2. Leibniz Summer School on Chronic Inflammation

Connecting Concepts of Chronic Inflammation

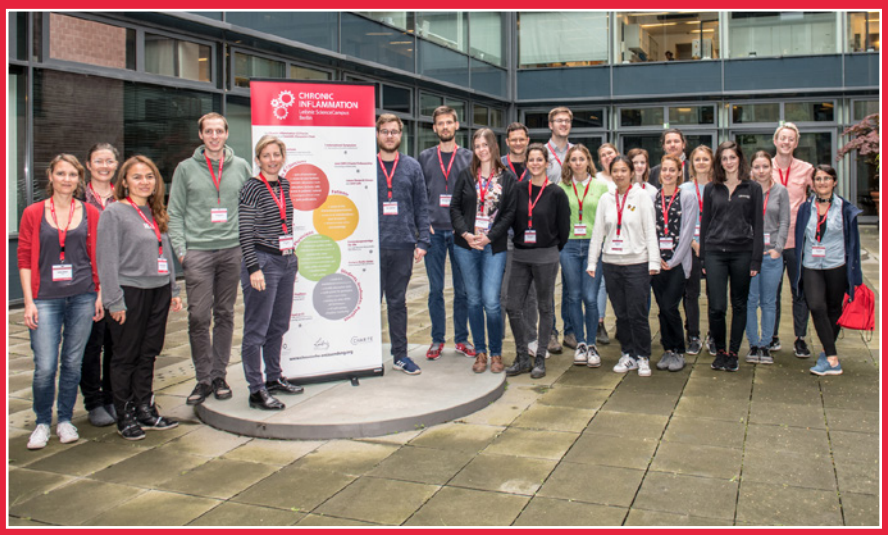
CHRONIC INFLAMMATION
Leibniz ScienceCampus
Berlin

16. - 17. May 2019
Berlin








Dear Summer school attendees,

It was a great pleasure to have you as participants in our 2nd Summer School on Chronic Inflammation. We are now happy to present our school booklet, with a short presentation of all participants and a recapitulation of the program.
Science is facing ever new challenges, and in an increasingly complex world science does not lack behind in complexity. In our 2nd summer school we tried to offer an update on current concepts on how to do science, familiarize the participants with current tools and share insights on the patient perspective. The focus of our discussion was to collaboratively develop strategies to tackle scientific problems and it was a great pleasure to see and have many lively discussions with all of you.
We hope that you will take the skills and knowledge developed in class and apply it in a new way or to a new situation not covered in this course.

Yours,

Helena Radbruch Philipp Enghard

2nd Leibniz Summer School on Chronic Inflammation

Organisation Committee

Ronja Mothes
Lennard Ostendorf
Helena Radbruch
Philipp Enghard
Marie Urbicht
Katrin Moser

Faculty

Philipp Enghard, Felix Fischer, Frederik Heinrich, Bob Jack, Magdalena Kraft, Elke Luger, Katrin Moser, Ronja Mothes, Lennard Ostendorf, Helena Radbruch, Clemens Schmitt, Axel Schulz, Ulf Tölch, Marie Urbicht, Dimitrios Wagner

Venue

Deutsches Rheuma-Forschungszentrum Berlin (DRFZ)
A Leibniz Institute
and Charité - Campus

Charitéplatz 1
10117 Berlin

Program

2nd Summer School of the Leibniz ScienceCampus Chronic Inflammation
Connecting Concepts of Chronic Inflammation

Thursday, May 16th

Time	Title	activity	location
09:30	**Introduction to the Leibniz Science Campus and welcome**		DRFZ Foyer at Virchowweg 12
10:00	**Scientific debate: Could Big Data be the end of theory in science?** Moderator**: Clemens Schmitt** **Learning objectives:** • Reflecting the possibilities and limitations of the data-driven and hypothesis driven approach • Practice a discussion or structured contest about an issue • Articulate perspectives on issues and dealing with controversial issues	Interactive session	01.050 at Virchowweg 3, Eduard Henoch Haus /Lernzentrum der Charité
11:00	**Coffee break**		DRFZ Cafeteria
11:15	**From cells to patients to concepts** **Start a translational medicine research proposal Part 1** Moderators**: Ronja Mothes, Lennard Ostendorf, Dimitrios Wagner** **Learning objectives:** • Get to know the road from bench to bedside guided by a real-life case of chronic inflammation • Practice collaborating over disciplines and cultures • Be able to reflect upon own role, expectations and the potential actions to make to achieve personal goals as a scientist	Group assignment & professional skills	01.046; 01.043; 02.041 at Virchowweg 3
12:30	**lunch**		DRFZ Cafeteria
13:30	**The challenges of Translational Medicine in chronic inflammatory diseases** **Personal experiences Part 2 in clinical setting** Moderators: **Magdalena Kraft , Helena Radbruch, Philipp Enghard** **Learning objectives:** • Importance of collaboration across disciplines and institutes • Discuss the role of a translational scientist	Professional skills	Derma, DRFZ and Neuro-pathology
15:30	**Coffee break**		DRFZ Cafeteria
16:00	**Flipchart Session and get together** Moderators: **Helena Radbruch, Philipp Enghard** **Learning objectives:** • Sharing contributions in translational research • Define and Discuss the essence of your thoughts about your research • express ideas to people from other disciplines and cultures	Interactive session	DRFZ SR 3
18:00-21:00	**Keynote meeting** evolution of the immune system with Bob Jack and **Get Together**	Interactive lecture	DRFZ seminar room 1/2

Program

2nd Summer School of the Leibniz ScienceCampus Chronic Inflammation
Connecting Concepts of Chronic Inflammation

Friday, May 17th

Time	Title	activity	location
9:15	**Keynote meeting: Reproducibility Crisis in biomedical research** Moderator: **Ulf Tölch (Quest Center BIH)** **Learning objectives:** • Identify roadblocks in translational medicine • Develop awareness of the need to rethink biomedical research and to initiate a culture change in academic biomedicine.	Professional skills	CCO Auditorium, Virchowweg 6
10:30	**From concepts to cure** Moderator**: Felix Fischer** **Learning objectives:** • Apply learning to develop solutions (clinical trials) • Discover the possibilities of design thinking	Professional skills	01.050 at Virchowweg 3
13:00	**lunch**		DRFZ Cafeteria
14:00	**The challenges of Translational Medicine in chronic inflammatory diseases** **Personal experiences Part 2 in Analytics** Moderators**: Axel Schulz, Frederik Heinrich, Marie Urbicht** **Learning objectives:** • Discover previously unknown, interesting patterns such as groups of data records (cluster analysis), unusual records (anomaly detection), and/or dependencies in real datasets • learn about the challenges and approaches/principles in analyzing high-dimensional single cell data	professional skills	02.035; 02.041; 02.045 at Virchowweg 3
16:00	**Coffee break**		DRFZ Cafeteria
16:30	**The challenges of Translational Medicine in chronic inflammatory diseases** **Personal experiences Part 3 in connecting concepts** Moderators: R**onja Mothes, Lennard Ostendorf, Dimitrios Wagner** **Learning objectives:** • evaluate differences and similarities in concepts of chronic inflammatory diseases • Present and discuss findings • translation of therapeutic concepts from one disease to another	Mixing of groups & professional skills	DRFZ SR 1/2
18:00	**Summary**	plenary session	DRFZ SR1/2
18:30	**Feedback and reflection**	Professional skills	DRFZ SR1/2

Speakers and Moderators

Philipp Enghard

Charité - Universitätsmedizin Berlin, Medizinische Klinik m.S. Nephrologie und Internistische Intensivmedizin

Philipp Enghard is a nephrologist and intensivist with a research focus on renal inflammation. Exploiting the urine as source for kidney invading cells his team is investigating the pathogenesis of human renal diseases, with an emphasis on lupus nephritis and acute kidney injury. Besides using urinary cells as "window" into the kidney, he and coworkers are establishing diverse subsets of cells in the urine as biomarkers for renal diseases.

