DBM FACTS

Periodisches Informationsblatt des Departementes Biomedizin
Universität Basel, Universitätsspital Basel und
Universitäts-Kinderspital beider Basel

Studying Host-Microbiota Interactions by Silencing Receptors of the Host | Christmas in Slovakia | "What is Hanukkah?"

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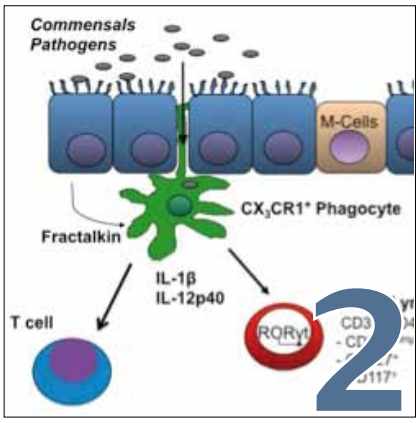
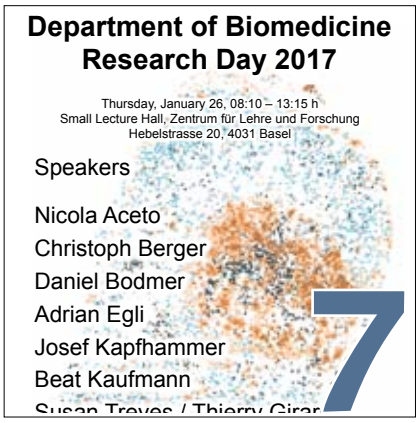
Departement Biomedizin Basel



Studying Host-Microbiota Interactions by Silencing Receptors of the Host
from Jan Hendrik Niess



DBM Research Day

IMPRESSUM

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Christmas in Slovakia
from Jakub Zmajkovic

"What is Hanukkah?"
from Moran Elbaz

UKBB Universitäts-Kinderspital beider Basel

Universitätsspital Basel

UNI BASEL

EDITORIAL

Radek Skoda
Leiter DBM

Liebe Leserinnen und Leser

2016 neigt sich seinem Ende zu. Ein herausforderndes, aber auch sehr erfolgreiches Jahr liegt hinter uns. Mohamed (Momo) Bentires-Alj wurde auf die Professur für „Experimental Surgical Oncology" berufen und er hat mit seinem Labor bereits seinen Platz im DBM gefunden. Rolf Zeller, Mohamed Bentires-Alj und Nicola Aceto erhielten dieses Jahr prestigeträchtige ERC Grants. Auch bei den Publikationen hat das DBM sehr gut abgeschnitten (siehe ab Seite 9). Am DBM-Hebelstrasse stieg die Zahl der Mitarbeitenden im Jahr 2016 erstmals über die magische Zahl von 500. Der PhD und Postdoc Club haben durch ihre Aktivitäten das ganze Jahr über jungen Forschenden viele Möglichkeiten geboten, sich mit dem wissenschaftlichen Umfeld auseinanderzusetzen und sich weiter zu entwickeln. Ich möchte allen danken, die unter immer anspruchsvolleren Bedingungen zum Erfolg des DBM 2016 beigetragen haben!

Die wissenschaftliche Artikelserie beschliessen wir dieses Jahr mit Jan Niess, der in der Dezemberausgabe die Forschungsaktivitäten seines Labors «Gastroenterology» vorstellt (ab Seite 2). Jakub Zmajkovic und Elban Moraz lassen das Jahr festlich ausklingen. Jakub feiert mit uns slowakische Weihnachten (ab Seite 30) und Elban nimmt uns mit in ihre Tradition des Hanukkah, das dieses Jahr auf das gleiche Datum wie Weihnachten fällt (ab Seite 34).

Viel Spass bei der Lektüre und schöne Festtage!

Dear Readers

2016 is drawing to a close. A challenging, but also very successful year lies behind us. Mohamed (Momo) Bentires-Alj was appointed as professor for Experimental Surgical Oncology and he and his lab have already found their places at the DBM. This year Rolf Zeller, Mohamed Bentires-Alj and Nicola Aceto were all awarded prestigious ERC grants. With respect to publications the DBM also finishes off very well (see page 9). In 2016 the number of staff at DBM-Hebelstrasse rose above the magical figure of 500 for the first time. Throughout the year the activities of the PhD and Postdoc Club have given young researches many opportunities to deal with the scientific environment and to develop further. I would like to thank all of those who have contributed to the success of the DBM in 2016 under constantly challenging conditions!

