Dieter G. Weiss, Rostock

Bader, Benjamin M.; Pielka, Anna-Maria; Ehnert, Corina; Jügelt, Konstantin; Gramowski-Voss, Alexandra; Weiss, Dieter G.; Schröder, Olaf H.-U.. Benjamin M. Bader1, Anna-Maria Pielka1, Corina Ehnert1, Konstantin Jügelt1, Alexandra Gramowski-Voss1, Dieter G. Weiss1, Olaf H.-U. Schröder. 1NeuroProof GmbH, Rostock, Germany, Benjamin M. Bader <benjamin.bader@neuroproof.com>, Anna-Maria Pielka <anna-maria.pielka@neuroproof.com>, Corina Ehnert <corina.ehnert@neuroproof.com>, Konstantin Jügelt <konstantin.juegelt@neuroproof.com>, Alexandra Gramowski-Voss <alexandra.voss@neuroproof.com>, Dieter G. Weiss <weiss.rostock@t-online.de>, Olaf H.-U. Schröder <olaf.schroeder@neuroproof.com>

Introduction:

Phenotypic screening has led to the majority of successfully launched CNS drugs in the last 15 years. In vitro models using primary cultures are widely used for testing drug candidates. Here, the use of human induced pluripotent stem cell-derived (hiPSC) neurons is presented as an *in vitro* drug discovery assay of increased predictability, sensitivity and specifici y.

Aim: It was our aim to establish a toxin-induced *in vitro* model of Parkinson's disease based on hiPSC-derived neuronal cultures and test its applicability for functional phenotypic screening of anti-Parkinson compounds by comparing it with the effects on conventional primary neuronal networks (1).

Methods:

We cultured dopaminergic hiPSC-derived neurons with ventral midbrain identity (PITX3, FOXA2, with 60-70 % TH positive cells) and primary embryonic mouse midbrain neurons on multiwell micro electrode arrays (mwMEAs) and recorded the spontaneous electrical activity of single cells during development of functional network communication patterns. We used a short pulse of the dopaminergic neuron-specifi toxin MPP+ (1-methyl-4-phenylpyridinium) to induce a strong functional but only small morphological impairment. We tested the known neuroprotective compound GDNF to prevent the functional impairment by a single pre-treatment. Using quantitative multi-parametric data analysis of the functional activity patterns, we calculated a read-out able to capture both, occurrence and prevention of impairment of the neuronal network activity.

Results:

We demonstrate that the two neuronal network cultures produce robust spontaneous electrical spiking activity showing a functional maturation into a communicating network within 3 weeks *in vitro*. Exposure to MPP+ causes a quantitatively defined and specific electrical activity pattern whose appearance is prevented by a single GDNF pre-treatment. We compare the results with those of primary mouse midbrain neuron/glia co-cultures (1) using the same experimental design and show that MPP+ induces similar functional effects in both systems which also are prevented by GDNF in both systems.

In summary, our functional phenotypic *in vitro* screening platform with human iPSC-derived dopaminergic neurons growing on mwMEAs allows the robust and reproducible screening of novel lead compounds or advanced drugs for future therapies of Parkinson's disease.

1. B. M. Bader, C. Fahrun, A.-M. Pielka, M. Winkler, A. Podßun, K. Jügelt, O. H. U. Schröder, A. Voss. A novel In vitro Parkinson's model for compound screening based on the functional rescue of dopaminergic neurons using challenged midbrain cultures growing on micro electrode arrays. Neuroscience Meeting 2013, San Diego CA, Program No. 419.20. Society for Neuroscience, 2013

Comparison of fl w cytometry samples using dimension reduction and binning

Kristen Feher, Berlin

Feher, Kristen; Giesecke, Claudia; Doerner, Thomas; Radbruch, Andreas; Kaiser, Toralf. DRFZ, Germany

Motivation: The automated analysis of multiple fl w cytometric dataset with respect to a covariate has been at the forefront of attention in the past 5 years. It is not a straightforward problem, as the data format does not fit into any priorly available statistical framework. Efforts have focused on clustering of the samples - either each individual sample or pooled. However, a clustering-based approach has the drawback of cluster validation.

We propose a scheme of inter-sample comparison that is based on binning dimension-reduced data using a uniform grid. For a given covariate, we choose the best dimensionality and grid size by training Support Vector Machines with half the data (randomly selected), validating with the test set and replicating this procedure 100 times for each parameter combination.

We demonstrate the approach on a dataset consisting of tonsil, bone marrow, spleen and blood samples (53 in total), and the selected model has a median classific tion rate of 85%. Additionally, this approach allows for the embedding of the samples in a vector space, highlighting the relationship between the samples and bins. Finally, this approach can be extended to regression in the case of a continuous covariate.

Notes

Session 6: Iberian Society Guest Session

4:00pm - 5:30pm   Paul-Ehrlich Lecture Hall

Chair: Andrea Cossarizza, Modena, Italy

Chair: Hyun-Dong Chang, Berlin

If there is a place where interactions among different people, ideas and perspectives are at the basis of the progress and knowledge, this is the world of Science and Medicine. Indeed, the magically fluorescent world of Cytometry has always been taking enormous advantage from interactions among scientists whose interests were in different fields, from Optics to Chemistry, from Informatics to Immunology, and from researchers with different cultural backgrounds and geographic areas of education. With this in mind, the fact that National Conferences are hosting Societies from different parts of the world is becoming not only a way to further open our minds of scientists, but a profound cultural enrichment for all who participate to such events.

The participation of renown leaders of the Iberian Society for Cytometry (SIC) to the Congress of the German Society for Cytometry (DGfZ) is thus an example of how the exchange of scientific knowledge and ideas has to be carried on in such a complex historical period. The former president of SIC, dr. Jordi Petriz, will review the topic of ABC multidrug transporters and stem cells, and discuss some new important data in the field. The Secretary of SIC, dr. Julia Almeida, will present recent advances and future challenges in the field of immunophenotyping of hematological malignancies. Additional data will be presented by the current President of SIC, Prof. Josè Enrique O'Connor, discussing the potential of real time flow cytometry.

The session, chaired by the President of DGfZ, dr. Hyun-Dong Chang, and by a "neutral" italian guest, myself, will thus see vibrant talks and discussion in some areas of relevant interest for all cytometrists attending the meeting.

Searching for the hematopoietic stem cells: Functional heterogeneity of CD34 positive and CD34 negative cells by co-staining with Vybrant® DyeCycleTM Violet (DCV) and an Alkaline phosphatase live stain.

Jordi Petriz, Barcelona, Spain

Jordi Petriz1, Laura García Rico1, Mª Dolores García-Godoy1, Cristian Núñez Espinosa2, Ginés Viscor2, (1) Josep Carreras Leukemia Research Institute. (2) University of Barcelona. Barcelona (Spain)

Background

Alkaline phosphatase (AP) is a phenotypic marker of pluripotent stem cells (PSCs), including undifferentiated embryonic stem cells, and induced pluripotent stem cells. While AP is expressed in most cell types, its expression is highly elevated in PSCs. Since most of stem cells express multidrug resistance transporters, we have validated APLS as a marker for stem-like CD34 negative Side Population (SP) cells based on the efflu of Vybrant® DyeCycleTM Violet stain (DCV). We previously developed a new method for counting CD34+ cells in whole blood samples, based on nucleic acid viable staining to discriminate erythrocytes and debris. We have adapted this protocol to study the heterogeneity of circulating CD34+ cells in peripheral blood.

Material and methods

In this study we have used rat peripheral blood cells and human brain astrocytoma cells (MOG- G-CCM, GOS 3, LN405, SW1783 and U87-MG) were used to study SP cells. In addition, DCV stain was used to discriminate nucleated cells from non-nucleated cells and debris. CD45-FITC and CD34-PE monoclonal antibodies were used in this study for simultaneous staining with DCV. Data were collected using the Attune® Acoustic Focusing Cytometer (Life TechnologiesTM)

equipped with two lasers operating at 405 and 488 nm. Filter combination for DCV analysis was: 500 DLP, BP 450/40 (blue), and BP 603/48 (red). DCV signal was measured using linear scale at 50 mW. Filter combination for APLS analysis was: 555 DLP, BP 530/30 (green).

Results and discussion

Current available AP substrates are toxic to the cells, which prevent them from propagating once stained. However, APLS can be used to easily distinguish primitive stem cells, since the stain maintains stem cell viability. In addition, APLS was not effl ed by multidrug resistance pumps, making feasible this stain for the identific tion of differentially expression within SP cells, and AP is overexpressed when SP cells were incubated at low O2 concentrations. Regarding CD34+ cells, panels of immunomarkers provide greater information in combination with functional analysis of unlysed whole blood specimens. The distribution of DCV-CD34+ cells identifies two main cluster groups, with significa tly different fluo escent intensities, allowing the identific tion of relevant and reproducible subsets of rat hematopoietic progenitors. Finally, using DCV and violet excitation, the SP displays almost identical distributions as for the Ho342 SP assay.

Flow cytometry immunophenotyping of hematological malignancies: recent advances and future challenges

Julia Almeida, Salamanca, Spain

J. Almeida1, Q. Lécrevisse1, C.E. Pedreira2, E. Mejstrikova3, V.H.J. van der Velden4, S. Böttcher5, J. Flores-Montero1, S. Matarraz1, A. W. Langerak4, T. Kalina3, A. Trinquand6, L. Martín-Martín1, A.J. van der Sluijs-Gelling7, D. Karsch5, L. Sędek8, M. Lima9, M. Gomes da Silva10, Elaine S. Costa11, G. Gaipa12, M. Roussel13, L. Campos-Guyotat14, B. Paiva15, N. Villamor-Casas16,R. Fluxa17, J. Verde17, G. Grigore17, L. Lhermite18, T Szczepański8, J.J.M. van Dongen4 and A. Orfao1, on behalf of the EuroFlow Consortium.
Institutions: 1 Department of Medicine and Cytometry Service (NUCLEUS), Cancer Research Center (IBMCC-CSIC/USAL) and Instituto de Investigación Biosanitaria de Salamanca (IBSAL), Universidad de Salamanca y Hospital Universitario de Salamanca, Salamanca, Spain. 2 Federal University of Rio de Janeiro - UFRJ COPPE - Systems and Computing Department . 3 Department of Pediatric Hematology and Oncology, 2nd Faculty of Medicine, Charles University (DPH/O), Prague, Czech Republic. 4 Department of Immunology, Erasmus MC, University Medical Center Rotterdam (Erasmus MC), Rotterdam, The Netherlands. 5 Second Department of Medicine, University Hospital of Schleswig Holstein, Campus Kiel, Kiel, Germany. 6 Department of Hematology, Hôpital Necker-Enfants-Malades (AP-HP) and UMR CNRS 8147, University of Paris Descartes, Paris, France. 7 Dutch Childhood Oncology GroupDEN HAAG, The Netherlands. 8 Department of Pediatric Hematology and Oncology, Medical University of Silesia (SUM), Zabrze, Poland. 9 Department ofHematology, CytometryLab, Centro Hospitalar do Porto, Hospital de Santo António, Universityof Porto, Porto Portugal. 10 Department of Hematology, Portuguese Institute of Oncology (IPOLFG), Lisbon, Portugal. 11 Department of Pediatrics, Faculty of Medicine, Federal Universityof Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil. 12 Centro RicercaTettamanti, Clinica PediatricaUniversitàdi Milano, Bicocca – Ospedale San Gerardo, MONZA Italy. 13 Hematology Laboratory CHU Pontchaillou, RENNES France. 14 Hematology Laboratory CHU de Saint-Etienne, SAINT-ETIENNE France. 15 Clinica Universidad de Navarra – Centro De InvestigacionesMedicasAplicadas (CIMA) Universidad de Navarra, PAMPLONA Spain. 16 Unitat d'Hematopathologia Hospital Clínic de Barcelona, Escala BARCELONA Spain. 17 Cytognos SL, Salamanca, Spain. 18 Department of Hematology, Hôpital Necker and UMR CNRS 8147, University of Paris Descartes (AP-HP), Paris, France

Flow cytometric immunophenotyping is currently recognized to provide essential information for the diagnosis, staging, classific tion and treatment monitoring of hematological malignancies. Accordingly, World Health Organization endorses a multiparametric approach to classify hematological malignancies, which combines immunophenotypic together with morphologic and genetic data, as well as clinical information. Despite this, fl w cytometry is still perceived as a technique highly dependent on expertise and it is regarded to have limited reproducibility in multicenter studies. This probably related to the increasing number of antibodies and fluo ochromes that have been used, among other technological developments that have taken place over the past decade; undoubtedly, these improvements have translated into a progressively higher number of markers that can be simultaneously evaluated on an increasingly high number of cells and consequently have allowed a more rational panel design for the study of leukemias and lymphomas, but in parallel, they have led to an increased complexity of fl w immunophenotypic data; further, interpretation of fl w cytometry data is still based on the experts' knowledge and experience, which may negatively influen e on the reproducibility of data analysis and, hence, on its clinical impact. In addition, the new reagents and improvements have not been accompanied by an appropriate standardization of the laboratory procedures and instrument settings. In order to address both the poor standardization and the complexity and subjectivity of analysis of fl w cytometry data, the EuroFlow Consortium started working in 2006, with the aims to develop and evaluate new antibodies, as well as to design novel multicolor immunostaining protocols in a standardized manner, and in parallel to design, develop and validate a high throughput system for the automated and standardized fl w cytometry data analysis for the diagnostic screening, classific tion and monitoring of hematological malignancies. The near 10-year extensive collaborative work already developed and under development in the EuroFlow centers will be presented here, to show it has provided innovative protocols and antibody panels for virtually all the WHO2008 categories, with particular focus on the software tools and procedures developed by the EuroFlow Consortium for data analysis and data visualization and interpretation, to be used for fully standardized diagnosis and classific tion of hematological malignancies.