Felix Fischer

Charité - Universitätsmedizin Berlin, BioThinking Programme

Felix Fischer studied industrial engineering and was cofounder of the start-up "Happy Pils" before he came to the Charité to build up a designthinking section at the BCRT. As there is a growing need for innovation and interactions across disciplinary boundaries, the Charité, in cooperation with the Hasso Plattner Institute (HPI) in Potsdam, developed a program to educate a new generation of scientists who are equipped to rise with the challenges of our times. The BioThinking approach is a worldwide unique program using design thinking as an innovation driver in biomedical sciences. Design thinking is a methodology for innovation that combines creative and analytical approaches, and requires collaboration across disciplines.

Frederik Heinrich

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

Freddy Heinrich is postdoc at the DRFZ and expert in the analysis of big data sets. Studied Biochemistry at the Free University of Berlin, and did his PhD in the lab of Alexander Scheffold at the DRFZ, working on the immune modulation of T-cells via the Notch signaling pathway. For his postdoc he joined the group of Farzin Mashreghi. There he is involved in the analysis, and visualization of big data, obtained from RNA sequencing experiments in cooperation with various groups. Recent work focused on single cell experiments, which incorporate transcriptional and clonal information of immune cells.

Bob Jack

Bob Jack studied Biochemistry in Edinburgh and went for his PhD to Cambridge, doing a lot of protein sequencing. The first contact with immunology was made in the lab of Klaus Rajewsky in Cologne. After some years there and in Basel in the lab of Walter Gehring, he became professor at the Immunology Institute in Greifswald. Now he is retired - good for us, so he has more time for lectures at the DRFZ. No one else can explain the immune system in such a thrilling and entertaining way.

Magdalena Kraft

Charité – Universitätsmedizin Berlin, Klinik für Dermatologie, Venerologie und Allergologie

Magdalena Kraft is a research associate and physician in 4th year residency at the Department of Dermatology, Venerology and Allergology at Charité - Universitätsmedizin Berlin. The scope of her research and clinical work are immediate-type immune reactions, and in particular anaphylaxis.

Ronja Mothes

Charité – Universitätsmedizin Berlin, Institut für Neurolpathologie

Ronja Mothes has recently finished her medical studies and is now doing her PhD at the Department of Neuropathology and the DRFZ in the field of chronic inflammation. At the moment she looks for long-lived plasma cells in different autoimmune diseases and antibody-mediated damage in brain tissue.

Lennard Ostendorf

Charité – Universitätsmedizin Berlin, Medizinische Klinik m.S. Rheumatologie und Klinische Immunologie

Lennard Ostendorf is a last-year medical student at the Charité - Universitätsmedizin Berlin. He is involved in research at the Department of Rheumatology and the Department of Neuropathology and his interests involve the borderlands between internal medicine and neurology as well as jazz music.

Helena Radbruch

Charité – Universitätsmedizin Berlin, Institut für Neurolpathologie

Helena Radbruch is a physician and research group leader at the department of neuropathology at Charité - Universitätsmedizin Berlin. Her group "chronic neuroinflammation" was established in 2013. The focus of the lab is to use innovative new imaging techniques to investigate mechanisms of neuronal tissue damage especially during chronic neuroinflammatory diseases as e.g. multiple sclerosis.

Clemens Schmitt

Universitätsklinik für Hämatologie und Internistische Onkologie, Kepler Universitätsklinikum Linz

Clemens Schmitt is cancer researcher and haemato-oncologist and just recently moved to Linz to become the Director of the University Clinic for Hematology and Internal Oncology Department. Before, he was Deputy Director of the Medical Department, Division of Hematology, Oncology and Tumor Immunology at the Charité Berlin and is still founding speaker of the Berlin School of Integrative Oncology (BSIO), a graduate school of the Charitè for graduate students and postgraduate scientists.

Axel Schulz

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

Axel Schulz studied Molecular Medicine at the Charité, did his PhD in the Immune Aging group at the BCRT, and is postdoc at the DRFZ since 2015. Since his PhD, he has been fascinated by the systems biological analysis of the human immune system, which is the reason why he joined the DRFZ mass cytometry lab of Dr Henrik Mei. Mass cytometry (CyTOF technology) enables him to decipher complex biological processes at single cell resolution, measuring up to 50 cellular markers simultaneously, thereby gaining a better understanding of the driving factors of autoimmune diseases.

Ulf Tölch

QUEST Center for transforming biomedical research at the Berlin Institute of Health

Dr. Ulf Tölch is a behavioral biologist by training but his focus now is Education, Training & Quality in Research at the QUEST (Quality|-Ethics|Open Science|Translation) Center of the BIH. The data sets on which scientific publications are based are becoming more and more complex and are correspondingly processed to an ever greater extent. This often happens at the expense of transparency and thus reproducibility. However, non-reproducible data are meaningless in science. Increasing the value and impact of biomedical research by maximizing the quality, reproducibility, generalizability, and validity of research is the mission of QUEST.

Marie Urbicht

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

Marie Urbicht studied biochemistry and is now doing her PhD in immunology. She is using mass cytometry to monitor immunophenotypic and signaling-related molecular changes in chronic inflammatory diseases in different organ systems. Besides that, she enjoys participating in events related to science communication, such as the Summerschool, the Long Night of Sciences or the Day of Immunology.

Dimitrios Laurin Wagner

BIH Center for Regenerative Therapies, Berlin Center for Advanced Therapies, Institute of Medical Immunology, Charité Universitätsmedizin Berlin

Dimitrios Laurin Wagner is a last year MD/PhD student at Charité Universitätsmedizin Berlin working on projects at the crossroad between immunology and biotechnology. In his research, he uses novel genome engineering technologies to develop new therapies for autoimmune diseases and cancer.

Participants

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Etanercept restores vasocontractile sensitivity affected by mesenteric ischemia reperfusion

S. Erpulat Ozis, Tamila Akhayeva, Sahika Guner, Sibel S. Kilicoglu, Arzu Pampal

Background
The aim of the study is to evaluate in vivo and in vitro effects of etanercept, a soluble tumor necrosis factor receptor, on the contractile responses of superior mesenteric artery in an experimental mesenteric ischemia and reperfusion model.

Material and methods
After obtaining animal ethics committee approval, 24 Sprague–Dawley rats were allocated to three groups. Control group (Gr C, n = 6) underwent a sham operation, whereas ischemia/reperfusion and treatment groups underwent 90 min ischemia and 24-h reperfusion (Gr I/R, n = 12; Gr I/R+E, n = 6). The treatment group received 5 mg/kg etanercept intravenously at the beginning of reperfusion. At the end of reperfusion, all animals were sacrificed, and third branch of superior mesenteric artery was dissected for evaluation of contractile responses. In vitro effects of etanercept on vasocontractile responses were also evaluated. The excised ileums were analyzed by using light microscope. Two-way analysis of variance following Bonferroni post hoc test was used to evaluate contractile responses.

Results
Endothelin-1 and phenylephrine-mediated vasocontractile sensitivity were found increased in Gr I/R when compared with Gr C. Both intravenous administration and organ bath incubation of etanercept decreased the sensitivity of contractile agents for Gr I/R. Mucosal injury, lamina propria disintegration, and denuded villous tips were observed in Gr I/R, whereas the epithelial injury and the subepithelial edema were found to be milder in Gr I/R+E.

Conclusions
Etanercept can be a promising agent in mesenteric ischemic reperfusion injury as it does not only inhibit inflammation by blocking tumor necrosis factor-α in circulation but also restores vascular contractility during reflow. These findings support an unexplained recuperative effect of drug beyond its anti-inflammatory effects.