This year we finish off the scientific article series with Jan Niess, who presents the scientific activities of his Gastroenterology laboratory in the December issue (page 2). Jakub Zmajkovic and Elban Moraz ring out the year on a festive note. Jakub celebrates the Slovakian Christmas with us (page 30) and Elban shares the tradition of Hanukkah, which this year falls on the same date as Christmas (page 34).

Enjoy reading this latest issue and happy holidays!

Studying Host-Microbiota Interactions by Silencing Receptors of the Host

"Ein Punkt nur ist es, kaum ein Schmerz, Nur ein Gefühl, empfunden eben; Und dennoch spricht es stets darain, und dennoch stört es dich zu leben."

First verse of "Beginn des Endes" from Theodor Storm

Introduction

Theodor Storm wrote his poem "Beginn des Endes" without a distinct biographical occasion twenty years before he died of gastric cancer. When an individual complains nowadays about abdominal pain, sophisticated imaging technologies are used to diagnose and treat gastrointestinal diseases. High-resolution endoscopes not only diagnose and predict the course of diseases in attempts to personalize medicine, but are also used to treat diseases by stopping bleedings, by dissecting pre- or even cancerous lesions or by draining extraintestinal cysts. Although the advanced imaging technologies have revolutionized the way in which gastro-intestinal diseases, such as inflammatory bowel diseases (IBD), are diagnosed and treated, the understanding of these conditions is still limited. 75,000 subjects and controls have been mapped to identify complex traits associated with IBD.[1] 163 genetic loci have been identified associated with IBD, which explains only less than 20% of the variance in disease risk. New technologies have also helped to investigate the intestinal microbiota, to describe 'who is there' and 'where they are' in defined compartments of the gut (i.e. upper versus lower gastro-intestinal tract, mucous layer vs. intestinal lumen) but 'what are they doing' has not been explored in detail. Our research program does not focus on examining the effect that one specific microorganism has on the host. Instead, we aim to understand how the host reacts to the intestinal microbiota and for this we use different approaches:

(a) Sterile germ-free animals can be challenged with defined groups of microorganism (gnotobiotics) to investigate the host response.

(b) Pattern recognition receptors (PRRs) that recognize pathogen-associated molecular patterns (PAMPs) expressed by microorganisms, such as the Toll-like receptors, have been identified more than a decade ago. Microbiota derived metabolites, such as bile acids, the tryptophan metabolite kynurenic acid, short chain fatty acids (SCFAs), retinoid acid etc. are also recognized by specific host receptors. These 'metabolite- or xenosensors' can be silenced by genetic means in animals and cells.

(c) Specific deletion of cells that recognizes constituents of the microflora. For example, CX_3CR1+ mononuclear phagocytes can be depleted by diphtheria toxin injection in CX_3CR1-DTR mice.

In this article, I will discuss what cell populations are in contact with constituents of the intestinal microbiota. I will also describe how recent findings opened new questions that guide the research within the Gastroenterology lab.

Importance of macrophages for mucosal immune responses

A physical barrier separates the luminal content from the host to avoid non-controlled immune responses in the gut.[2] Intestinal epithelial cells that are sealed by tight and adherence junctions form this barrier. The

complete surface of intestinal epithelium renews every 4 – 5 days. All the intestinal epithelial cells originate from the stem cells that are located at the base of the intestinal crypts. Intestinal stem cells differentiate into antimicrobial peptides producing Paneth cells, mucus secreting goblet cells, IL-25 secreting Tuft cells, antigen-uptaking microfold (M) cells or the absorptive epithelial cells. The mucus that covers the epithelial surface can be divided into a sterile inner layer and an outer layer, which serves as a specific niche for constituents of the microflora. The mucus increases in size from the upper to the lower gastrointestinal tract. Within the epithelial cells the intraepithelial lymphocytes are located, a heterogeneous cell population that express a γδ or a αβ T cell receptor (TCR) to protect the epithelium in response to pathogen-induced stress responses or against inflammation caused by infiltrating leucocytes. Beneath the epithelium the connective tissue layer containing macrophages that sample the luminal content is called lamina propria.