Analysis by Real-Time Cytometry of the Interaction between Nitric Oxide and Superoxide Anion in Hematic and Tumor Cells

Jose-Enrique O'Connor, Valencia, Spain

O'Connor, Jose-Enrique (1); Balaguer, Susana (1); Herrera, Guadalupe (2); Jàvega, Beatriz (1); Salvador, Alicia (1); Silla, Salvador (1); Sulbarán, Jesús-Américo (1). 1: Laboratory of Cytomics, The University of Valencia, Spain; 2: Service of Cytometry, UMIM, Incliva Foundation-University of Valencia, Spain

Nitric oxide (NO) plays an important role as signaling molecule in vascular physiology, but also NO-related reactive nitrogen (RNS) and oxygen (ROS) species are crucial in immune responses against pathogens and in inflamm tory conditions and cancer. A nodal point to both processes is the strong oxidant peroxynitrite (ONOO), arising from fast reaction between NO and superoxide anion. ONOO is involved in ROS- and RNS-cytotoxicity and causes protein nitrosylation. In this context, we have set up and validated a novel real-time fl w cytometry assay to detect and follow up the NO- and superoxide-driven generation of ONOO in peripheral whole blood and isolated blood cells, as well as in tumoral cell lines.

Whole blood samples and isolated leucocytes preparations were stained with CD45 and CD14 antibodies plus each of a series of fluo escent probes sensitive to RNS, ROS or glutathione (GSH), including 4-Amino-5-Methylamino-2′,7′-difluo ofluo escein diacetate (DAF-FM DA), dihydrorhodamine123 (DHR), MitoSOX Red, Dihydroethidium (DHE) and 5-chloromethylfluo escein diacetate (CMFDA) plus a viability marker. Tumoral cells (N13 rat hepatoma and U-937 human monocytic leukemia) were stained only with the indicated fluo ochromes. Samples were exposed sequentially to NOR-1, a NO donor, and to different superoxide donors, while analyzed in real time by kinetic fl w cytometry. Relevant kinetic descriptors, such as the rate of fluo escence change were calculated from the kinetic plot in erythrocytes, leucocyte subpopulations (gated on light scatter and CD45/CD14 expression) and tumoral cells.

The real-time generation of ONOO, which consumes both NO and superoxide, led to a decrease in the intensity of the cellular fluo escence of the probes sensitive to these molecules in all leucocytes from samples preincubated with superoxide donors and then treated with NOR-1. In addition, the NO generation in samples treated with NOR-1 was rapidly reversed by the addition of superoxide donors. Further analysis with fluo escent probes sensitive to ROS and GSH showed striking differences among lymphocytes, monocytes and granulocytes and in tumor cells, regarding the response to NO and superoxide, indicating a different regulation of oxidative pathways in those cells.

This is a fast and simple assay that may be used to monitor the intracellular generation of ONOO and to explore ROS and NOS interactions in a variety of relevant cell types.

A novel phagocytosis assay in whole blood by fl w- and imaging cytometry with GFP-expressing bacteria

Francisco Sala-de-Oyanguren, Valencia, Spain

Sala-de-Oyanguren, Francisco (1); Tamarit, Jesús (1); Herrera, Guadalupe (2); O'Connor, José-Enrique (1). 1: Laboratory of Cytomics, The University of Valencia, Spain; 2: Service of Cytometry, UMIM, Incliva Foundation-University of Valencia, Spain

Phagocytosis is essential for the clearance of pathogens, apoptotic bodies and necrotic debris. Defects in the phagocytic process are diagnostic and prognostic biomarkers in deficiencies of the innate immune system, including disorders such as chronic granulomatous disease. Also, novel cell-based assays may complement current approaches for *in vitro* immunotoxicology. We have developed a fl w cytometric method based on the use of green fluo escence protein (GFP) labeled Escherichia coli (GFP-E. coli) and dihydroethidine (DHE) to assess phagocytosis and the subsequent oxidative burst in human whole blood samples and the human monocytic cell line U937. For the whole blood study, GFP-E. coli was added at different bacterium:leukocyte ratios (1, 0.5 and 0.25) to 50 mL of heparinized whole blood incubated with 2.5 mL of CD45-APC antibody. After incubation at 37ºC for 30 min, 1 mL of BD FACS lysing solution was added. For the U937 study, GFP-E. coli was added to U937 cells at bacterium : leukocyte ratio of 30. After incubation at 37ºC for 90 min, 400 mL of RPMI was added. For oxidative burst quantitation in both assays, 2 mL DHE at 1 mg/mL was added prior incubation at 37ºC. Positive controls were samples stimulated with PMA. Negative controls included cytochalasin A and 0ºC incubation. Samples were analyzed in three different cytometers: FACSVerse, Accuri C6 and Amnis ImageStream. A bacterium:leukocyte ratio as low as 0.25 is enough to trigger phagocytosis and oxidative burst by neutrophils in whole blood samples. The capability of GFP –E. coli to elicit radical oxygen species is nearly that induced by PMA. Contrarily, cytochalasin A decreased markedly the phagocytic potential of blood and U937 cells. Analysis by imaging fl w cytometry of U937 cell line and whole blood samples allowed us to discriminate between surface-adherent and internalized bacteria. This assay has been applied successfully to estimate the phagocytic potential of leukocytes in whole blood samples of normal subjects and it may provide a fast, simple and accurate protocol for diagnosing phagocytic disorders and toxic effects on phagocytic cells.

Notes

Session 7: Immunology

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

Chair: Gergely Toldi, Birmingham, UK

Chair: Günter Valet, München

Technological advances have significantly enhanced the potential to discriminate and functionally characterize immune cells. The session will cover recent progress in the characterization of multiple myeloma cell populations as well as in the recognition of changes in immune cells during ageing. Technical progress provides better insight into early processes of T-cell activation, increased discrimination amongst T-regulatory cells and closer follow-up of receptor clustering on B-cell membranes

Few Parameter Cytometry – How to survive in the Century of Big Data

Andreas Thiel, Berlin

Berlin-Brandenburg Center for Regenerative Therapies (BCRT), Charité - Universitätsmedizin Berlin

The recent introduction of modern multidimensional conventional or mass cytometry technologies in biomedicine gives hypothesis driven research a hard time. Big data studies and their results start to dominate the fiel . But while shining on the first sight, distinct findings remain rare and particularly new results seem difficul to get incorporated into routine clinical diagnostics or treatments. One major reason for this may be its costs; although prices for individual cytometric reagents did not increase, the numbers of analyzed parameters substantially did.

Only about a century ago 17-color cytometry was introduced as the ultimate tool to "unravel the immune system". Nowadays, mass cytometry theoretically allows up to 100 parameters analyzed. Calculating low prices as 200 US$ for single reagents lead to panel prices of at least 10.000 US$ for a 50 parameter analysis of about 20 samples. Defini ely such experiments cannot be integrated into routine procedures and more dramatically for us, conventional grant funding cannot finan e them.

Where do we go from here? Currently, we experience a déjà vu: a kind of triumphal procession of omics technologies takes over biomedical science. Remember? Already, the praised introduction of global gene expression analysis in late 90´ promised the beginning of a golden century of effici t personalized medicine. Some promises were kept; but finally only few results made it into the clinics. ,

With the recent introduction of new cytometric technologies enabling multidimensional combined functional and phenotypic profiling directly on single cell level, we may face similar troubles. How can we avoid ending up with empty hands (and pockets) and nice colorful publishable but not transferable results?

We should not forget about hypothesis-driven research that can push projects and we should not loose our ability to restrict ourselves to employ also fewer parameter analyses in our studies. We also have to include firs -hand bioinformatic expertises in our projects from the beginning on. To make it simple: results from a 30 parameter analysis that cannot be repeated a second time because of a blasted budget are worse than results from a 10 parameter analysis validated in three independent experiments. Secondly, bioinformatics will be needed to extract valid patterns and distinct signatures already starting from 10-parameter cytometric analysis. Without we will not be able to survive in the forthcoming Century of Big Data.

Multiple myeloma – from phenotypes to relationships

Leo Alexander Hansmann, Berlin

Berlin-Brandenburg Center for Regenerative Therapies (BCRT), Charité - Universitätsmedizin Berlin

Multiple myeloma is an incurable malignancy of the B lineage characterized by the accumulation of clonal plasma cells in the bone marrow. Current models of pathogenesis involve genetic aberrations of the malignant cells themselves and their interactions with bone marrow and immune cells rendering multiple myeloma a prime example for tumor-microenvironmental interactions. Although substantial alterations in the bone marrow environment could be assumed to affect circulating immune cell compositions efforts to detect disease-related phenotypes in peripheral blood have not been conclusive.

Recent advances in cellular immunology technologies, particularly cytometry by time-of-flig t (CyTOF), which supports 40-parameter analysis, and next generation sequencing (NGS) of immunoglobulin and T cell receptor genes, have greatly increased the possible depth of analysis at the single cell level and tremendously expanded the resolving power for the detection of (immune-) phenotypes and cellular relationships. We show that a B cell population presenting mixed characteristics of naïve and memory B lymphocytes was expanded in the peripheral blood of patients with active multiple myeloma raising questions about clonal expansion and relationships within different B lineage populations in multiple myeloma bone marrow. Our novel combination of FACS single cell sorting, next generation sequencing, and computational tools allows the precise and high-dimensional phenotypic tracking of different B lineage clones in human bone marrow.

Malignant multiple myeloma cells showed phenotypic intra-clonal heterogeneity while expanded normal-phenotype B lineage clones were molecularly unrelated and most likely the result of antigen-driven convergent expansion. Our data highlight the power of CyTOF and high-dimensional immune phenotyping in combination with NGS for the detection of aberrant and unexpected immune phenotypes as well as cellular relationships on the single cell level.

Reduced fluoescence versus forward scatter time-of-flig t and increased peak versus integral fluoescence ratios indicate receptor clustering in fl w cytometry

Dirk Mielenz

Barbara G. Fürnrohr1,2,3, Merle Stein1, Benjamin Rhodes3,4, Prabhjoat S. Chana3, Georg Schett1, Timothy J. Vyse3, Martin Herrmann1, Dirk Mielenz1. 1University of Erlangen-Nürnberg, Germany; 2Medical University Innsbruck; 3King's College London; 4University Hospitals Birmingham NHS foundation trust

Clustering of surface receptors is often required to initiate signal transduction, receptor internalization and cellular activation. To study the kinetics of clustering we developed an economic high-throughput method employing fl w cytometry. The quantific tion of receptor clustering by fl w cytometry is based on the following two observations: firstl , the fluoescence signal length (FL time-of-flig t (ToF)) decreases relative to the forward scatter signal length (FSc ToF) and secondly, the peak fluoescence (FL-peak) increases relative to the integral fluoescence (FL-integral) upon clustering of fluoescence labeled surface receptors. Receptor macroclustering can, therefore, be quantified using the ratios FL-ToF/FSc-ToF (method ToF) or FL-peak/FL-integral (method Peak).