Keywords
Etanercept, Tumor necrosis factor-α receptor, Mesenteric ischemic reperfusion injury, Transactivation.

Abstract

Tabea Borde

Objective
To study and compare the anti-tumoral effects of transarterial chemoembolization (TACE) with idarubicin-loaded 40 µm and 100 µm Oncozene® drug-eluting beads (DEBs) on the liver tumor microenvironment using multi-parametric MR imaging (mpMRI).

Methods
12 New Zealand White rabbits with orthotopically implanted left-hepatic VX2 tumors were assigned to undergo DEB-TACE with either 40 (n=5) or 100 µm (n=4) Oncozene® microspheres (Boston Scientific, Maple Grove, MN, USA) or used as untreated controls (n=3). Blood samples were obtained pre-procedurally, as well as 5 min, 24 h and 48 h post-TACE to evaluate laboratory parameter toxicity. All animals underwent mpMRI within 4 days after TACE including dynamic contrast enhanced MRI (DCE-MRI), diffusion weighted imaging (DWI), as well as biosensor imaging of redundant deviation in shifts (BIRDS) for in-vivo assessment of the extracellular pH (pHe) including quantitative assessment of all acquired parameters. Histopathological ex-vivo analysis included fluorescence confocal microscopy imaging to evaluate idarubicin tissue penetration and immunohistochemistry stainings for proliferation, cell death, and hypoxia (hematoxilin and eosin [H&E], proliferating cell nuclear antigen [PCNA], terminal deoxynucleotidyl transferase dUTP nick end labeling [TUNEL], hypoxia-Inducible factor 1 alpha [HIF-1α], and pimonidazol).

Results
DCE-MRI demonstrated more devascularization in embolized tumors as compared with controls (mean arterial enhancement [%] 8 ± 12 vs. 36 ± 51, p = 0.07), which was predominantly seen in the tumor rim, without significant difference between the treatment groups. In DWI, controls showed significantly higher levels of cellularity in the tumor rim than tumor core (apparent diffusion coefficient [ADC x10-3 mm2/s] 2.34 ± 0.18 vs. 1.55 ± 0.09, p = 0.006) whereas after TACE, diffusion significantly increased homogenous in both treatment groups suggestive of tumor cell necrosis (1.89 ± 0.18 vs. 1.28 ± 0.21, p < 0.0001). BIRDS imaging demonstrated profound tumor acidosis in untreated tumor tissue, with significantly lower pHe values within the tumor as compared with the liver parenchyma (mean pHe 6.79 ± 0.08 vs. 7.13 ± 0.08, p = 0.02), which did not change post-embolization with either DEB size (6.8 ± 0.06 for tumors vs. 7.1 ± 0.04 for liver tissue, p < 0.01). On fluorescence imaging, 40µm microspheres showed central vascular and deep parenchymal tumor penetration with non-target extra-tumoral deposition. In contrast, 100 µm beads were primarily deposited within the peri-tumoral vasculature without significant non-target embolization. Idarubicin elution coverage was seen within a radius of 100 µm from the embolized vessels for both bead sizes. Tissue analysis revealed complete inhibition of tumor cell proliferation, induction of apoptosis, and profound hypoxia within the tumors after embolization in both treatment groups.

Conclusions
The use of non-invasive mpMRI demonstrated profound changes to the tumor microenvironment after DEB-TACE with Oncozene® microspheres of either tested size. The sustained tumor acidosis, increased hypoxia and rapid induction of necrosis after complete devascularization suggest ischemia as the predominant mechanism of action, while idarubicin elution resulted in limited peri-vascular distribution of the drug. Overall, combining various mpMRI techniques (DCE, DWI, and BIRDS) could serve as an important tool to monitor tumors longitudinally and assess tumor response.

Impact of intestinal localization on inflammatory response

Zuzanna Borek

Background

Inflammatory bowel disease (IBD) is a complex, multifactor disorder where immune, microbial, genetic and environmental cues lead to prolonged inflammation and disease progression. Beside complexity, IBD is also highly heterogeneous disorder - it varies in severity, age of onset, aetiology, localization and number of inflammatory sites. The complexity and heterogeneity of IBD make it difficult to study, as more factors should be considered while comparing the immune response between individuals. All of those factors impact disease progression and treatment outcome, but until now they are not utilized in treatment decisions.

Aim

We aim to investigate the impact of intestinal tissue heterogeneity and localization imprinting on the inflammatory response in IBD mouse model.

Method/approach

We analysed publicly available dataset (GEO100833) that comprises of human intestinal biopsies from IBD patients with localization of the biopsy marked. Based on our results, we built up the hypothesis that inflammation trail differs between distinct intestinal localizations. To investigate this topic further, we collected intestinal samples of seven distinct gut localizations from different types of mice (wildtype, germ-free, colitic mice). We will perform bulk RNA-Seq transcriptomic analysis, histological stainings and compare abundance of different myeloid and lymphoid cells for all samples of distinct intestinal localization.

Conclusion/perspective

By integrating and overlaying results from all the methods, we aim to characterize the effect of local environmental imprinting on inflammation. We will validate our findings on samples derived from IBD patients. Finally we will translate our results to improve treatment decisions in IBD patients.

Abstract

Thomas Breakell

Background

Multiple Sclerosis (MS) is the most common cause of neurological disability in young adults (1) and is not yet curable. Various research with drugs modulating B cells exist, but all current MS drugs that target function or migration of peripheral immune cells essentially fail to halt MS progression (2): Current B cell depleting monoclonal antibodies (mAb) Rituximab (RTX) and Ocrelizumab have been able to reduce the frequency of relapses and thus slow the progression of MS, but as the blood brain barrier (BBB) becomes permeable for immune cells during relapses, inflammation in the central nervous system (CNS) can persist after the integrity of the BBB has been restored. Current drugs are limited in targeting these cells, resulting in a merely moderate benefit for secondary progressive MS (SPMS) patients. Thus, a strategy for SPMS could be to directly target the B cells compartmentalized in the CNS.

2. Hypothesis/Goal

The aim of this project is to increase brain exposure of experimental B cell therapies (3) in order to deplete ectopic B cell aggregates and halt further neurodegeneration (4). Additionally, we aim to study whether next generation B cell therapies are more efficient than RTX in the acute and chronic MP4-induced experimental autoimmune encephalomyelitis (EAE) model.

3. Project Plan

The study will be conducted with male C57BL/6 transgene huCD20xHIGR3 mice. This modified mouse line by Roche has the advantage of possessing both the murine and human form of the B cell associated CD20-molecule. Additionally, these mice are able to produce human antibodies, which makes future therapeutic trials with the humanized antibodies we will then be using easier.

Prof. Kürten has developed a new myelin antigen immunization which gives rise to one of the few animal models for MS that is dependent on B cells (5): The first step will be the induction of EAE: The mice (aged six to eight weeks) will be injected s.c. with 200 µg MP4 (a fusion protein between MBP (myelin basic protein) and a genetically engineered form of PLP) in complete Freud-Adjuvans (6). On the day of immunization and 48 h later, the mice will be boosted with i.p.-injections of 200 ng pertussis toxin. The clinical development of EAE will then be scored daily according to the standard EAE scale.

In order to judge whether the formation of B cell infiltrates during acute disease can be prevented by the administration of the first dose will be injected intravenously at the peak of the EAE (i.e. between days 14 and 25 after immunization). For the chronic model, treatment will be started accordingly on day 50. There will be further injections every three days for a total of nine days. The treated mice will then be compared to isotype control-treated mice and mice treated with Rituximab. The mice in the acute model will be killed with CO2 on day 30 and the mice in the chronic model on day 62 after immunization.

Depletion of CNS-resident B cells will be studied by IHC analysis (immunohistochemistry) of the cerebellum. Peripheral and germinal centre B cells will be analysed using flow cytometry.