Intestinal macrophages positioned beneath the intestinal epithelium can extend processes into the epithelial layer. These macrophages processes are reaching into the intestinal lumen to sample luminal content in the terminal ileum. These cells secrete IL-1ß and IL-23 to support the production of IL-22 by innate lymphocytes. IL-22 facilitates the production of the antimicrobial peptides RegIIIß and RegIIIγ by epithelial cells.

Intestinal macrophages also influence adaptive immune responses. Studies with *E. coli* expressing Ovalbumin (OVA) tagged to a fluorescence reporter indicated that intestinal macrophages deliver sampled *E. coli* to dendritic cells (DCs) in the lamina propria. These DCs transport *E. coli* derived antigens to mesenteric lymph nodes, where T cells are primed. The effector T cells home back to the lamina propria, where macrophages might activate them (Figure 1).

Macrophages also express pathogen recognition receptors, such as the Toll-like receptors (TLR) 9 and 4 that recognize pathogen associated molecular patterns – highly conserved structural motifs of molecules that are common to a wide range of microbes. Recent work indicated that not only the recognition of bacterial motifs but also of bacterial-derived metabolites modulate the immune response of the host. Myeloid cells, for example, express the G-protein coupled receptors GPR35 and GPR120 and the purinergic receptors P2Y and P2X. GPR120 recognize long chain fatty acids with aliphatic tails of 13 to 21 carbons, which inhibits the production of TNFα and IL-6. GPR35 is the receptor for the chemokine CXCL17 and also for the tryptophan metabolite kynurenic acid.[3] The T-type amino acid transporter 1 (TAT1) transports the essential amino acid tryptophan across the intestinal barrier, where tryptophan is metabolized by indoleamine-2,3-dioxygenase (IDO) to kynurenine and kynurenic acid. Gut bacteria convert tryp-

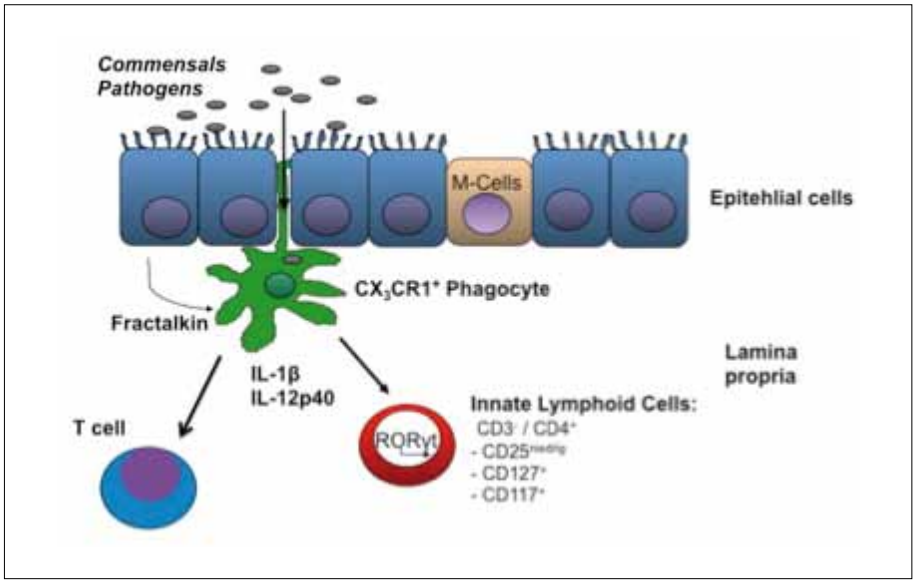


Figure 1. Intestinal macrophages sample parts of the microbiota and initiate innate and adaptive immune responses

tophan by aerobic degradation (kynurenine pathway) to kynurenate. Other genera of anaeroebic bacteria, such as members of the *Bifidobacterium* genus lack tryptophanse activity and may be used as a probiotic to treat inflammation.