We have used these methods to analyze clustering of two immune receptors known to undergo different conformational and oligomeric states: the B-cell receptor (BCR) and the complement receptor 3 (CR3), on murine splenocytes, purified B cells and human neutrophils. Engagement of both the BCR and CR3, on immortalized as well as primary murine B cells and human neutrophil, respectively, resulted in decreased FL-ToF/FSc-ToF and increased FL-peak/FL-integral ratios. Manipulation of the actin-myosin cytoskeleton altered BCR clustering which could be measured using the established parameters. To confi m clustering of CR3 on neutrophils we applied imaging fl w cytometry. Since receptor engagement is as a biological process dependent on cell viability, energy metabolism and temperature, receptor clustering can only be quantifie by gating on viable cells under physiological conditions. In summary, with this novel method receptor clustering on non-adherent cells can easily be monitored by high-throughput conventional fl w cytometry.

Automatic measurement of early T cell activation via Flow Cytometry FRET

Kerstin von Kolontaj

Kerstin von Kolontaj1, Gabor Horvath2, Eicke Latz2, Martin Büscher1. Miltenyi Biotec, Germany; 2University Hospital Bonn, Germany

Protein-protein interactions play a key role in almost all biological functions. The activation of human T cells, for example, results in a structural rearrangement of the cell surface receptors. Their clustering at the immunological synapse is essential for downstream signaling of activation signals inside the cell.

Förster resonance energy transfer (FRET) is a well-suited method to identify protein-protein interactions in cells. However, the determination of the FRET efficie y is usually very complex and time laborious.

Here, we established an automatic FRET-based program, which allows for the identific tion of protein interactions and receptor clusters on the cell via fl w cytometric analysis.

Using this automatic FRET-based method, we could measure early human T lymphocyte activation both via the clustering of the T cell markers CD3 with CD4 and via the homoclustering of the CD3 receptor on the cell surface after activation by a strong superantigen. We could demonstrate that the FRET efficie y increases along with the formation of these clusters. Therefore, the FRET assay is able to detect T cell activation even faster than conventional calcium influ assays.

For that reason, this novel FRET-based method is a powerful tool which allows to acquire spatiotemporal information of protein clusters. It is furthermore adaptable to many systems and easy to use even in high throughput screenings.

Lack of CD154 expression discriminates stable from instable Foxp3-expressing CD137+ Treg

Anna Nowak

Anna Nowak1, Petra Bacher2, Thordis Hohnstein2, Katrin Vogt3, Birgit Sawitzki3, Andreas Thiel4, Alexander Scheffold1,2.
1German Rheumatism Research Centre (DRFZ) Berlin, Leibniz Association, Berlin, Germany; 2Department of Cellular Immunology, Clinic for Rheumatology and Clinical Immunology, Charité - University Medicine, Berlin, Germany; 3Institute for Medical Immunology, Charité - University Medicine, Berlin, Germany; 4Berlin-Brandenburg Center for Regenerative Therapies (BCRT), Charité - University Medicine, Berlin, Germany

Natural Foxp3+ regulatory T cells (Treg) maintain self tolerance and control inflamm tory reactions and are therefore promising therapeutic targets for the treatment of many immune mediated diseases, e.g. by adoptive transfer of *in vitro* expanded Treg. For safe and effective treatment the functional stability, i.e. maintenance of the suppressive potential, is essential. Current clinical isolation protocols are mainly based on CD25 (+/- CD127) expression resulting in highly variable Treg stability upon expansion, as measured by Foxp3 expression and demethylation of the Foxp3 TSDR region. However, currently the further purific tion of stable Treg from expanded Treg is not possible due to the lack of suitable surface markers.

Converse expression of CD137 and CD154 has previously been described to identify *in vitro* antigen-activated Treg. We show here, that the CD137+CD154- phenotype is a general characteristic for stable Treg, i.e. Foxp3+ with a highly demethylated TSDR region. CD137+CD154- Treg can readily be identified and sorted even after prolonged *in vitro* culture and retain their stable phenotype upon further *in vitro* expansion, including low expression of inflamm tory cytokines and high suppressive capacity.

In contrast, CD137+CD154+ T cells, although they express Foxp3, are characterized by a rather methylated TSDR as well as a rapid loss of Treg phenotype and suppressive activity.

Therefore, CD137 and lack of CD154 expression provides unique possibilities to identify and sort stable Treg for diagnostic or therapeutic applications.

Session 8: Emerging Technologies

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

Chair: Leoni Kunz-Schughart, Dresden

Chair: Wolfgang Göhde, Nottuln

Technological innovations made available to the scientific community by industry has always allowed the scientific community to address biological questions in novel ways, analyze more and different parameters per single cell, more analytes in smaller samples, more cells in less time. In this session, selected companies will not only introduce novel and up-and-coming technologies, but also show examples how these technologies have been applied in science, and enter into a dialogue with the academic participants of the session.

Multiple Cytokine Detection: three dimensional assay – two dimensional read-out

Thomas Bauer, AYOXXA

The detection of multiple targets in parallel has become essential to understand complex biological processes and immune response. This requires not only a sensitive detection method but should also comprise a large dynamic detection range to allow for coverage of a broad expression protein profil . AYOXXATM Biosystems has developed the revolutionary LUNARISTM platform technology, enabling the detection of multiple cytokines in various sample types using 3µl sample volume only. The detection of targets is hereby based on an immunoassay which is realized by the use of beads. The beads are individually coated with a specific targeting antibody and are afterwards sequentially loaded on a bio- chip. After each deposition of the target specific beads, the location of the beads on the chip is recorded. As a user the scientist receives the already bead-loaded chip and bead-map. The assay is simply performed by an ELISA like protocol and a chip read out using fluo escent microscopy. As a result you qualify and quantify the targets of interest down to pg/ml in a 3-4Log dynamic range. LUNARISTM herby represents a unique method of combining outstanding robustness and sensitivity in a 3 dimensional assay with a simple planar readout.

Beckman Coulter CytoFLEX Research Analyser, Exceptional Performance In A Surprisingly Small Instrument

Michael Braun, Beckman Coulter

Providing quality and performance at any configu ation, the CytoFLEX system provides powerful sensitivity and resolution for the simple to the most challenging applications. CytoFLEX delivers and surpasses capabilities expected in top tier analyzers, with excellent performance and nanoparticle resolution. In this presentation we will discuss the new technology including the WDM module and FADP used in the CytoFLEX and show data of some special applications like microparticle measurement as well as functional assays like phagozytosis.

20 Colors And Beyond

Joachim Brenner, tBD Life Sciences

We would like to share a first look t our new generation of high performance cell analyzes. Thanks to a brand-new electronics these BD LSR Fortessa TM based instruments will be capable to detect more than 20 parameters with a superior resolution.

In combination with our ever growing family of BD Horizon BrillantTM dyes, it will be possible for the first time to look at more than 18 fluo escence colors simultaneously, and furthermore to design better and therefore more sensitive multicolor experiments.

We will discuss laser options with the potential benefits in these high pe formance systems. We will also show some preliminary data with new BD Horizon BrilliantTM dyes.

Assessment of early kinetics of sequential gag mRNA and protein expression following HIV-1 latency reversal using PrimeFlow RNA technique

Glòria Martrus, Hamburg

Glòria Martrus1, Annika Niehrs1, Marcus Altfeld1,2
1Heinrich-Pette-Institut, Leibniz Institute for Experimental Virology, Hamburg, Germany; 2The Ragon Institute of MGH, MIT and Harvard, Cambridge, USA

Background
Upon infection, HIV-1 establishes a pool of persisting latently infected cells. Although antiretroviral treatment can effici tly target HIV-1 replication, it cannot eradicate the established reservoir. New drug therapies are focusing on reactivating the latently infected cells. Nevertheless, it remains uncertain to what extent latently infected cells are reactivated. We established a new technique to evaluate the molecular kinetics of HIV-1 latency reactivation on the single cell level, distinguishing cells in which only viral mRNA is expressed from cells in which viral proteins are produced.

Methods
J89 cells were used as an HIV-1 latency reactivation model. Cells were treated with different concentrations of hTNFα cytokine and an HDAC inhibitor, Romidepsin (RMD), for defined time periods. Using a newly established technique that allows for simultaneous detection of mRNA targets and intracellular proteins by multiparameter fl w cytometry, combined intracellular staining for Gag p24 protein and Gag p24 mRNA was performed.

Results
Following stimulation with 10ng/ml of hTNFa or 5nM RMD for 24hrs, three distinct cell populations were distinguishable: single-positive p24 mRNA+ cells, double-positive p24 mRNA/protein+ cells and double-negative cells. p24mRNA+ cells were detected 3hrs post-stimulation (hTNFa) and 6hrs post-stimulation (RMD) in the absence of intracellular Gag protein detection. With increasing time, the single-positive p24mRNA+ population shifted towards a double-positive p24 mRNA+/protein+ population.

Conclusion
Using a novel fl w cytometry based method, the kinetics of HIV-1 mRNA and protein synthesis upon latency reactivation were determined, and the consequences for the expression of specific su face markers that might enable immune recognition of HIV-1-infected cells.

Imaging Flow Cytometry Enhances the Detection of Small Particles and Rare Events Enabling Emerging Applications in Immunology and Oncology

Peter Rhein, Merck Millipore

Detection of small particles and rare events by Flow Cytometry is often hampered by the limited amount of information that can be gathered from light scatter signals and fluo escence. Small particles like extracellular vesicles (EVs) have recently gained increased interest as they are physiologically and diagnostically relevant. The small but variable size and abundance made analysis of single EV difficult on   aditional fl w cytometers.

For rare events detection discrimination of relevant events and artifacts is absolutely mandatory and requires complex and challenging experimental design for traditional fl w cytometers. One example are circulating tumor cells (CTCs), released into the bloodstream from primary and metastatic cancers and tumor cells persisting during therapy (minimal residual disease, MRD) that have important prognostic and therapeutic implications and are valuable tools for understanding tumor biology.

The ImageStream® is a multispectral imaging fl w cytometer that helps to overcome these obstacles by combining the statistical power of fl w cytometry with imaging content of microscopy in one system. Creating bright field and fluo escence images of each single cell, the researcher can objectively quantify hundreds of features based not only on fluo escence intensity or scatter light but also related to morphology, probe localization or co-localization of probes. A dedicated side scatter laser and the use of morphology characteristics of bright fiel and fluo escence images enhances the detection of small particles and rejection of artifacts in rare event analysis. Using the ImageStream, we can combine fl w cytometric and microscopic assays like immunophenotyping and fluo escence in situ hybridization (FISH), which can significa tly improve the detection and functional analysis of rare events like CTCs and MRD cells.

High-Content Cytometry for Translational Research

Jörg Schlegel, Zellkraftwerk

Single cell analysis has become increasingly important for cellular biologists in basic and translational research as it allows studying cell-to-cell variation within a cell population. Chipcytometry is a new technology not competing with flow cytometry, but complementing it in areas where sample analysis with flow cytometry is difficult, e.g. when more than 16-18 biomarkers need to be analyzed per sample or for multicenter studies of samples collected from different continents.

Chipcytometry uses disposable microfluidic chips for long-term biobanking of cell suspensions and tissue sections. In this way each cell can be called individually again and again over a period of at least 20 months and a virtually unlimited set of markers can be analysed per cell.

At CYTO 2014 Zellkraftwerk has launched a 55-plex panel of validated antibodies for human immune cell phenotyping which has grown continuously since. Here we now present a 71-plex immonophenotyping panel and give an overview on validation data and applications for Chipcytometry. Results shall be presented on applications such as high-content immunophenotyping, studies on subsets of immune cells, signaling events on many cells simultaneously and phenotyping of cells infiltrating into tissue, supporting research in immunology, immuno-oncology, neuroimmunology, autoimmunity and precision medicine.