My responsibilities will include the analysis of spinal cord pathology using electron microscopy, the establishment of a cytokine profile in the three different groups of mice and the measurement of MP4-specific antibodies in serum samples using ELISA. In addition, I will assist with the perfusion and sample preparation.

For EM, we will perfuse the mice with 4% paraformaldehyde (PFA) in 0.1 M phosphatebuffered saline (PBS). The tissue will then be extracted, post-fixed for 24 h and embedded in EPON.

Subsequently, 80 nm thick sections will be cut and analysed via transmission electron microscopy (TEM), which makes it possible to evaluate the status of the axons of the spinal cord and quantify the amount of damage made to them. Analysis will include the counting of intact and axolytic axons per area and their percentage. In addition, the degree of myelin pathology will be assessed.
For ELISA, we will draw blood from the inferior vena cava prior to perfusion. After the serum is extracted by centrifugation and diluted, it can be tested for the presence of anti-MP4-IgGs. The cytokine profile will be established using commercially available ELISA kits.

Literature

(1) Kingwell E et al. BMC Neurol. 2013 ;13:128.
(2) Lehmann-Horn K et al. Ther.Adv.Neurol.Disord. 2013 ;6:161 ; Weber MS et al., JAMA Neurology 2015 ;72:1407
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(6) Elliott EA et al.: J Clin Invest. 1996 ;98(7):1602-12.

Response-time modeling of T-helper cell differentiation

Philipp Burt, Kevin Thurley

CD4+ T cell differentiation is a key element of the adaptive immune system driving appropriate immune responses by selective recruitment and activation of effector immune cells. The decision on T cell differentiation into specific subtypes, such as Th1 or Th2 cells, is made by interacting immune cells in a collective process. Such immune cell communication is part of an extensive network involving multiple feedback mechanisms on the intracellular and intercellular level. The complexity of these cell-cell interaction networks confounds intuition and complicates quantitative, systems-level analysis. Here, employing response-time modeling (Thurley et al. 2018, Cell Systems 6:355), we develop and analyze data-driven models of T cell activation and decision-making. We find that response-time modeling of such differentiation circuits reveals qualitative and quantitative properties regarding robustness, reaction times and magnitude of the T cell response, which go beyond analysis with traditional rate equation models. We envision using response-time modeling to derive large-scale data-driven models of cell-cell communication circuits in autoimmune diseases, to elucidate decision-making processes and to derive testable predictions regarding therapeutic opportunities.

Abstract

Alexander Fiedler

2 Beschreibung des Projektes

In meiner Promotion beschäftige ich mich, im Zeichen der translationalen Forschung im Bereich der Medizinphysik, mit der effizienten Nutzbarmachung und Anwendung von Fluoreszenz im Rahmen der Entwicklung von Bildgebungsverfahren für klinische Zwecke.

Es soll sich mit der Optimierung der Fluoreszenzlebensdauer-Mikroskopie (FLIM) im allgemeinen beschäftigt werden , sowie mit der Validierung eines neu aufgebauten Systems für zeitaufgelöste Fluoreszenzmikroskopie in der Frequenz-Domäne (FLIM-Zytometer) [1] . Außerdem soll eine neuartige Methode zur optischen Bildgebung (von myeloiden Immunzellen) entwickelt werden. Es handelt sich dabei um eine Kombination aus FLIM und einem intravitalen Mikroendoskopie-Verfahren mithilfe des am DRFZ entwickelten LIMB-Implantates [2] . Mit dieser Methode kann erstmalig intravital der Ge-

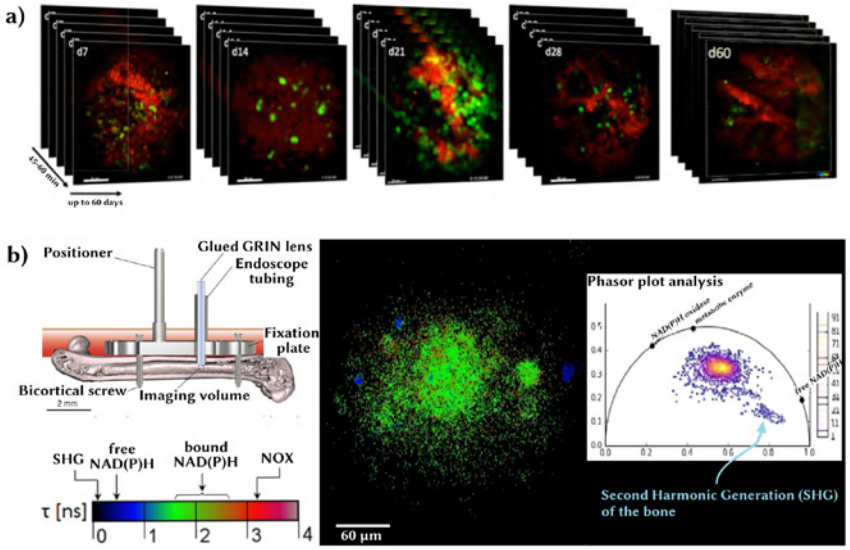

Abb 1: **a)** Bildaufnahmen einer LIMB Versuchsreihe mit 45-60 minütigen Mess-Sessions am 7., 14., 21., 28. und 60. Tag nach der LIMB-Implantation. Blutgefäße wurden, durch intravenöse Injektion von QuantumDots, vor jeder Mess-Session kontrastiert. Die hier grün dargestellten Immunzellen (B-Lymphozyten), der verwendeten CD19:tdRFP Mäuse exprimieren genetisch den roten Fluoreszenzfarbstoff *red fluorescent protein* (RFP), Quelle: [2] **b)** Qualitative Pilot-Messung zur Erprobung der LIMB-FLIM-Methode. Links oben ist eineschematische Darstellung des Limb-Implantats, auf der rechten Seite ein farbkodiertes Fluoreszenzlebensdauer-Bild mit zugehörigem Phasor-Plot zu sehen. , Quelle: [3]

webeumbau und Neoangiogenese während der Knochenheilung nach einer Osteotomie (s. Abb 1 a)) und gleichzeitig die metabolisch-enzymatische Aktivität von (myeloiden) Immunzellen, beteiligt an der Knochenheilung, untersucht werden (s. Abb 1 b)).

Die beschriebenen Methoden sollen angewendet werden, um neue Erkenntnisse über die immunologischen Prozesse bei der Knochenregeneration auf zellulärer Ebene zu gewinnen . Im Vordergrund steht die Untersuchung des NAD(P)H-Metabolismus in myeloiden (Stamm-)Zellen (z. B. Osteoclasten) sowie die myeloide Zelldifferenzierung und deren Einfluss auf die Knochenregeneration im altersbedingten Kontext. Die Hypothese, dass sich der Metabolismus in myeloiden Zellen, sowie deren Zelldifferenzierung mit zunehmenden Alter verringern und letzlich in einer Beeinträchtigung der Knochenheilung resultieren, soll erstmalig quantitativ geprüft werden.

Literaturverzeichnis

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Dermatomyositis is associated with a strong type I IFN driven immune activation in blood and skin

Kristina Fischer[1], S. Meisterfeld[1], M. Aringer[2], S. Beissert[1], C. Günther[1]

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Dermatomyositis (DM) is a rare autoimmune disease with proximal muscle weakness and characteristic cutaneous violaceous erythema in sun-exposed areas. Patients have variable inflammatory pulmonary involvement and an enhanced risk of malignancies if the disease starts in adulthood. Dermatomyositis is characterized by a strong type I interferon (IFN) mediated inflammation and by specific autoantibodies such as Mi-2, transcriptional intermediary factor 1 γ (TIF1γ), nuclear matrix protein 2 (NXP2), small ubiquitin-like modifier-activating enzyme heterodimer (SAE1/SAE2) and melanoma differentiation-associated antigen 5 (MDA5). In a cohort of 22 dermatomyositis patients we found that patients with TIF1γ antibodies had an enhanced risk for a chronic recurrent disease course that was associated with continuous upregulation of the type I IFN signature in blood and an enhanced Stat1 expression in monocytes. The expression of type I IFN stimulated genes (ISGs) correlated with the extension of skin involvement measured by the CDASI-score. Type I IFN induced genes were also elevated in primary cutaneous fibroblasts from patients and associated with a cellular stress response. These data indicate that inflammation in dermatomyositis is mainly driven by type I IFNs in blood and skin.