Outlook and future perspective

Our group has identified a fundamental pathway by which the microbiota interacts with the mucosal immune system. Phagocytes that express the fractalkine receptor CX3CR1 have processes reaching into the intestinal lumen to sample luminal content. How this pathway is regulated and how it shapes the immune system of the host is only partially understood. These cells not only recognize parts of the microflora but also their metabolites, as well as food components that all may shape their function. The current work of the gastroenterology research group focuses (i) on the cytokine IL-19, member of the IL-10 cytokine family, (ii) the G protein coupled receptor GPR35, which serves as a receptor of the chemokine CXCL17 and kynurenic acid, (iii) on CD98, heterodimer composed of SLC3A2 and SLC7A5 that forms the large neutral amino acid transporter (LAT1) and (iv) the NOD-like receptor family pyrin domain containing 6 (NLRP6), an intracellular protein that plays a role in recognizing viral and microbial derived products (Figure 2). This means that our group focuses on projects elucidating the effector function of intestinal macrophages and characterizes their receptors required for the recognition of metabolites and microbes.

(1) Function of macrophage derived IL-19 on mucosal immune responses

Recent discoveries indicated that members of the IL-20 cytokine subfamily are expressed by macrophages. Anna Steinert (Ph.D. student in our lab) is working on this project. She could demonstrate that patients with active inflammatory bowel disease (IBD) express higher levels of IL-19, IL-20 and IL-24. Also, there is higher IL-19 expression in animals with Dextran Sodium Sulfate (DSS) induced colitis. Gnotobiotic mice that cover the five major phyla of prokaryotes present in the gut are characterized by an accelerated colitis. In these mice IL-19 expression could not be detected during colitis. This indicates, that in part, the expression of IL-19 is modulated by the intestinal microflora. The injection of Lipopolysaccharide (LPS) leads to the up-regulation of IL-19. We are currently testing the importance of IL-19 for the development of colitis with a newly generated IL-19tdTomato reporter mouse strain.

(2) Recognition of microbiota derived metabolites by intestinal myeloid cells

Before myeloid cells can serve as effector cells in the mucosal immune system, they must recognize potentially danger signals. These cells express receptors required for the recognition of microbial derived metabolites and structural motifs. One potential receptor

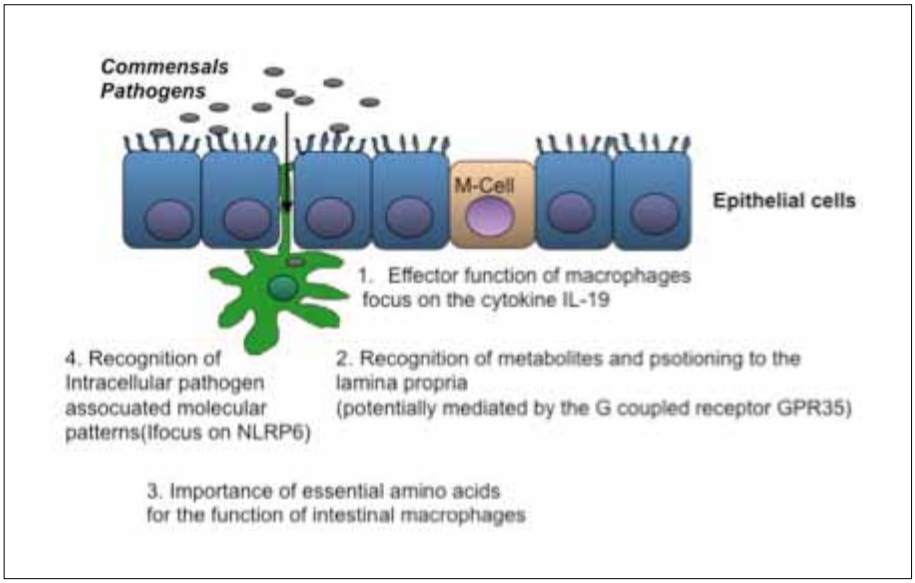


Figure 2. Focus and projects of the Gastroenterology Research Group.

for the recognition of the tryptophan metabolite kynurenic acid is GPR35, which also serves as a receptor for the chemokine CXCL17. Berna Kaya (Ph.D. student) currently investigates the expression pattern of GPR35 and aims to identify the cell that expresses GPR35. Her work will define, what cell will sense parts of the intestinal metabolome and may contribute to our understanding how the microbiota interacts with the host.