Session 9: Klaus Görttler Session

2:00pm - 3:30pm   Paul-Ehrlich Lecture Hall

Chair: Anna Rao, Stockholm, Sweden

Chair: Alexander Grünberger, Jülich

Bet-hedging in bioprocesses deciphered by on-line fl w cytometry

Frank Delvigne, Gembloux, Belgium

University of Liège, Gembloux Agro-Bio Tech, Passage des Déportés, 2 5030 Gembloux, Belgium

Microbial phenotypic heterogeneity is known to be naturally present in isogenic population and can be attributed to the stochastic nature of the biochemical reactions. An important question at this level was to determine whether such stochastic behavior exhibits some functionality, i.e. how single cell heterogeneity leads to population level strategies [1]. One of this strategies, called bet-hedging, is known to give a competitive advantage to the population, by leading for example to a persistent phenotype able to survive to antibiotics exposure. Persistence has been recently recognized to occur during diauxic shift of several microorganisms [2][3], a mechanism often encountered during industrial bioprocesses. Additional experiments have pointed out a significa t impact of microbial phenotypic heterogeneity at the level of the metabolic flu es [4][5]. Taken altogether, these results point out the fact that microbial phenotypic heterogeneity plays significa t role on bioprocesses performances and robustness [6]. In order to highlights the occurrence of microbial phenotypic heterogeneity during bioprocesses, on-line fl w cytometry will be used in order to investigate the fitness of different E. coli single deletion clones. These clones have been deleted at the level of their capacity for synthesizing porins. Porins ensure the transport of nutrients at the level of outer membrane of E. coli and their synthesis is modulated in front of substrate availability [7]. By using on-line fl w cytometry and propidium iodide staining, it will be shown that outer membrane permeabilization can be considered as a bet-hedging strategy displayed by E.coli in bioprocessing conditions.

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The human toponome project: translating the spatial protein network code (Toponome) into effici t therapies.

Walter Schubert, Magdeburg

Walter Schubert 1,2 1 Head Molecular Pattern Recognition Research group, Otto-von-Guericke-University, Magdeburg, Germany, 2 Visiting professor for Toponomics, BIOSS center, University of Freiburg, Germany

The spatial protein network code (toponome) driving diseases can be directly assessed in morphologically intact tissue by using 3D imaging cycler microscopy (ICM). 3D ICM co maps thousands of multi-protein complexes at a time. They are visualized as large geometric objects, or landscapes, uncovering unexpected/unknown multicellular disease mechanisms with hierarchical lead proteins, controlling the underlying protein network: blockade of lead proteins results in protein network disassembly and loss of (dys) function. Experimentally this has been shown to effici tly interfere with the membrane toponome of cancer cells leading to loss of spatial orientation. Similarly, proof of concept has shown that clinical predicitions from such in situ toponomes (1999) were correct: stopp of disease progression of appr 30% of amyotrophic lateral sclerosis patients as concluded from a current US clinical trial. The underlying disease mechanisms of neuronal damage have been decoded within the human toponome project in our lab for translation into more effective future therapies. Similarly, the human toponome project has been established internationally GER, UK and USA in other field .„Extreme" 3D ICM imaging is a hypothesis free approach which appears to be critical for findin unknown disease mechanisms and valid drug targets with high degree of certainty.

The success of ICM approach relies on the fact that natural protein networks *in situ/in vivo* are directly recognised by their spatial code.

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(5) Schubert W. Advances in toponomics drug discovery: Imaging cycler microscopy correctly predicts a therapy method of amyotrophic lateral sclerisis . Cytometry A. 2015 Apr 13, 87: 696-703. Review

Editorials

(1) Murphy F. Putting proteins on a map. Nat Biotechnol. 2006 Oct;24(10):1223-4 (comment on ref 1, above)

(2) Sage, L The molecular face of prostate cancer. J. Proteome Res.2009; 8, No 6, 2116 (editorial to a corresponding publication in this issue)

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- OPEN TO ALL -

Keynote: „Meet the Expert"

3:30pm - 4:30pm   Paul-Ehrlich Lecture Hall

Chair: Hyun-Dong Chang

Professor Paul Frenette is the chair and director oft he Ruth L. and David S. Gottesman Institute for Stem Cell and Regenerative Medicine Research at Albert Einstein College of Medicine, and he is adjunct professor of medicine, hematology and medical oncology at Johns Hopkins University.

A hematologist and cell biologist, Prof. Frenette is interested in the behavior of stem cells *in vivo*, particularly he focuses on the role of hematopoietic stem cell regulators in blood diseases, and on the differences in the development of normal and cancerous human stem cells. In his talk, he will present his groundbreaking work on the analysis of hematopoietic stem cell niches in situ.

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Notes

Dissecting hematopoietic stem cell niches

Paul S. Frenette

Ruth L. and David S. Gottesman Institute for Stem Cell and Regenerative Medicine Research, Albert Einstein College of Medicine, New York, USA

The identific tion of niche cells in the bone marrow has proved to be a challenging undertaking due to the complexity of its cellular constituents, the paucity of specifi markers to accurately separate stromal cells, and its poorly accessible location in calcifie bone. Several recent studies have indicated that the vasculature of the bone marrow may form major niches for maintaining the delicate balance between self-renewal and differentiation of hematopoietic stem cells (HSCs). Our laboratory has been interested to dissect the constituents of the stem cell niche. Our previous studies have found that nerve fibers from the sympathetic nervous system (SNS) regulate circadian HSC migration, indicating that the stromal cells targeted by nerves may play important roles in the niche. Nerves are closely associated with blood vessels in many tissues, including the bone marrow. Rare stromal cells marked by Nestin-GFP containing mesenchymal stem cell activity form a niche as they are significa tly associated with HSCs, and express high levels of "niche factors". Further studies have revealed distinct Nestin+ cell subsets wherein the NG2+ pericyte-like arteriole-associated fraction harbors dormant HSCs whereas the reticular-like sinusoid-enriched LepR+ subset is associated with activated HSCs. HSC progeny also regulates HSC fate, including the macrophage which, by contrast to SNS signals that reduce niche factor-mediated HSC attraction to the niche, exerts an opposite influen e on the niche. Additionally, megakaryocytes contribute

to HSC quiescence in a distinct niche via CXCL4-mediated feedback loop where they regulate their numbers. These data suggest a complex interplay and feedback among stromal and hematopoietic cells. We will discuss the implications in hematopoietic malignancies and present recent studies about the identific tion of a portal vessel-associated HSC niche supporting fetal liver hematopoiesis.

Notes

Poster Session

Auditorium Cross Over

Chair: Leoni Kunz-Schughart, Dresden

Chair: Torsten Viergutz, Dummerstorf

1:00pm - 1.:30pm Poster Session Track A

1.:30pm - 2:00pm Poster Session Track B

A01   IL-10 induction in human naïve and memory T cell subsets by the Notch signaling pathway

Jonas Ahlers

Ahlers, Jonas (1,2); Mantei, Andrej (1); Hohnstein, Thordis (1,2); Mocke-Tenbrinck, Nadine (3); Bacher, Petra (1,2); Scheffold, Alexander (1,2,3). 1: DRFZ Berlin, Germany; 2: Department of Cellular Immunology, Clinic for Rheumatology and Clinical Immunology, Charité - University Medicine Berlin, Germany; 3: Miltenyi Biotec GmbH, Department of Research and Development, Bergisch Gladbach, Germany

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 define . 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 in murine T cells activation of the Notch pathway induces the selective induction of IL-10 in differentiating and established Th1 cells which was *in vivo* dependent on the Notch-ligand Delta-like4 (Dll4). Now we demonstrate that recombinant Dll4 or Dll4 expressing plasmacytoid dendritic cells potently induce the expression of IL-10 in human naïve and memory T cells including their major subsets Th1, Th2, Th17, Th1/17 and Th22. In naïve T cells Dll4 requires co-stimulation by a cytokine cocktail consisting of IFN-alpha, IL-6 and IL-21. In contrast Dll4 is suffici t to induce IL-10 in memory T cells but the expression is enhanced by IFN-alpha, IL-6 and IL-21.

A02   Biobanking of blood and cytometric analyses: establishment of a stabilizing protocol allowing long-term storage of whole blood samples and their immunophenotyping by fl w and mass cytometry

Patrizia Batel

Batel, Patrizia; Hirseland, Heike; Schulte- Wrede, Ursula; Baumgart, Sabine; Mei, Henrik; Radbruch, Andreas; Grützkau, Andreas. DRFZ Berlin, Germany, AG Grützkau

The possibilities for long-term storage of whole blood samples with respect to biobanking and clinical research applications are very limited so far. Freezing of PBMC's is a commonly used strategy, but typically all granulocytes will be removed by density gradient centrifugation. Therefore, a detailed immunophenotyping of the entire leukocyte compartment will be not possible. This study aims to establish a new commercially available protein-stabilizing reagent ("SmartTubeTM proteomic stabilizer"") to freeze human blood samples that will be analyzed after thawing by multiparametric fl w and mass cytometry. For this purpose the influen e of the SmartTubeTM reagent on epitope preservation, fluo escence properties of the fluo ochromes used and possible non-specific binding artefacts were examined. In total 82 antibody conjugates were compared on fresh blood cells and on blood samples stored for a period of at least 24 hours and at the longest up to 21 months at -80°C. It could be shown that light scatter parameters of main leukocyte populations, such as neutrophils, monocytes and lymphocytes have been changed after fix tion, freezing and thawing, so

that a clear discrimination between neutrophils and monocytes was not possible according their light scatter characteristics alone. While most of the antibodies investigated still recognized their particular epitope a marked increase in autofluo escence especially of the granulocytes was observed. Therefore, all antibodies used must be thoroughly titrated using SmartTube-fi ed blood samples. Here we show data for a successful establishment of an 11-parameter staining allowing detection of T- and B-cell subsets, neutrophils and eosinophils, NK-cells and monocytes in stabilized, frozen/thawed blood cell samples analyzed by conventional fl w and mass cytometry (CyTOF). In addition, we could show firs data for a long-term storage of blood samples frozen for 21 months, in which all leukocyte subsets investigated, were successfully assessed in a qualitative and quantitative manner. These data are encouraging that biobanking of whole blood cells is possible with respect to cytometric analyses, especially if barcoding protocols will be applied to measure multiple samples in parallel by mass cytometry. Therefore, this procedure could be an interesting option to monitor multicentric clinical studies in the field of ch onic inflamm tion

A03   A biomarker discovery approach for urinary cells in Lupus Nephritis

Sabine Baumgart

Baumgart, Sabine (1); Bertolo, Martina (1); Peddinghaus, Anette (1); Enghard, Philipp (2); Radbruch, Andreas (1); Hiepe, Falk (3); Grützkau, Andreas (1). 1: DRFZ, Germany; 2: Department of Nephrology and Intensive Care Medicine, Charite University Hospital, Berlin, Germany; 3: Department of Rheumatology and Clinical Immunology, Charite University Hospital, Berlin, Germany

Background:
Systemic lupus erythematous (SLE) is a multisystem inflamm tory autoimmune disease. 50-80% of SLE patients develop end-stage renal failure (lupus nephritis (LN)) that is one of major causes of morbidity and mortality. Currently, histopathological results from renal biopsy are the basis for categorizing patients according to the ISN/RPS classific tion scheme into LN classes I to VI and for defining therapeutic plans. Renal biopsy is an invasive procedure not free from risk and it is not applicable to monitor LN activity over time. The goal is to detect and define cell-based urinary abnormalities and signatures circumventing a critical intervention. Recently, conventional fl w cytometry was used to detect immune cells in urine of LN patients. We apply mass cytometry technology to urine samples of LN patients to accomplish a more comprehensive immunophenotyping.
Methods:
Urine and heparin anti-coagulated blood samples were simultaneously collected from 7 LN patients and prepared for mass cytometry analysis. All patients had clinically active SLE and had already started an immunosuppressive therapy. Urine samples were adjusted with 30% v/v PBS/BSA immediately after collection to preserve cell viability. Peripheral blood cells were obtained after erythrocyte lysis. Two panels including 54 surface marker in total were used for staining procedure with additional live/dead discrimination (Pt/Ir intercalators). Cells were analyzed on CyTOF1 instrument and data analyzed by manual gating using FlowJo 10.0.7 software.

Results:

Mass cytometry allows a comprehensive immunophenotyping of immune cells washed out in urine circumventing problems regarding sample size, autofluo escence-induced artefacts and spectral overlap inherent in conventional fl w cytometry. Neutrophils and monocytes/macrophages followed by T cells represent the dominant leukocytes populations in urine samples of SLE patients. The elevated CD8 T cell count was used as biomarker for proliferative LN among SLE patients. Although T cells were below 3% of CD45+ cells, we were able to observe a decreased CD4/CD8 ratio as already described in the literature for LN blood. Additionally, a T cell subpopulation expressing the activation marker CD69 and CD38 was identified in urine but not in blood. Active LN patients show a raised urinary monocyte/macrophage population (CD14+CD36+HLA-DR++). Among this population there were M1/M2 macrophage marker observed. Almost no B cells, NK cells or DC's have been detected in urine samples.