Impact of epigenetic imprinting on the perturbation of intestinal inflammation and dysplasia

Elif Gelmez

Aberrant alterations in histone modifications have been seen in several diseases such as cancer, mental retardation, autoimmune diseases and diabetes. The inability of the turn off acute intestinal inflammation results with chronic inflammation, which then further cause the development of colitis-associated cancer (CAC) via inducing histone modifications. Covalent histone modifications have important role on the regulation of gene transcription via changing the compaction of DNA. Therefore, we aim to learn that which kind of transcriptional changes occur via histone modifications induced by chronic inflammation in intestinal epithelial cells (IEC), and how it can cause the perpetuation of intestinal inflammation, epithelial barrier dysfunction and finally progression of CAC. Therefore, we are planning to address the following topics in IEC i) Generate genome wide map for the H3 lysine 27 trimethylation and H3 lysine 27 acetylation (H3K27me3&H3K27ac) ii) Correlate this map with the transcriptional profile of the same cells iii) Correlate alterations in histone modifications with progression of intestinal inflammation. In all, the proposed project can help to clarify role of histone modifications on progression of CAC and show diagnosis, prognosis and novel therapeutic targets.

Maintenance of bone marrow-resident memory T lymphocytes

Lukas Heiberger

The current paradigm is that memory T lymphocytes, in the absence of antigen, are maintained over time by homeostatic proliferation, driven by cytokines like interleukin-7 (IL-7) and interleukin-15 (IL-15). Recently however, our group could show that bone marrow-resident memory T lymphocytes, which confer memory to systemic antigens, are not proliferating at all. Instead, they are resting individually in contact to bone marrow stromal cells. My hypothesis is that the memory T lymphocytes receive critical survival signals from the stromal cells, in particular signals activating the PI3K/AKT/FOXO pathway, preventing apoptosis induced by metabolic stress. To identify these signals and potential further survival signals from the bone marrow environment, I have set up a hypoxic in vitro co-culture system of ST2 stromal cells and memory T lymphocytes isolated ex vivo. In this co-culture system, survival of the memory T lymphocytes is indeed dependent on the presence of stromal cells, but not on IL-7 and IL-15. Signal transduction and apoptosis pathways involved are currently under investigation.

Abstract

Jiachen Hu

Tricellular tight junction (tTJ) refers to the contact of three or more cells among tight junction (TJ) strands, which converge and then extend to the basal direction forming a central tube. This point has long been assumed to be a structural weak point of paracellular barrier. Previously, it has been shown that tricellulin (Tric) and angulins localize at this part. Tric is a barrier-forming TJ protein playing an important role in maintaining the overall TJ network structure, especially in passage of macromolecules, which relate to the mechanism of paracellular antigen uptake.
Inflammatory bowel disease (IBD) could emerge when integrity of intestinal barrier is compromised. Ulcerative colitis (UC) and Crohn's disease (CD) are two major subtypes of IBD. To date, the exact pathogenesis of IBD is still unknown, yet there is no doubt that immune system malfunction plays a crucial role in every stage of the disease. Interleukin-13 is a Th2 family cytokine involved in a variety of mucosal inflammation, including UC, asthma, and several fibrotic diseases like idiopathic pulmonary arterial hypertension and systemic sclerosis. So far, there are two types of IL-13 receptors known. IL-13 receptor α1 (IL13R α1) usually composes a complex with the interleukin-4 receptor and regulate via MAPK and PI3K signaling pathways, while IL-13 receptor α2 (IL13Rα2) possibly has a higher affinity and thus could make IL13Rα1 unavailable to bind IL-13 and aggravate inflammation.
In a previous study, IL-13 was found to mediate Tric downregulation via IL13Rα2 through subsignaling pathways ERK1/2, JNK and AP-1 in UC. AP-1 is a common transcription factor that regulates gene expression in response to a number of stimuli, usually assembled a homodimer or heterodimer by proteins from jun and fos family. Sub-proteins analysis illustrated an upregulation of c-Fos and c-jun, along with their downregulation of phosphorylation level.
In CD, even though Tric protein level showed no difference compared to controls, the predominant localization of Tric appeared to shift from the crypts toward the surface epithelium. This regulation of Tric localization and the underlying components has yet not been studied in detail. The angulin family, another main component of tTJs, consisting of angulin-1(used to call LSR), angulin-2 and angulin-3 (former named ILDR1 and ILDR2), has been proven to have the properties to recruit Tric to tTJ and may be involved in the observed changes in CD.
This project is going to investigate detailed mechanism for IL-13-mediated Tric downregulation and to analyze function and protein expression level of biopsies from remission UC patients and healthy controls to identify whether Tric downregulation inclines to pathogenesis or consequence of UC. As for CD, the underlying regulation of shifted localization of Tric will be explored.

Ilaria Luperi

Untersuchung zum Auftreten extraintestinaler Immunphänomene und zur Prädiktion (prediction) des Therapieerfolgs anhand Integrinexpression und -funktion auf T-Lymphozyten bei CED-Patienten unter Vedolizumab (VEPREDEX-Studie)

Eleni Mantzivi

Zusammenfassung:
Unter der Blockade von α4β7 durch den darmselektiven Integrin-Antagonisten Vedolizumab treten bei Patienten mit chronisch entzündlichen Darmerkrankungen vermehrt extraintestinale Symptome, insbesondere Gelenk- und Hautbeschwerden, aber auch andere Immunphänomene auf. Auf α4β7- α4β1-co-exprimierenden T-Zellen wird durch Vedolizumab ein Shift innerhalb der Integrin-Untereinheiten in Richtung von α4β1 induziert. In dieser Studie soll die durch Vedolizumab getriggerte veränderte Expression mehrerer Integrin-Untereinheiten (u.a. α1, α2, α4, αL, αM, β1, β2 und β7) auf Leukozyten von Patienten mit chronisch entzündlichen Darmerkrankungen mittels Durchflusszytometrie untersucht und ihre Assoziation mit dem Auftreten spezifischer extraintestinaler Manifestationen sowie dem intestinalen Ansprechen analysiert werden.
Dies ist eine multizentrische, prospektive Beobachtungsstudie, in die alle CED-Patienten, die aus einer klinischen Indikation heraus Vedolizumab erhalten, eingeschlossen werden sollen. Es werden volljährige Patienten mit aktivem Morbus Crohn (MC) oder Colitis ulcerosa (CU) eingeschlossen. Die Patienten werden über einen Zeitraum von 30 Wochen (sechs Gaben Vedolizumab bei CU bzw. sieben Gaben Vedolizumab bei MC) beobachtet.

Abstract

Lil Meyer-Arndt

Pro-inflammatory cytokines and other endogenous mediators are assumed to play an important role in inflammation-associated neurodegeneration in active multiple sclerosis (MS) by stimulating the immune response, attracting immune cells into the CNS and triggering their activation. However, little light has been shed on whether these cytokines may directly induce cytotoxic target organ damage or even give rise to protective mechanisms including immune-regulatory processes.

To gain a better understanding of the distinct role of pro-inflammatory and immune-regulatory mediators linked to MS, we investigated the effects of IL-17, IFN-g, TNF-a, glutamate and IL-10 on in-vitro human neuronal stem cell-derived neuron cultures.