(3) Importance of amino acid transporters for intestinal macrophages

Intestinal macrophages have processes that reach between intestinal epithelial cells into the intestinal lumen, where macrophages sample constituents of the microflora. Macrophages are also exposed to nutrients present in the gastrointestinal tract. However, there is not much information on how the intestinal content shapes the function of macrophages. Philipp Wuggenig (Ph.D. student) is focussing on the potential importance of branched-chain and aromatic amino acids. He utilizes a conditional knock out mouse strain, which lacks the glycoprotein CD98 that forms the large neutral amino acid transporter LAT1. Philipp will investigate if CD98 is required or the development of intestinal macrophages.

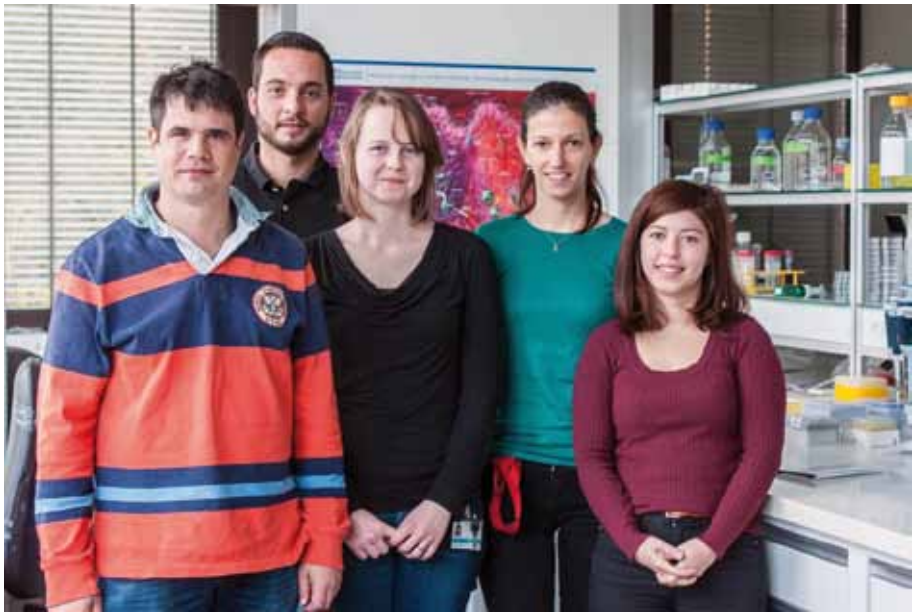
(4) Nucleotide-binding and oligomerization domain (NOD)-like receptor (NLR)

Nucleotide-binding and oligomerization domain like receptor (NLR) are intracellular sensors for the recognition of pathogen-associated patterns (PAMPs) that have entered the macrophage by phagocytosis. The occurrence of dysbiosis has recently been described in mice lacking the nucleotide-binding and oligomerization domain like receptor NLRP6. Intestinal epithelial cells, macrophages and T cells, express NLRP6 but the function of NLRP6 for a respective cell population has not been studied in detail. Katarina Radulovic (PostDoc) will investigate to find the cellular compartment that is required for the effects of NLRP6 on the gut microflora.

Conclusion

Understanding principles required for the communication between the host and the intestinal microbiota is essential to study gastrointestinal diseases, which may range from abdominal discomfort, such as meteroismus to inflammatory bowel disease and colorectal cancer. Since intestinal macrophages are in direct contact with the microbiota and initiate innate and adaptive immune responses we focus our studies on this cell population by silencing receptors required for the recognition of microbiota derived metabolites and structural motifs of microorganisms.

Jan Hendrik Niess and team



The Gastroenterology Group in November 2016

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CHRISTMAS 2016

Die Geduldigen

Die Pinie scheint zu horchen, die Tanne zu warten: und beide ohne Ungeduld. Sie denken nicht an den kleinen Menschen unter sich, den Ungeduld und seine Neugierde auffressen.