Conclusion:

First patient data shown indicate that leukocytes detectable in urine of LN patients are promising biosensors refle ting chronic inflamm tion in the kidneys. Obviously, the LN histopathologic heterogeneity seems to be better refle ted by urinary cell populations than by peripheral blood. Therefore, larger patient cohorts and additional epithelial markers will be analyzed to identify robust biosignatures that can be used diagnostically for a classific tion of lupus nephritis.

A04 Single-cell microbiology in controlled extracellular environments

Christian Dusny

Dusny, Christian; Schmid, Andreas. Helmholtz Centre for Environmental Research - UFZ, Leipzig

The macroscopic output of any community- or population-based process employing whole cells as living catalysts comprises the cumulative activity of single cells. However, isogenic microbial populations exhibit significa t phenotypic heterogeneity at the single-cell level, encompassing the hierarchical layers from genome to phenome, yet implying heterogeneity in the catalytic capacity of individual cells. The extent of this functional intrapopulation heterogeneity is inextricably connected to frequency and amplitude of physicochemical changes in the extracellular environment. Hence, the ability to analyze single-cell dynamics in controlled environments is a fundamental requirement to mechanistically understand cellular processes.

We met the challenge of investigating single-cell physiology in controlled microfluidi environments with the Envirostat microbioreactor [1]. In combination with novel analytical concepts for the holistic description of single-cell traits, we demonstrated specific growth rates of single cells being decisively dictated by the extracellular environment. We could prove this principle to be valid for single cells of one bacterial and two unicellular eukaryotic yeast strains. All strains exhibited growth rates that exceeded population growth rates by more than 120% [2]. Furthermore, uncoupling environment and metabolic activity of single cells with the Envirostat revealed a *hitherto* underrated ultrasensitivity of the industrially important MOX promoter in the yeast *Hansenula polymorpha* to carbon catabolite repression [3]. Concerning the question whether the analysis of promoter regulation is biased by physicochemical

changes due to metabolic activity, it could be clearly demonstrated that results from apparently controlled bulk cultivations underrate effects arising from population activity and can be misleading. This underpins the importance of uncoupling environment and cellular physiology with microfluidic single-cell analysis. With regards to the MOX promoter and its industrial application in protein-secreting cell factories, the here obtained results are of high value for rationally improving population-based protein production processes. Finally, the firs multi-platform comparison of (microfluidic) single-cell cultivation technologies demonstrated possible technological bias on biological key parameters and confi med the Envirostat as an indispensable tool for elucidating single-cell dynamics beyond the bulk [4].

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A05 Direct *ex vivo* characterization of autoreactive T cells provides no evidence for autoantigen-based high affin y selection and expansion in autoimmune patients

Stefanie Gamradt

Gamradt, Stefanie (1); Hahn, Franziska (1); Rosche, Berit (2); Assenmacher, Mario (3); Bacher, Petra (1); Scheffold, Alexander (1). 1: Dep. of Rheumatology and Clinical Immunology, Charité-Universitätsmedizin Berlin, Germany; 2: Dep. of Neurology and Experimental Neurology, Charité-Universitätsmedizin Berlin, Germany; 3: Miltenyi Biotec, Bergisch Gladbach, Germany

Introduction:
Autoreactive CD4+ T cells are considered to contribute to the development and chronific tion of various autoimmune disorders, such as Multiple Sclerosis, Diabetes or Rheumatoid Arthritis. However, the characteristics of autoreactive T cells in health and disease remained so far elusive due to a lack of tools suitable for their direct identific tion. By applying a method for an unbiased, direct *ex vivo* characterization of MS-associated antigen-specific CD4+ T cells we aimed at elucidating their relevance in this disease.
Methods:
We combined the enrichment of antigen-activated T cells expressing the activation marker CD154 (CD40L) after stimulation with MS-relevant antigens (MOG, MBP1, PLP) with multicolor FACS analysis to characterize the frequencies, cytokine expression and phenotype of autoantigen-specific T helper cells directly from the peripheral blood of healthy individuals and patients. Furthermore, autoantigen-specific T cell clones and lines were generated to

confi m their specifici y and to study the functional avidity of these cells.

Results: CD154 identified naïve and memory-type autoreactive T cells with high specifici y in healthy donors and MS patients. Autoantigen-reactive memory T cells, in particular with a Th1/Th17 phenotype, were slightly overrepresented especially in younger patients. A higher proportion of these cells were polyfunctional and displayed a higher production of total IFN-γ- and IFN-γ+GM-CSF+TNF-α when compared to healthy controls. However, the memory/naive ratio as well as the functional avidity of MOG-specific memory T cells was low in both groups when compared to a recall antigen.

Conclusions:

The CD40L-enrichment technology enabled a rapid quantitative and qualitative characterization of autoantigen-specific CD4+ naïve and memory T cells in healthy individuals and MS patients. Although frequencies did not differ, these cells displayed a more inflamm tory and polyfunctional phenotype in patients. The low functional avidity of the autoantigen-reactive memory T cells and the minimal differences with regard to naïve/memory status, however, argue against an autoantigen-driven high affin y selection in MS patients. Instead, autoantigen-specific memory T cells may largely result from the broad cross-reactive memory repertoire present in adult blood donors.

A06   Sorting of vancomycin BODIPY FL labeled *S. aureus* from infection experiments for further mass spectrometric analyses

Petra Hildebrandt

Hildebrandt, Petra (1,2); Surmann, Kristin (1,2); Depke, Maren (1,2); Gawron, Sophia (1,2); van Dijl, Jan Maarten (3); Völker, Uwe (1); Schmidt, Frank (1,2). 1: Universtätsmedizin Greifswald, Interfakultäres Inst. f. Genetik und Genomforschung, Abt. Funktionelle Genomforschung; 2: ZIK-FunGene JRG "Applied Proteomics", Fr.-L.-Jahn Straße 15A, 17487 Greifswald, Germany; 3: Department of Medical Microbiology, University of Groningen, University Medical Cemnter Groningen, The Netherlands

In order to comprehensively monitor host-pathogen interactions of *S. aureus* and human cells on the molecular level an increasing number of omics studies are being conducted. Such analyses allow simultaneous profiling of the response of the host to pathogen invasion as well as the response of the pathogen to the new environment. However, due to often relatively low numbers of internalized bacteria together with a prevailing number of host proteins, for proteomics studies it is necessary to enrich the bacteria from such an infection setup after lysis of the host cells. Within this study we optimized a labeling strategy originally published by Oosten et al. 2013 for proteomics applications to successfully quantify and enrich internalized *S. aureus*, a pathogen which is responsible for skin infections but also severe diseases such as pneumonia, endocarditis, and septicaemia.

Fluorescence labeling of *S. aureus* cells allows counting and separation of bacteria from lysed host cell debris after infection experiments by fluo escent activated cell sorting (FACS). Permanent labeling of microorganisms can e.g. be achieved by transfection with a plasmid coding for the expression of a fluo escent protein (FP). Another possibility is to stain viable bacteria with either DNA-specific dyes, e.g. Syto, or with carboxy fluo escein diacetate succinimidylester (CFDA-SE), a substrate that displays fluo escence after cleavage

by cytoplasmic esterases. Syto as well as CFDA-SE applications are suitable to stain bacteria from pure cultures, e.g. for counting purposes, but not for counting or sorting from infection settings, because the unspecific back round in the region of interest is too high.

Therefore, we investigated the possibility to stain the cell wall of *S. aureus* with the commercially available glycopeptidic vancomycin BODIPY FL (VMB) conjugate at sub-inhibitory concentrations of vancomycin. In preceding experiments the VMB concentration which resulted in the highest fluo escent intensity was determined. Applying a low concentration of 0.2 µg/ml we found only few unspecific proteome changes in the labeled bacteria compared to non-labeled bacteria. Moreover, cell sorting provoked stronger changes compared to the staining. Having tested the suitability of VMB labeling for proteomics, we first applied this dye for counting labeled *S. aureus* HG001 by fl w cytometry – a method faster and more reliable than counting of colony forming units (CFU). We counted a pure bacterial culture as well as bacteria recovered from lysed human cell lines 2.5 h and 6.5 h p.i. and obtained comparable results to a *S. aureus* HG001 strain which continuously expresses plasmid-encoded GFP. Also bacterial enrichment in a FACSAria Ilu cell sorter was possible due to a clear separation of labeled bacteria and host cell debris.

Within this study we provide a staining strategy which allows the separation of *S. aureus* from infection experiments and which is, moreover favorable compared to plasmid-encoded GFP when working with clinical isolates since genetic manipulations which are technically difficu in these strains are avoided.

A07   From monitoring to steering microbiomes based on single cell analysis

Christin Koch

Koch, Christin; Müller, Susann; Harnisch, Falk.,Helmholtz Centre for Environmental Research - UFZ, Germany

Currently, bioreactors exploiting natural microbial communities (microbiomes) for bioenergy production or waste removal are almost exclusively operated based on bulk parameters and empirical expert knowledge. The microbiomes of these bioreactors often remain a "black box" as their composition and function are only analyzed retrospectively (mostly in a case of failure). Here, on-time microbiome analysis can allow a proactive process management. Cytometric finge printing represents an excellent method to monitor microbiomes with high resolution and in a fast and cheap manner as it is cultivation independent and detects changes in presence, abundance as well as in the activity of individual cells and subcommunities. A fast detection of dynamics is required for process monitoring and control and can be provided by cytometric finge printing applying established protocols [1]. The combination with multiparametric correlation analyses finally allows recovering structure-function relationships within microbiomes.

The potential of cytometric finge printing for successful monitoring functional community changes is demonstrated on selected examples of bioenergy production and wastewater treatment.

[1] Koch, C., Günther, S., Desta, A.F., Hübschmann, T., Müller, S., Cytometric finge printing for analyzing microbial intra-community structure variation and identifying sub-community function, 2013, Nat. Protoc. 8 (1), 190-202.

A08   Surface marker expression of memory T helper cells in chronic inflamm tion

Patrick Maschmeyer

Maschmeyer, Patrick (1); Westendorf, Kerstin (1); Grün, Joachim (1); Durek, Pawel (1); Häupl, Thomas (2); Radbruch, Andreas (1); Chang, Hyun-Dong (1)   1: German Rheumatism Research Center (DRFZ) Berlin, Germany; 2: Department of Rheumatology and Clinical Immunology, Charité Universitätsmedizin Berlin, Germany

Chronic inflamm tory diseases cannot be cured using state-of-the-art immunosuppressive therapies. Thus, a better understanding of pathogenic cells in chronic inflamm tory diseases is required. Since memory CD4+ T helper (Th) cells are critical contributors to chronic inflamm tion in rheumatic diseases, we aimed at characterizing the surface marker expression profile of disease-maintaining memory Th cells. For this, we isolated CD45RO+CD4+ Th cells from synovial fluids of patients with rheumatic diseases and compared their transcriptomes to those of CD45RO+CD4+ Th lymphocytes from the peripheral blood of healthy individuals. Transcriptome analyses revealed differences regarding surface marker expression patterns between pathogenic memory Th cells from patients and memory Th cells from healthy individuals. We are currently using fl w cytometry to validate the expression of surface markers on Th lymphocytes from independent clinical samples on the single cell level. In addition, we are planning to investigate whether these markers are suitable targets for the selective depletion of pro-inflamm tory Th cells by using pre-clinical mouse models of chronic inflamm tion.

A09   Barcoding of live PBMC for multiplexed mass cytometry analyses

Henrik E. Mei

Mei, Henrik E; Leipold, Michael D; Schulz, Axel R; Cariad, Chester; Maecker, Holden T. The Human Immune Monitoring Center (HIMC), Institute for Immunity, Transplantation and Infection, Stanford University School of Medicine, Stanford, CA 94305, USA

Mass cytometry allows massively multiparametric and high-resolution immunophenotyping on a single-cell level. We present here a sample barcoding approach for live human PBMC, allowing for identical conditions during immunostaining, fix tion, and permeabilization of PBMC, as well as during acquisition on the CyTOFTM instrument.

Using unique combinations of 3 out of 6 different anti-CD45 conjugates, we barcoded and combined up to 20 different samples.

In addition to previously established In-113 and In-115 conjugates, we generated new anti-CD45 conjugates labeled with isotopically purified palladiums and platinums, which permitted CD45 barcoding with no or negligible interference with lanthanide-conjugate-based immunophenotyping panels.