We described neuronal reactions to inflammatory stress by performing various tests, i.e. fluorescence microscopy, metabolic cell viability assays and functional calcium imaging. Reproducible parameters were established: Neuronal damage associated with a loss in axonal and dendritic integrity and network density was detected by immunostaining for class III beta-tubulin. We identified neuronal cell death by staining for the apoptotic marker cleaved caspase-3. Cell viability was quantified by masuring cellular ATP production, which signals the presence of metabolically active cells. Neurons were treated with inflammatory mediators (IL-17, IFN-g, TNF-a, glutamate and IL-10) or a control condition, i.e. medium as negative control and staurosporine and H2O2 as positive control for induced neuronal damage. Subsequently, we evaluated the effects of these pro-inflammatory and immune-regulatory mediators on changes in neuronal RNA expression profiles.

Neurite density was reduced in samples treated with IL-17 and glutamate without affecting cell survival; IL-10 induced higher neurite density while IFN-g and TNF-a did not show an effect on these parameters. Our results demonstrate differential effects of distinct cytokines on neuron-like cells in an in-vitro human cell culture based CNS- like model.

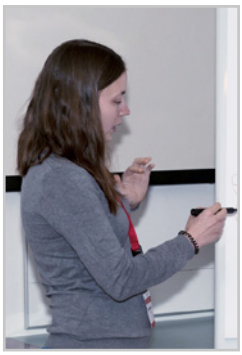
ILCs detection and characterization in the skeletal muscle tissue in WT mice at steady state

Chiara Panicucci, C. Sthele, E. Gazzerro, C. Romagnani.

A chronic inflammatory status characterizes a considerable number of neuromuscular disorders, such as some muscular dystrophies. In those disorders a primitive muscular membrane instability leads to a leakage of pro inflammatory mediators in the extracellular space, thus inducing a sterile chronic inflammatory response mainly sustained by macrophages and neutrophils and to a lesser extent by lymphocytes, which worsen the disease progression. Nevertheless, alongside the detrimental role of the type 1 immune response in worsening the pathology, the immune system plays also a pro regenerative and anti-inflammatory role acting via M2 macrophages and type 2 response axis. Furthermore it has been shown that a population of immune cells, namely Foxp3+CD4+ regulatory T cells rapidly accumulates in acutely injured skeletal muscle as well as in the mouse model of Duchenne Muscular Dystrophy (mdx mice) and ameliorate muscle tissue damage, possibly via production of IL-10 and the epithelial growth factor family Amphiregulin. Also, in the last years, it emerged that lymphocytes, both adaptive and innate, are not exclusively recirculating in secondary lymphoid organs but some populations mainly reside in different tissues and organs, where they do not only contribute to the defence against pathogen infections but also modulate tissue homeostasis and repair. A major emerging family of tissue resident lymphocytes is represented by innate lymphoid cells (ILCs). In particular, a putative ILC2 population has been identified in muscle tissue even if the phenotypic and functional characterization of this population as well as its role in regulating inflammation in the muscle remains unclear.

Here we show the detection of ILCs populations in skeletal muscle tissue in WT mice at the steady state (n=3). We found that the CD45+CD90+ TCRb-TCRgd- population counts for 37,8% (SD=2,92) of the total muscle infilatrating leucocyte. Within this population the NK and ILCs subsets were analyzed as follow: the NK population was marked as NKp46+CD127- and counted for the 8,27% (SD=4,15); the ILC1 subset marked as NKp46+CD127+ was almost abstent; the ILC2 and ILC3 subsets was detected as NKp46-CD127+ and represented the 6,57%. Within the NKp46+CD127+ population the ILC2 subset was detected as RORgt-GATA3+ and counted for the 16,9% (SD=3,29) and the ILC3 subset, marked as RORgt+GATA3-, counted for the 5,08% (SD=3,46). Furthermore the KLRG1 expression was tested on NK and ILC2 cells. We found the 32% (SD=5,41) of the NKp46+ cells and the 76% (SD=9,22) of the GATA3+RORgt- to be positive for KLRG1.

In conclusion we found the NK and ILC2 subsets to be the most represented innate lymphocytes within the muscle tissue in WT mice at the steady state. Further characterization of these populations is needed to better define their phenotype and role in the skeletal muscle tissue.



Abstract

Isabel Schobert

Abstract: Quantitative inflammatory biomarkers for the prediction of tumor response to DEB-TACE in HCC

Purpose: To investigate the prognostic value of quantifiable laboratory markers for tumor response in hepatocellular carcinoma (HCC) treated with drug-eluting beads transarterial chemoembolization (DEB-TACE).

Material and Methods: This IRB-approved retrospective study included 51 patients with treatment-naïve HCC (m/f 41/10, 64.6±9.6 years) who received DEB-TACE (2012-2018). All patients underwent a comprehensive laboratory work-up prior to treatment, including complete and differential blood count, liver function tests, and alpha-fetoprotein levels. Neutrophil-to-Lymphocyte-Ratio (NLR) and Platelet-to-Lymphocyte-Ratio (PLR) were calculated based on the differential blood count. Tumor response was assessed according to 3D quantitative European Association for the Study of the Liver (qEASL) criteria and correlated with laboratory markers. Statistics included Pearson correlation and linear regression with alpha level adjusted to multiple testing.

Results: Baseline immunologic scores were predictive of tumor response to DEB-TACE. Specifically, patients with increased NLR, PLR, or alkaline phosphatase levels were less likely to respond to therapy ($p=0.016$, $p=0.005$, $p<0.001$, respectively).

Conclusion: This study demonstrates the prognostic value of quantitative laboratory and particularly immunologic biomarkers at baseline to predict tumor response to DEB-TACE.

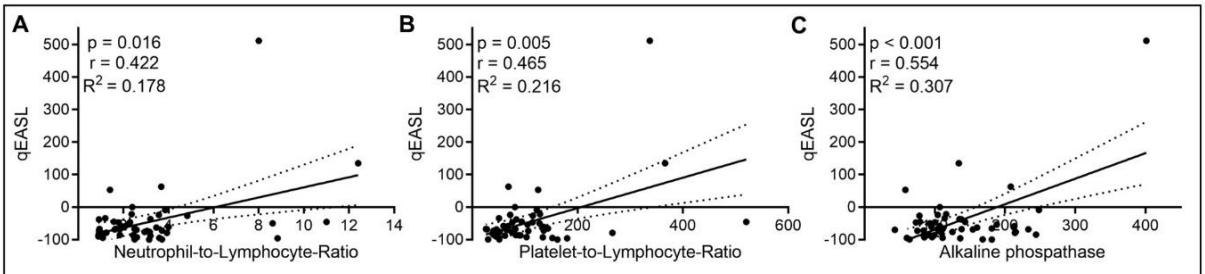


Figure: Correlation of pre-treatment immunologic ratios with tumor response in patients with HCC after DEB-TACE. Pre-treatment Neutrophil-to-Lymphocyte-Ratio (A), Platelet-to-Lymphocyte-Ratio (B), and alkaline phosphatase (C) showed a significant correlation with tumor response according to three-dimensional enhancement-based quantitative European Association for the Study of the Liver (qEASL) criteria.

The impact of antibodies against Angiotensin II type 1 receptor on delayed allograft function of kidney transplantation

Kaiyin Wu

Background.
Delayed graft function (DGF) is a failure of the transplanted kidney to function immediately after transplantation mainly with causes of ischemia-reperfusion and immunological injury. The kidney graft from donor with high level of angiotensin II (Ang II) measured during procurement is significantly more likely to develop DGF. Ang II type I receptor (AT1R) mediates most physiologic and pathophysiologic actions of Ang II, autoantibodies to AT1R (anti-AT1R) are implicated in several vascular pathologies. The impact of anti-AT1R on clinic outcomes of DGF grafts was evaluated in this study.

Methods.
We reviewed the records of all consecutive adult recipients who received single kidney transplantation and clinically management between Jan.2006 and Dec.2009 in our centre. The serum binding levels of anti-AT1R were measured by a recombinant-receptor-based sandwich ELISA and a cutoff of 15 units was used to distinguish high from low binding.

Results.
Three hundred and seventy-seven recipients were enrolled. The overall presence of DGF was 31%, and 12% recipients had high binding anti-AT1R, the incidence of DGF in patients with high binding anti-AT1R was significant higher than patients with low binding of anti-AT1R (42% vs 29%, p=0.03). In addition, more female recipients, longer duration of renal replacement therapy, higher resistance index (RI) of allograft and more severe acute tubular injury/acute tubular necrosis were observed in DGF recipients with high binding anti-AT1R (AT+DGF) comparing with DGF recipients occurring low binding anti-AT1R (AT-DGF). Under the administration of angiotensin-converting enzyme inhibitors (ACEi) or Ang II receptor blockers (ARB), the RI of allograft and graft function in AT+DGF recipients could be ameliorated as well as one-year graft survival and death censored graft survival was similar between two groups.

Conclusions.
Presence of high binding anti-AT1 R had detrimental impacts on initiation and development of DGF.

Key words: antibodies against angiotensin II type I receptor; delayed graft function; kidney transplantation; ischemia-reperfusion injury

Results I: From cells to patients to concepts - Start a translational medicine research proposal