There was good correlation of cell population frequencies derived from single, successively acquired samples, and from deconvoluted barcoded sample data. Using three and only three out of the possible six antibodies per barcode facilitated electronic removal of cell aggregates that expressed more than three labels. This addresses an important limitation of

mass cytometry data and its interpretation. The aggregate removal during data deconvolution allowed for less restrictive gating on DNA and cell length parameters, thus allowing more events to be analyzed. As a result, electronic cell recoveries after denconvolution of barcoded samples and separately acquired samples were similar. In summary, CD45-barcoding speeds sample acquisition, allows aggregate removal, minimizes antibody use, and improves data accuracy by harmonizing sample preparation and acquisition conditions.

A10   Assessment of mast cell/basophil degranulation by cell surface protein analysis and intracellular histamine measurement

Zane Orinska

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Allergies are among the most common chronic conditions worldwide. Allergy symptoms range from making life miserable to risk for life-threatening reactions in case of severe asthma or anaphylaxis. Pseudo-allergic IgE-independent reactions against polycationic compounds such as non-steroidal neuromuscular blocking drugs or antibiotics of fluo oquinolone family - are the second type of hyper-responsiveness described for MCs so far. Mast cells and basophils are considered as primary effector cells in allergies. Local or systemic MC-degranulation is responsible for pseudo-allergic conditions. Clinically relevant mast cell/basophil degranulation can be assessed by the measurement of both secreted mediators and translocation of secretory granule membrane proteins to the plasma membrane. Histamine is preformed in mast cell/basophil granules and immediately released from cells e.g. upon activation through cell surface antigen-specific IgE bound to high affin y FcepsilonRI or polycationic compounds. Here we assess mast cell/basophil functionality on single cell level by a combination of multiparametric cell surface protein analysis with intracellular histamine measurement. Murine peritoneal mast cells (PMCs) were enriched, loaded with IgE and degranulated by specific antigen. Activation dependent cell surface expression of granula membrane proteins (CD107a, CD63) was combined with measurement of histamine-specific mesoionic acid fluo ide - Histamine Blue (HB) - fluo escence. In IgE/antigen-activated PMCs a decrease of HB fluo escence correlated with CD107a and CD63 upregulation and   beta-hexosaminidase release. Subcellular live-cell analysis of PMCs indicated different pattern of HB fluo escence in resting and activated PMCs. Furthermore, upon mast cell activation HB fluo escence was changing in stimulus-dependent manner. Human basophils were purifie , activated and subsequently, HB-fluo escence was measured. In human basophils HB staining indicated heterogeneity in histamine content; basophil activation also induced reduction of HB-fluo escence. Thus, the analysis of receptor expression/translocation and measurement of intracellular histamine content will complete degranulation-related mast cell/basophil signature, provide additional information about stimuli-specific degranulation requirements and dissect new functional populations of mast cells and basophils.

A11   Micropatterned arrays of defined ligands to assess in real time the dynamics of single platelets

Raghavendra Palankar

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We report a strategy to generate by electron beam lithography high fideli y micropatterned arrays to assess the interaction of single platelets with immobilized ligands. The main aims were i) creating a surface which does not non-specifically activate platelets; ii) immobilizing immune complexes to this surface without altering their antigenic epitope properties; iv) measuring platelet binding to these immune complexes and, v) analysis of single platelet activation. As a proof-of-principle we functionalized the microarrays with platelet factor 4 (PF4)-heparin-IgG complexes. We embedded biotinylated water-soluble quantum dots (QD) into polyethylene glycol (PEG)-coated micropatterned arrays and functionalized them via streptavidin to bind biotinylated ligands, here biotinylated-PF4/heparin complexes. The integrity of the PF4/heparin-complexes was shown by binding of anti-PF4/heparin antibodies. Ligand density was quantified by immunofluo escence and immunogold antibody labelling. Real-time calcium imaging was employed for read-out of single platelets activated on micropatterned surfaces functionalized with PF4/heparin-IgG complexes. With the smallest micropatterns (0.5x0.5µm) we show that single platelets become strongly activated by binding to surface-immobilized PF4/heparin-IgG, while on larger micropatterns (10x10µm), platelet aggregates formed. These findings that HIT antibodies can cause platelet activation on microarrays illustrate how this novel method opens new avenues to study platelet function at single cell level. Generating functionalized microarray surfaces to which highly complex ligands can be bound and quantified has the potential for platelet and other cell function assays integrated into high-throughput microfluidic devi es.

A12   A whole blood nine-colour fl w cytometry panel for the evaluation of human primary immunodeficiencie

Ulrich Salzer

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Introduction:
In many primary immunodeficiencies (PID) and especially primary antibody deficien y syndromes (PAD) the composition of peripheral blood B-cell subsets is disturbed. Thus the detailed analysis of B-cell subpopulations becomes increasingly important in the differential diagnosis of PID patients in general and in the diagnosis and monitoring of PAD patients in particular. Here we present the results of a nine-colour whole blood fl w cytometry protocol for the analysis of B-cell subsets evaluated in 54 healthy donors and selected PID / PAD cases. Methods: Samples were prepared either with Ficoll density gradient centrifugation or EDTA

whole blood was lysed with ammonium chloride buffer. Cells were stained with antibodies against CD10, CD19, CD21, CD27, CD38, IgA, IgD, IgG and IgM. Samples were run on a Beckman Coulter Navios fl w cytometer.

Results:

The whole blood staining method improved and facilitated the workfl w compared to traditional Ficoll isolation while yielding comparable results. The used markers allowed the reliable identific tion of 12 B-cell subpopulations, including also low frequency populations like immature, transitional B-cells, activated CD21low B cells and plasmablasts. In the nine colour / marker setting, combined logical gating allowed more precise discrimination, determination and quantific tion of individual B-cell subsets. Normal ranges were calculated from 54 samples from adult healthy donors. Selected PID patients (CVID, HIGM, CID) were analysed to prove the applicability of the protocol in the diagnostic setting.

Conclusions: We established an effici t nine-colour whole blood fl w cytometry protocol useful in the diagnosis of patients with PID."

A13 NK cells as biosensors for responsiveness to Etanercept in ankylosing spondylitis

Ursula Schulte-Wrede

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Background:

Ankylosing spondylitis (AS) is a chronic inflamm tory rheumatic disease which preferentially affects the axial skeleton and is strongly associated with the pro-inflamm tory cytokine tumor necrosis factor alpha (TNF). Therapeutic targeting of TNF is approved to be highly effective in patients who fail to respond to conventional anti-inflamm tory drugs. However, only around two-thirds of anti-TNFa treated AS patients show an adequate response according to the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) independently from the biological used. Therefore, there is an urgent need for biomarkers which would aid in treatment choice and treatment outcome separating responders and non-responders to such expensive therapies and to avoid side effects induced by ineffici t drugs. Thus, the aim of this study was to identify cell-based biosensors in peripheral blood by multiparametric fl w cytometry that can be used for an early treatment stratific tion of AS patients.

Methods:

A multiparametric fl w cytometric approach, including 50 monoclonal antibodies combined to 10 staining cocktails, was applied to identify useful baseline predictors in 38 AS patients with active disease before treatment with the TNF blocker Adalimumab, Etanercept, Golimumab or Inflixima . BASDAI response criteria were used to determine therapeutic success after 1 to 6 month. Automated clustering of fl w data, correlation analysis and receiver operator characteristics were accomplished to appoint auspicious candidate phenotypes.

Results: Out of multiple potentially significa t parameters, which are involved both in acquired and adaptive immunity the NK cell compartment revealed most promising subsets that are predictive for a successful therapy response to Etanercept in AS. Correlation analyses showed an errorfree classific tion of responders and non-responders for Etanercept but not for Adalimumab-treated patients.

Conclusions:

This is the first study demonstrating that the composition of the NK cell compartment has predictive power with respect to classify AS patients whether they will respond or fail to the treatment by Etanercept. These results also shed some new light on the mode of action comparing TNF-alpha neutralizing antibodies and soluble TNF alpha-receptors. In conclusion, these data make it reasonable to assume that monitoring of particular NK cell phenotypes can be used in terms of a companion diagnostic to realize the concept of personalized medicine in the field of rheumatology. But we are aware that the results presented have to be validated by independent and larger patient cohorts.

A14   Defining the heterogeneity of murine mesenchymal bone marrow stromal cells

Daniel Schulz

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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 refle ts surface marker expression but difficul due to low numbers and yield in efficie   y. Thus, an effici   t protocol and subsequent _ex vivo_ characterisation 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 mice were dissected to obtain the bones. 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 and 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 suffici t numbers for gene expression analysis was feasible. Expression data from total VCAM-1+ stromal cells or an IL-7+ subset confi m the overall heterogeneity of bone marrow stromal cells.

Conclusion:

Ultimately, we developed a protocol investigate the stromal heterogeneity in the bone marrow _ex vivo_ by using an enzymatic digestion followed by fl wcytometrical analysis and sorting to gain insights into the role of stromal cells in the generation and maintenance of immunological memory."

A15   B7 COSTIMULATION AND INTRACELLULAR INDOLEAMINE 2,3-DIOXYGENASE (IDO) EXPRESSION IN UMBILICAL CORD BLOOD AND ADULT PERIPHERAL BLOOD

Gergely Toldi

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Objectives:

Alterations in the expression of B7 costimulatory molecules and their receptors as well as differences in the tryptophan catabolic pathway may influen e immunological reactivity of umbilical cord blood (UCB) compared to adult peripheral blood (APB) T lymphocytes.

Methods:

We determined the frequency of activated (CD11b+) monocytes expressing B7-1, B7-2, B7-H1, and B7-H2, and that of T cells and CD4+ T helper cells expressing CD28, CTLA-4, PD-1, and ICOS in UCB and APB samples using fl w cytometry (BD FACS Aria). We also examined the intracellular expression of indoleamine 2,3-dioxygenase (IDO) applying fl w cytometry and plasma levels of tryptophan (TRP), kynurenine (KYN) and kynurenic acid (KYNA) using high-performance liquid chromatography.

Results: The level of CTLA-4 expression on CD4 cells was higher in UCB compared to APB, indicating that the possibility of CD28-mediated costimulation may be decreased. The level of the corresponding costimulator molecule, B7-2 was also elevated. Therefore, this inhibitory relation may function to a higher extent in UCB than in APB. The plasma KYN to TRP (K/T) ratio was two-fold higher in UCB compared to APB. However, the capacity of UCB monocytes compared to APB monocytes was lower to produce IDO, and reverse signalling via B7-2 in UCB monocytes was found to be immature, which suggests that the observed increase in K/T ratio may be due to placental rather than fetal overexpression of IDO in competent cells.

Conclusion:

These factors may all contribute to the previously observed reduced reactivity of UCB T lymphocytes compared to APB T cells."

B01   Implementation and validation of a neutrophil purifi ation approach

Alina Bartholomäus

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"Since their discovery at the end of the 19th century, neutrophils were long considered as simple inflamm tory killer cells that are involved in the first line of de ense against infectious diseases.

With the discovery of sophisticated mechanisms of how neutrophils locate and eliminate pathogens and thereby regulate immunity and inflamm tion, this view has been changed dramatically. Neutrophils have been linked to shape adaptive immune responses by recruiting and activating cells like monocytes, dendritic cells and lymphocytes as well as they were found to be involved in physiological and pathological processes beyond the immune system.

Thus, there is a growing interest for studying and understanding neutrophil functions with regard to diverse research field .

One limiting factor is to isolate neutrophils without activating them. The gold standard for neutrophil purific tion methods is the density gradient centrifugation, which allows for the isolation of untouched neutrophils with good purities. However, this method is very laborious and time-consuming and it is difficult o achieve purities over 95%.

Therefore there is a high need for alternative purific tion protocols. Besides magnetic-activated cell sorting with microbeads, fl w sorting could offer a promising alternative. However, up to now there is no optical method available to sort untouched neutrophils. Conventional droplet sorters have several properties that might lead to an activation of neutrophils and impairment of function.

The MACSQuant Tyto is a micropchip-based cell sorter which requires no sheath fluid and droplet formation but instead uses low pressures to sort the sample in a disposable cartridge. The cells enter the sorting chip from the input chamber and are interrogated and sorted by a mechanical valve that redirects a target cell in the positive fraction chamber. With these characteristics the MQ Tyto offers gentle cell conditions and is a well-suited candidate instrument for neutrophil isolation.