Group I

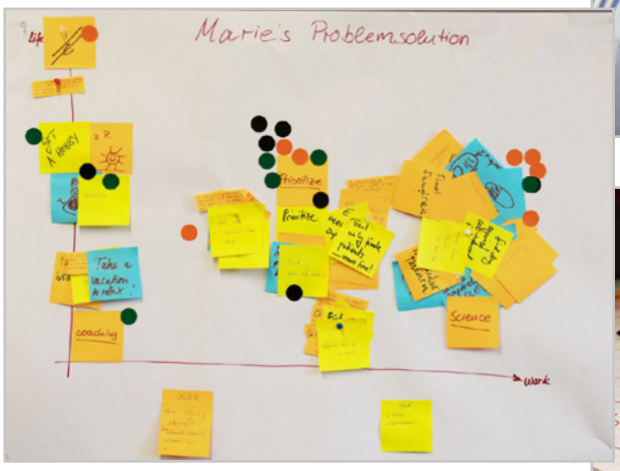
Group II

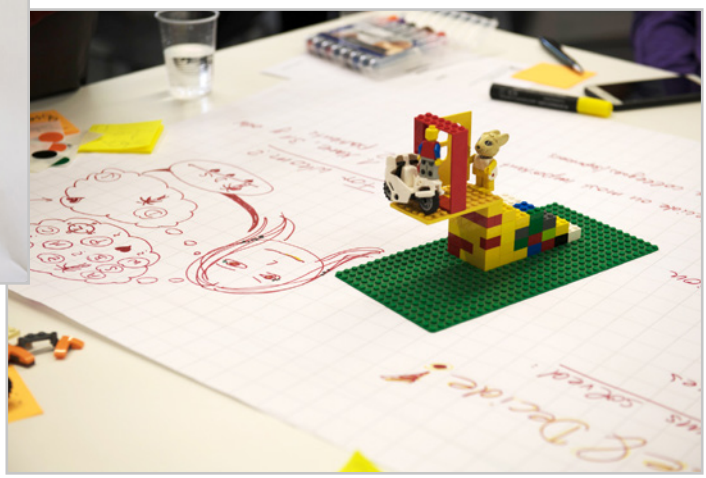
Group III

Results II: From concepts to cure

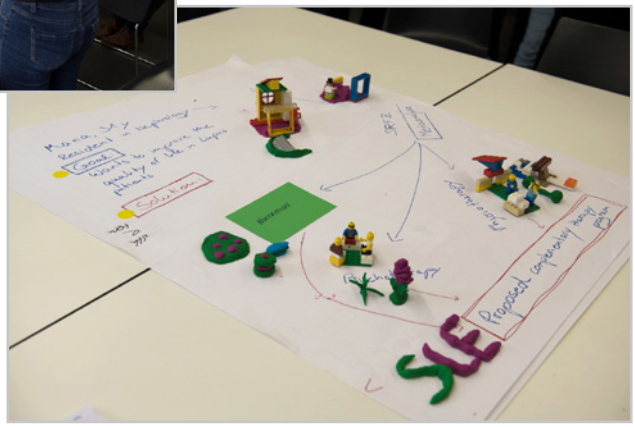
Felix Fischer, Berlin

Group I

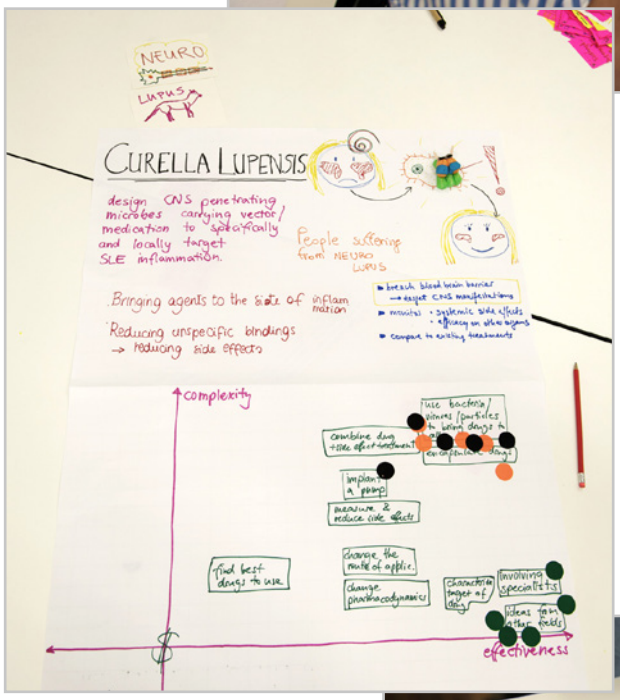




Group II

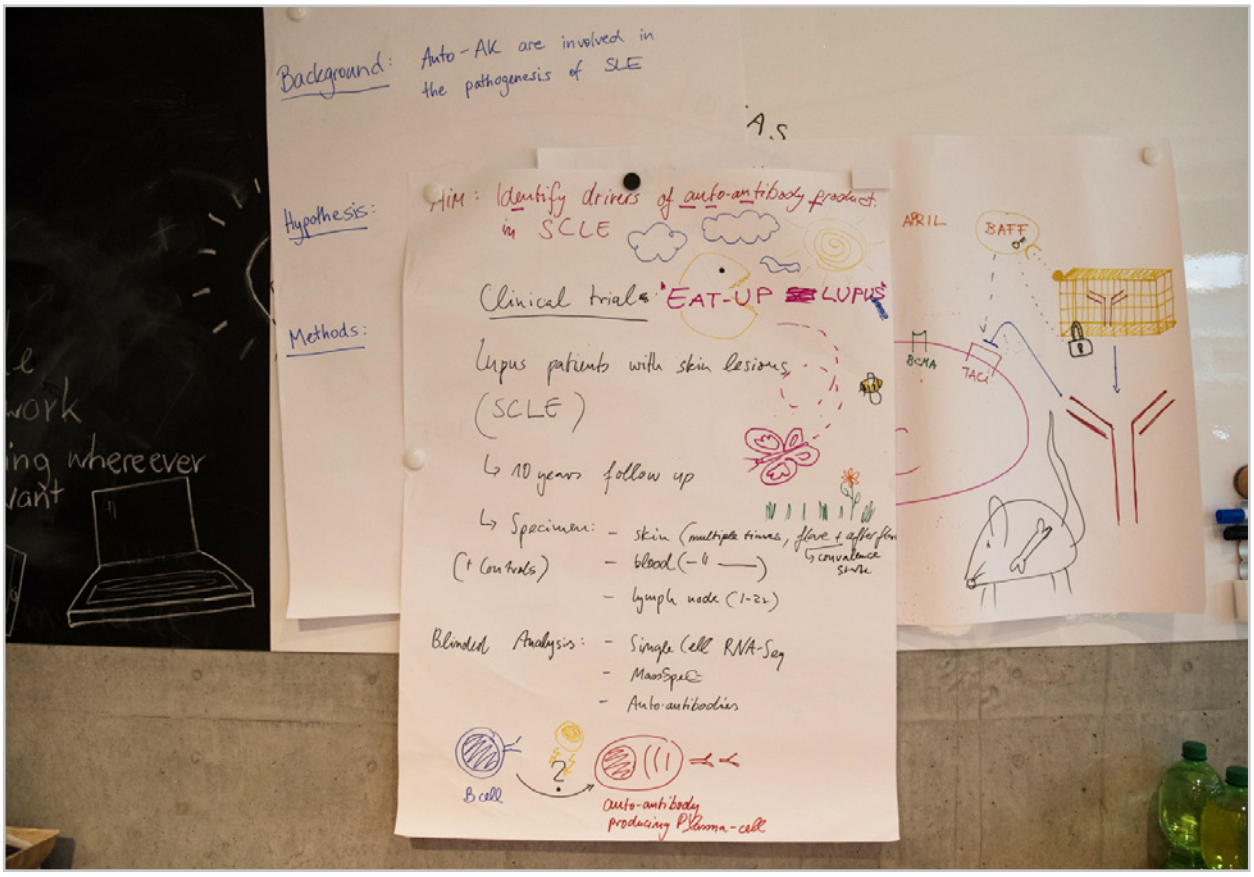


Group III


NEURO
LUPUS
CURELLA LUPENSIS
design CNS penetrating microbes carrying vector/ medication to spatially and locally target SLE inflammation.
People suffering from NEURO LUPUS
Bringing agents to the side of inflammation
Reducing unspecific bindings
→ reducing side effects
complexity
effectiveness


CURELLA LUPENSIS

Results III: The challenges of translational medicine in chronic inflammatory diseases - connecting concepts / Summary



First prize with star for flip chart presentation

Alexander Fiedler

First prize for flip chart presentation

Thomas Braekell

Seminar: Could Big Data be the end of theory in science?

Clemens Schmitt, Charité Berlin, Universität Linz

Keynote lecture: Evolution of the immune system

Bob Jack, Berlin

Seminar: Reproducibility Crisis in biomedical research

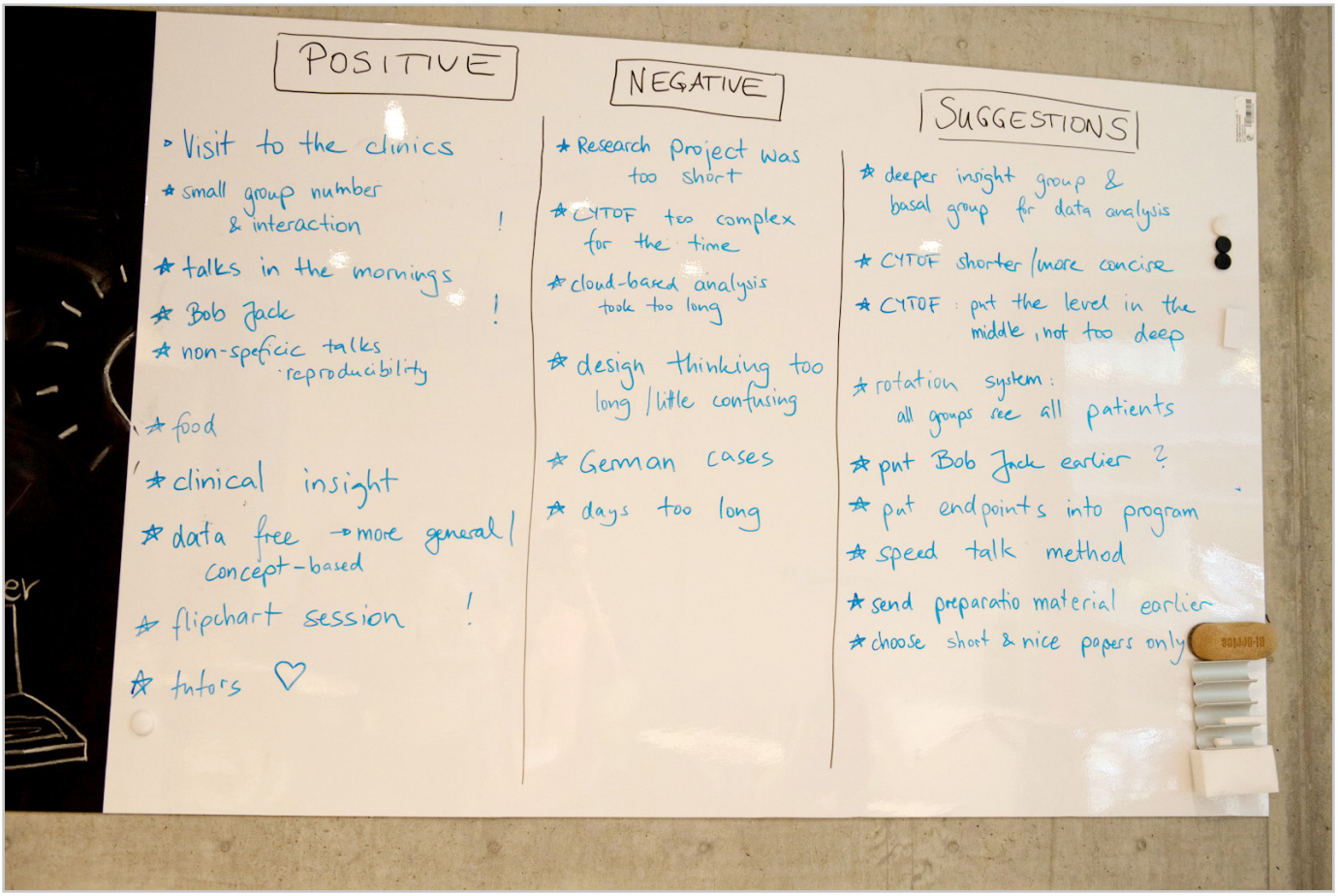
Ulf Tölch, Quest Center, Berlin Institute of Health

Seminar: The challenges of translational medicine in chronic inflammatory diseases - Analytics

Axel Schulz, Marie Urbicht, DRFZ Berlin

Feedback


POSITIVE
NEGATIVE
SUGGESTIONS


POSITIVE
Visit to the clinics
small group number & interaction
talks in the mornings
Bob Jack
non-specific talks reproducibility
food
clinical insight
data free → more general / concept-based
flipchart session
tutors
NEGATIVE
Research project was too short
CYTOF too complex for the time
cloud-based analysis took too long
design thinking too long / little confusing
German cases
days too long
SUGGESTIONS
deeper insight group & basal group for data analysis
CYTOF shorter / more concise
CYTOF: put the level in the middle, not too deep
rotation system: all groups see all patients
put Bob Jack earlier ?
put endpoints into program
speed talk method
send preparatio material earlier
choose short & nice papers only

2nd Leibniz Summer School on Chronic Inflammation

Organisation Committee

Ronja Mothes
Lennard Ostendorf
Helena Radbruch
Philipp Enghard
Marie Urbicht
Katrin Moser

Faculty

Philipp Enghard, Felix Fischer, Frederik Heinrich, Bob Jack, Magdalena Kraft, Elke Luger, Katrin Moser, Ronja Mothes, Lennard Ostendorf, Helena Radbruch, Clemens Schmitt, Axel Schulz, Ulf Tölch, Marie Urbicht, Dimitrios Wagner