We compared the sort performance of the isolation of human neutrophils with the MACSQuant Tyto to neutrophil separation with magnetic beads and Percoll density gradient centrifugation and analyzed the purity, viability, functionality and activation status of the cells, thereby illustrating the abilities of the MACSQuant Tyto.

In addition, we developed a novel metric to compare the sort performance with regard to the number of sorted cells and cell purity, as well as the time it took to process the sample material. With this sort performance index it is now possible to quickly compare distinct experimental setups, different sorting instruments or even different sorting technologies.

B02   Resident versus circulating memory T cells in Humans

Carla Cendon

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Memory T cells are an essential component of immunological memory. They have been identified both in blood circulation and in tissues such as the bone marrow, gut and skin. There is compelling evidence suggesting that they form separate populations, showing differences in their phenotype, antigen receptor repertoires and mechanisms of survival. Their differences in lifestyle may explain why, the number of memory T cells in blood specific for a given pathogen or vaccine decline slowly over time, while they are maintained for lifetime in the bone marrow. It is known that interleukin-7 (IL-7) is essential for the maintenance of T cell memory, and previous studies have shown that murine memory T cells of the bone marrow are docking onto IL-7 expressing stromal cells. Here we compare the survival mechanisms of memory T cells according to CD69 expression from bone marrow versus memory T cells circulating in the blood. Moreover, we have recently shown that, in humans, bone marrow is home to polyfunctional memory T cells which are resting in terms of proliferation, transcription and mobility. These memory T cells maintain memories to childhood pathogens and vaccines through lifetime, even when such memory T cells can no longer be detected circulating in the blood. It is enigmatic how bone marrow memory T cells are reactivated and mobilized to mount secondary immune responses. Here we show the rapid increase in frequencies and numbers of antigen-reactive memory T cells in the blood after systemic vaccination of healthy volunteers, suggesting mobilisation of tissue resident memory T cells, probably from the bone marrow. In the long run, however, those memory T cells generated in secondary immune reactions are maintained in the bone marrow again, at least the multifunctional ones, as evident from the analysis of elderly donors.

B03   ImmuDrug   discovery: Identifi ation   of   novel immunoregulators by high throughput fl w cytometry screenings

Stefan Frischbutter

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Autoimmune diseases like rheumatoid arthritis or multiple sclerosis are characterized by strong and chronic inflamm tion leading to cartilage or tissue destruction. Under such aberrant conditions, immune control mechanisms like regulatory T cells (Tregs) or anti-inflamm tory cytokines that normally limit self-reactive responses are overrun by effector cells secreting pro-

inflamm tory cytokines. In order to reconstitute the immunological balance during unwanted (auto) immune reactions we search for novel modulators that support immune-regulatory components like Tregs and/or cells producing regulatory cytokines like IL-10.

To identify compounds with immunomodulatory potential we developed a phenotypic micro cell culture assay using primary immune cells from transgenic mice containing green fluo escent protein (GFP) reporter for either Foxp3 (master transcription factor of Tregs) or IL-10 expression. After culturing the cells in the presence of compounds, frequencies of GFP expressing cells were analyzed using a specialized high throughput fl w cytometry platform capable of acquiring and analyzing samples from 384 well plates.

In collaboration with FMP Berlin we screened a library of 40.000 small molecules and identifie and validated one compound inducing Foxp3 and twenty compounds inducing IL-10. We further investigated the immunomodulatory potential of the hit compounds by generating cytokine expression profiles of anti-inflamm tory cytokines (e.g. IL-10, IL-27) or effector cytokines (e.g. IL-6, IL-4, TNF-α) induced by the compounds using multiplex cytokine arrays.

These phenotypic screening approaches allow identifying immunomodulatory agents useful to study immunological processes and might help the development of novel therapeutic approaches."

B04   Implementation of a System for Full Pulse Shape Analysis

Andre Gosselink

Gosselink, Andre; Krieg, Jürgen; Büscher, Martin. Miltenyi Biotec GmbH, Germany

In many research fields fl w cytometry is utilized to identify multiple complex cell populations. The methods for cell characterization were improved by increasing the numbers of fluo ochromes, discovery of cell markers and increasing the numbers of fluo escence channels. However, the spatial information about dye-distribution on and in cells still has no read-out in conventional fl w cytometry. With recent developments in electronic signal processing and increasing storage capacities on computer systems, extracting and exploring information about dye distribution is becoming possible with commonly available technology. Herewith we are presenting an approach where we use fast analog-to-digital conversion in combination with appropriate laser shaping optics in order to extract 1-dimensional dye distribution data from multiple fluo escence channels simultaneously. We derive multiple scalar values from this improved fl w cytometry setup and compare them to read-outs commonly used in imaging such as morphology, symmetry and polarity of cells. Extracting spatial information from conventional raw data in fl w cytometry is opening up the available parameter space significa tly, thus increasing performance and efficie y without increasing system complexity.

B05   Histological multiplex analysis of the spatial complexity of memory plasma cell microenvironments in the bone marrow

Karolin Holzwarth

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Stable antibody titers, which provide humoral immunity over extended periods of time, are maintained by memory plasma cells (mPC). While this mechanism serves to protect an individual in the case of repeated exposure to the same antigen, these mPCs can also contribute to disease in chronic inflamm tion, for example in humoral autoimmunity. In secondary lymphoid organs, activated B cells differentiate into plasmablasts and then, ultimately, into PCs. A fraction of these plasmablasts, which are mostly generated in secondary immune reactions (boost immunization), migrate to the bone marrow (BM), guided by the chemokine CXCL12. In the BM mPCs could become sessile while residing in specialized microenvironments called memory plasma cell survival niches. The exact composition of the survival niches as well as the mechanism of plasmablast immigration into the BM and their settlement in the niche is not yet fully understood, mainly due to the cellular and molecular complexity of the niche and a lack of tools to analyze this complex microenvironment in histological sections. In addition, BM is not only a tissue, which harbors immune memory cells; it is also the site of hematopoiesis in adults. Similar to immunological memory, hematopoiesis is organized by dedicated niches, such as the hematopoietic stem cell (HSC) niche. Some factors that are known to play a role in survival of HSCs are also important for the formation of memory niches, but the similarities and differences between various niches are not fully understood yet.The aim of this work is to analyze this complexity by using multiple-epitope-ligand-cartography (MELC), because this method allows the detection of >100 histological stainings on one single tissue cryosection. Furthermore, we will track the process of PB migration from venous sinusoids to BM parenchyma to elucidate not only the spatial but also the temporal contribution of various microanatomical environments.

B06   Dynamics of microbial communities in bioreactors

Zishu Liu

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There is still missing one perfect method to describe the structure and dynamics of natural microbial communities. Flow cytometry (FCM) can be a potential alternative to sequencing approaches for microbial community analysis.

Based on individual cell information, for example forward scatter and DAPI excitation, FCM finge printing can be used to describe community dynamics. The method has the advantages

that it is fast and cheap, and samples could be analyzed at any time and stored for longer time periods. The resolution and reliability of the community data is high and bioinformatics tools for data interpretation are available.

The purpose of the study was to check if natural microbial communities can be grown in bioreactors and whether their structure will change by the artificial procedure. One bioreactor was set up as contamination control, while three further reactors were inoculated with activated sludge. Both the design of the reactors and the culture condition were identical during the whole experiment, with a feeding rate of 0.4ml/min medium, air supply of 150ml/min, and stirring at 350 rpm. Media was composed by synthetic wastewater and 2% peptone as substrate. The sampled cells were fi ed and stained with DAPI solution before FCM measurement. The analyzed data were evaluated with the fowCybar tool (http://www.bioconductor.org/packages/release/bioc/html/fl wCyBar.html).

We found high dynamic variations in community structure over time but also between the reactors. The findings point to the fact that communities may change their composition much faster as was regarded in the past and that underlying mechanisms may not have been investigated till now. "

B07   Cell-to-cell communication in chordoma cell culture

Beate Rinner

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Chordomas are rare malignant tumors that develop from embryonic remnants of the notochord. Up to the present there is no specific or effici t chemotherapy available. Chordomas at the skull base are especially difficul to maintain in long-term culture, which would be a prerequisite for developing and testing new therapeutics. The establishment of a clivus chordoma cell line, named MUG-CC1, was characterized by morphology, immunohistochemistry and growth kinetics. During establishment Multiplex analysis using Luminex® xMAP-technology of HGF, SDF-1, FGF2 and PGFD was performed. Furthermore, a spontaneously formed lymphoblastoid cell line (termed: MUG-CC1-LCL) was characterized by immunohistochemistry, fl w cytometry and RT-PCR. The cell line showed gains of the entire chromosomes 7, 8, 12, 13, 16, 18 and 20, and high level gains on chromosomes 1q21-1q24 and 17q21-17q25. During cultivation, significa t expression of HGF and SDF-1 compared to continuous cell lines was detected. The MUG-CC1-LCL cell line is positive for CD19 and EBV. Thus, a new and well-characterized clivus chordoma cell line as well as a spontaneously immortalized B-cell line was created in this study. In order to detect potential communication between established chordoma cell lines and the lymphoplastoid cell line we established co-cultures and investigated the release of microvesicles by fl w cytometry and electron microscopy. A mix of fluo esent beads of varied diameters (Megamix-Plus; Biocytex) was used as a standard to achieve cytometer setting specific for microvesicle analysis. Additionally, microvesicles were stained through binding of

fluo escently labeled Annexin V. Preliminary data indicated that supernantant of MUG-CC1-LCL presented significa tly more Annexin positive microvesicles compared to MUG-CC1.

In summary, this cell system may serve as a tool for future research in the field of chordoma treatment strategies and in particular may serve as a model system for future studies on cell-to-cell communication.

B08 The LIFE-Adult-Study: Age- and gender matched reference information for leukocyte subsets determined by 10-color fl w cytometry

Susanne Melzer

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Introduction:

The LIFE study is a large population-based study in Leipzig, where 20,000 randomly selected subjects undergo comprehensive medical examinations and biomaterial sampling [1]. The cohort consists of 10,000 healthy adults with (age 40-79 years) and 10,000 individuals of different disease cohorts, such as head-and-neck cancer, depression and cardiovascular diseases. The overall aim is to find new molecular and cellular markers associated with frequent civilization diseases, health status and life style. Age- and gender matched reference intervals for peripheral blood leukocytes that are essential for identific tion of risk phenotypes, characterization of disease states and individualized therapy, were determined by 10-color fl w cytometry.

Methods:

Blood samples of over 600 healthy German adults (44% males and 56% female) were in-depth immunophenotyped. Experimental setup was detailed elsewhere [2]. Gender differences of the cell counts were analyzed using the Mann-Whitney U test, and age dependent differences using log-linear regression analysis (R-3.0.3), with p-values < 0.05 considered as significa t [3].

Results:

Gender related differences were found for monocytes, T-cells, NK cells and B-cells: Males had significa tly higher non-classical (p<.001) and classical (p=.005) monocyte counts, and higher NK cells counts. Women had higher counts for B-cells (p<.001) and CD4+ T-cells (p<.001), as well as for CD4+ regulatory T cells (p=.008). The strongest age-related changes were found for T-cell subsets. CD4+ CD8+ T cells (p=.002) and CD4+ regulatory T cells (p=.009) significa tly decreased with age in males, while these trends were only marginally in females. Other age effects, such as a decrease of neutrophils with advanced ages in females (p=.022) and B cells in males (p=.089) were only weak. But, for both genders with increasing age a decrease in CD8+ T cells (8-9% within fi e years; p<.001) and an increase in NK cells (males 4 % within fi e years; p=.014 and females 3 %; p=.061) were observed.

Conclusions:

The significa tly higher absolute NK cell counts in healthy elderly of the LIFE study is in accordance with the literature. Especially the differences between both genders might indicate different abilities to respond to infections. In the LIFE Follow-up these ageing effects (T cell drop and NK cell increase) will be investigated and confi med in longitudinal studies.

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B09   Microfluidic Singl -Cell Analysis with Affin   y Beads

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The functioning of biological cells relies on a complex cellular biochemical network. Important in this context are concentrations of cellular components like proteins and their changes in response to extracellular stimuli influencing the development of individual cells and eventually whole organisms. Knowledge of the mechanisms governing cellular physiology comes to a great extent from averaged biochemical analysis of ensembles of cells, although it is known that such ensemble measurements mask important functional heterogeneities between individual cells. For a comprehensive understanding of higher-level functions in cellular networks like tissues and organs and of the disease-specific dysfunctions that may occur in these networks, it is therefore vital to investigate the underlying biomolecular characteristics at the single-cell level.To address this problem, two major hurdles have to be overcome: First, one or several molecular species have to be specifically quantified in each cell. Second, a large number of cells has to be processed to sample also rare cell phenotypes. Single-cell analysis techniques such as fl w cytometry and fluo escence microscopy are only applicable if specific labeling of target analytes in whole cells is possible and cellular processes do not interfere with analyte detection.Therefore, methods with broader applicability have emerged in the past comprising the lysis of a single cell, separation of target analytes from other potentially interfering contents in the cellular lysate, e.g., by capillary electrophoresis, and laser-based detection of analytes. Integration of these labor-intensive procedures into microfluidic devices has recently enabled promising sample throughputs of up to several cells

per minute. However, during release of intracellular analytes into comparably bulky capillaries as well as transport of these analytes over large separation distances, samples are heavily diluted through diffusion, dispersion and nonspecific adsorption. In addition, biomolecules are prone to degradation and might undergo irreversible denaturation upon their interaction with the various materials used in construction of separation components. These factors significa tly reduce the sensitivity of current systems. In order to overcome these limitations, we have developed a single-cell analysis method taking advantage of versatile micrometer-sized affin y beads: They serve as substrates for capturing target analytes and act as stationary phase and as vehicles for analyte separation and transport. In a first step, the affin y beads were coincubated with and internalized by living cells via phagocytosis and brought into contact with the cytosol following light-induced phagosomal rupture; the beads carried specific capture agents, allowing effici t accumulation of intracellular target proteins in their native environment. In a second step, we used microfluidics and optical tweezers to rapidly extract the beads with the bound analytes from single cells under cell-lysing conditions, to separate them from the cell lysate and to transport them to the analysis site. Based on fluo escence readout, our method is currently able to resolve two analytes in parallel at a throughput of 1–2 cells min−1. Target proteins on the beads were detected either directly by their intrinsic fluo escence or after on-chip incubation of extracted beads with a fluo escent antibody demonstrating the potential of this method to detect also nonfluo escent proteins. In contrast to other single-cell methods based on cell lysis, we here demonstrate that target analytes could maintain a high local concentration throughout the entire protocol including lysis, separation, and transport processes. We envision our approach has broad applications ranging from screening of intracellular molecular recognition events, isolation and analysis of low abundance and unstable biomolecules from single cells, single cell autopsy for clinical diagnostics and investigation of host-pathogen interactions at single cell regimes.

B10 Identifi ation of differential surface marker expression between murine naive and memory B lymphocytes by a statistical approach to fl w-cytometry data analysis

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Splenic B cells were magnetically isolated and surface stained with a cocktail of antibodies for the identific tion of naive and IgG2b memory B cells and in a large-scale assay screened for the expression of more than 200 surface markers by staining with phycoerythrin (PE)-conjugated antibodies for the comparison of surface marker expression
For the automated comparison of expression levels, a composite index for the evaluation of the degree of similarity between two sets of frequency distributions of PE fluo escence signals was devised. The index allows ranking the distribution of different markers between two populations of cells based on the degree of similarity taking into account not only mean fluo escence values or the range of a frequency distribution. The conventional approach of visually comparing histograms produces observer-biased results, comparisons of mean

fluo escence intensity lack defini ve measure of difference between populations, whereas simple statistical measures for comparative statistics can be overly sensitive and identify even rather small differences between groups and, depending on the test, be obstructed by vast differences in the size of the populations to be compared. The composite similarity index was developed by evaluation of a test set of markers, which based on previous measurements were known to be or not be differentially expressed between naive and memory B cells. The similarity index ranges from 0 to 1, where 1 denotes a high degree of similarity and a score converging 0 indicates high dissimilarity.

The automated comparison between memory and naive B cells can identify differential expression of several surface molecules, e.g. CD80 or CD73, and represents a suitable method for the identific tion of differential marker expression in comparative screening studies.

B11   Synthesis of the physiological bone marrow memory plasma cell niche

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In the bone marrow, longlived memory plasma cells (mPC) are maintained in dedicated survival niches, provided by mesenchymal stromal cells and accessory cells, like eosinophilic granulocytes. Memory plasma cell survival and functional integrity depends on redundant and non-redundant signals from their environment. We are currently synthesizing this niche from its hypothetical elements, for a molecular understanding of the maintenance of mPC, i.e. the persistence of humoral memory. Since mPC die very fast, when removed from their niche, we had to develop a fast, gentle and effici t method to purify viable mPC, for a start. As mesenchymal stromal cells we used cells of the ST2 cell line, which express VCAM and CXCL12, but do proliferate *in vitro*, unlike their physiological counterparts *in vivo*.

When isolated bone marrow plasma cells are co-cultured with ST2 stromal 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 plasma cell 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. Survival of mPC is dependent on APRIL and also on VCAM1 – VLA4 interactions between plasma cell (VLA4) and stromal cell (VCAM1). Thus survival and function of mPC seems to be dependent on high expression of anti-apoptotic proteins like mcl-1, induced by BCMA signaling and activation of PI3K/AKT pathway for inhibition of pro-apoptotic proteins and induction of NFkB survival pathway, as induced by VLA-4 signaling.

B12 Low thymic activity and dendritic cell numbers determine the human immune response to primary Yellow Fever 17D vaccination in the elderly

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With advancing age a progressive decline in immunological competence, resulting in increased susceptibility to infection and impaired responses to vaccines, can be observed. Underlying mechanisms remain largely unclear in the human context, as complex, individual systemic immune properties render them challenging to investigate. In our study, we explored for the first time age-related changes in human immunity during a primary virus infection, experimentally induced by vaccination with live-attenuated yellow fever (YF) vaccine YFV-17D. Immunizing 11 young (median age: 26) and 12 elderly (median age: 60) volunteers, we assessed individual viral burden, serum cytokines and humoral and cellular immunity by advanced FACS analysis on numerous time points before and up to 2.5 years after vaccination. We discovered, that aged subjects developed fewer neutralizing antibodies, mounted diminished YF-specific CD8+ T-cell responses, showed quantitatively and qualitatively altered YF-specific CD4+ T-cell immunity and had reduced systemic IFNγ levels. A significa tly delayed peak in YFV-17D viremia indicated impaired infection control and viral clearance in the elderly. Among numerous immune signatures, low in-vivo numbers of naive CD4+ recent thymic emigrants (CD4+ RTE) and peripheral dendritic cells (DCs), translated into reduced acute responsiveness and altered long-term persistence of human cellular immunity to YFV-17D vaccination. Hence, we reveal here that essential elements of immune responses such as RTEs and DCs affect productive immunity in the elderly, explaining conclusively diminished responsiveness to vaccination with neo-antigens and infection with de-novo pathogens in the aged population.

B13 Memory CD8+ T cells colocalize with IL-7+ stromal cells in bone marrow and rest in terms of proliferation and transcription

Özen Sercan Alp

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Memory CD8+ T cells are important components of immune responses against recurrent infections and understanding their biology is crucial to improve their protective capabilities. It is believed that memory CD8+ T cells are maintained in secondary lymphoid tissues, peripheral

tissues and bone marrow by homeostatic proliferation. Their survival has been shown to be dependent on interleukin-7 (IL-7), but it is unclear where they acquire it. Here we show that in murine bone marrow, memory CD8+ T cells individually colocalize with IL-7+ reticular stromal cells. They are resting in terms of global transcription and do not express markers of activation, e.g. 4-1BB (CD137), IL-2 or IFNγ, despite the expression of CD69 on about 30% of the cells. These CD69+ T cells do not express Klf2 and S1PR1, and hence, resemble tissue-resident memory T cells. 95% of the memory CD8+ T cells in bone marrow are in G0 phase of cell cycle and do not express Ki-67. Less than 1% is in S/M/G2 of cell cycle, according to propidium iodide staining. While previous publications have estimated the extent of proliferation of CD8+ memory T cells on the basis of BrdU incorporation, we show here, that BrdU itself induces proliferation of CD8+memory T cells. Taken together, the present results suggest that CD8+ memory T cells are maintained as resting cells in the bone marrow in dedicated niches with their survival conditional on IL-7 receptor signaling.

B14   Comparison study of various fl w cytometers using a novel ultra stable calibration light source

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Characterization, calibration and monitoring a fl w cytometer are essential for high quality data acquisition. Suitable procedures are necessary, since a certain instrument employs specific hardware components (lasers, mirrors, fil ers etc) and is used under various conditions. Especially for long term studies or large comparison studies involving various clinics or fl w 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 present a comparison study of the various different cytometers available in the Core Facility of the DRFZ employing a novel calibration light source. This device is commercially available and implements ideas from Steen and Wood. With this device we pave the way to an even more precise calibration referring to an absolute scale for the number of detected photo electrons. Furthermore the effort one has to put into the calibration is substantially lowered.

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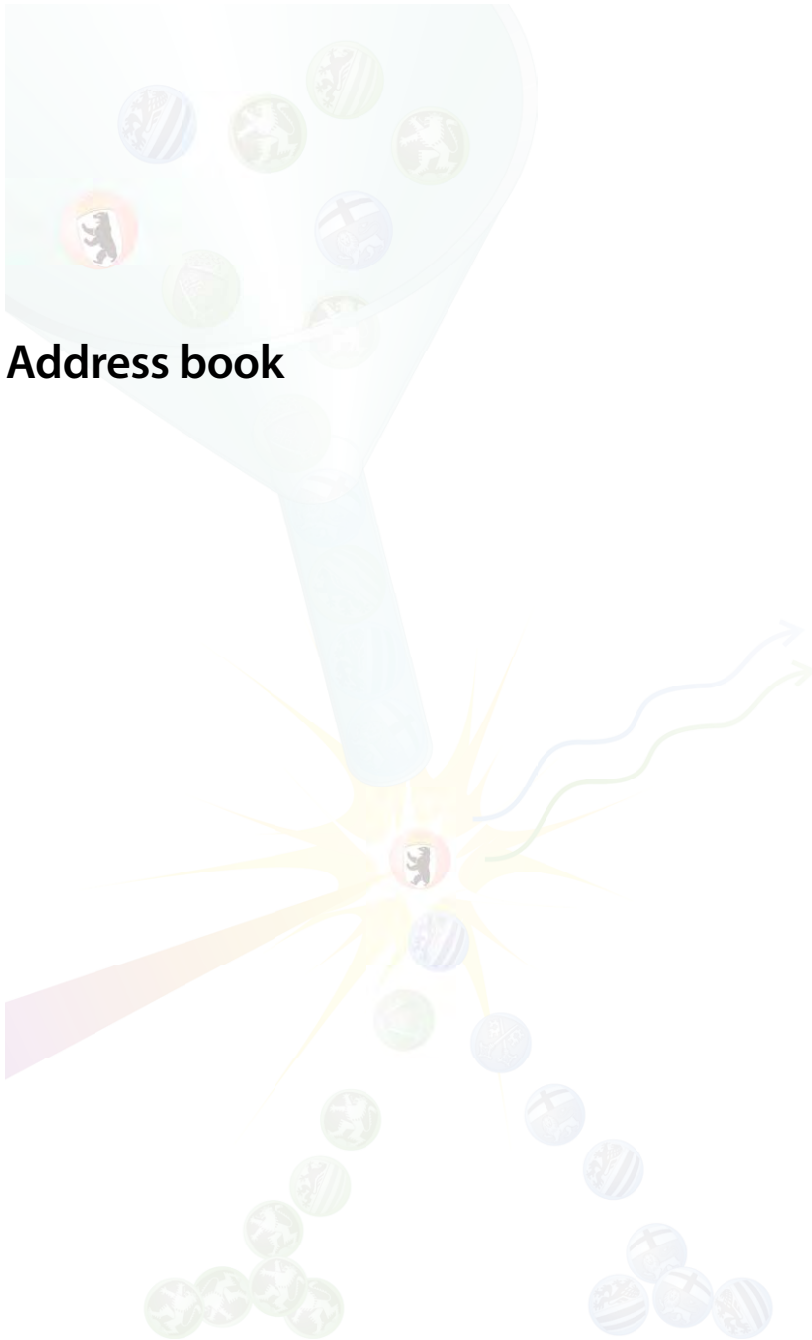
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Notes

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