Friedrich Nietzsche

Department of Biomedicine Research Day 2017

Thursday, January 26, 08:10 – 13:15 h
Small Lecture Hall, Zentrum für Lehre und Forschung
Hebelstrasse 20, 4031 Basel

Speakers

Nicola Aceto

Christoph Berger

Daniel Bodmer

Adrian Egli

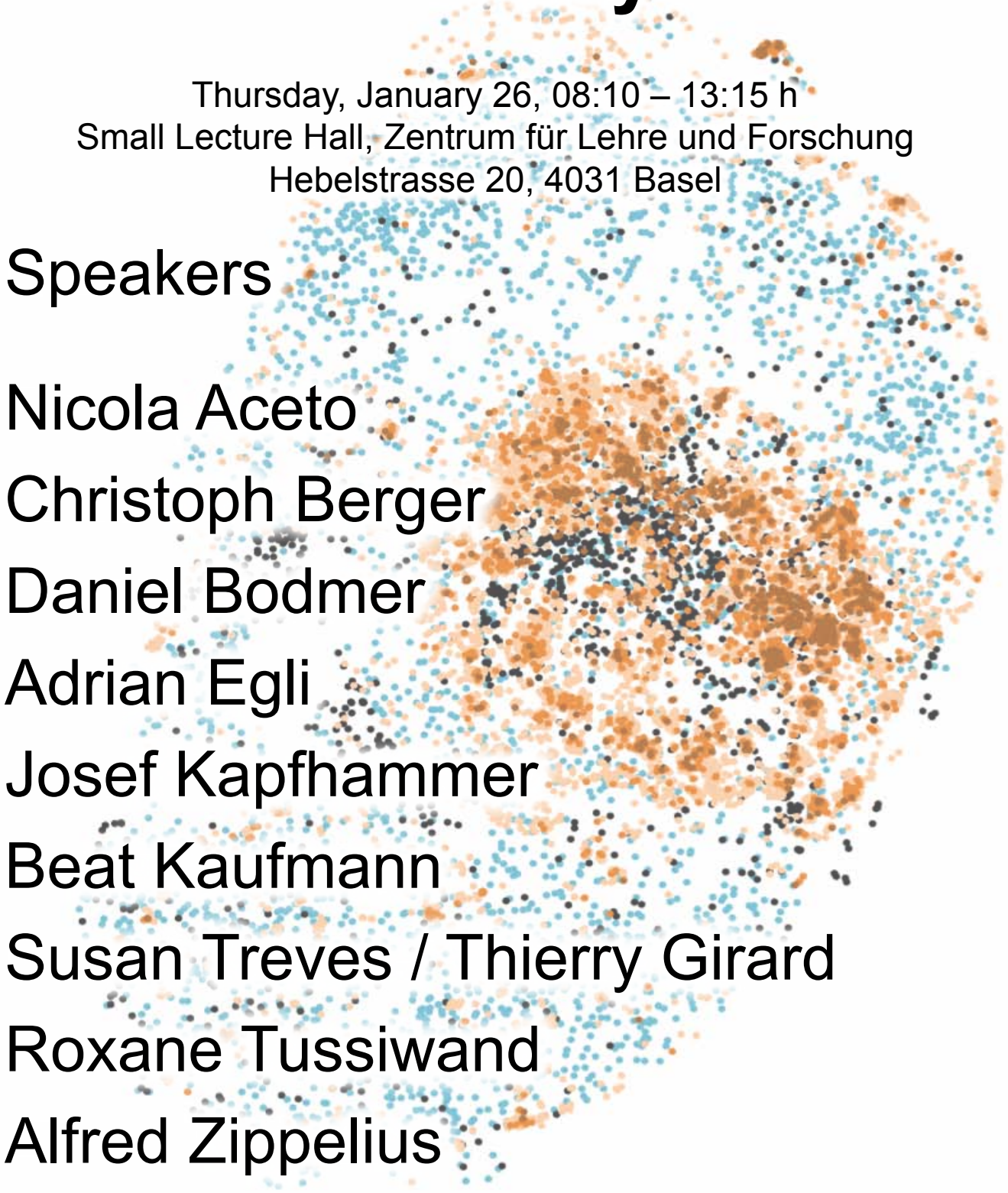
Josef Kapfhammer

Beat Kaufmann

Susan Treves / Thierry Girard

Roxane Tussiwand

Alfred Zippelius



Departement Biomedizin Basel

Courtesy of Romain Amante / Bentires-Alj lab

Dissertationen

Am 10. Mai 2016 konnte **Ruben López Dicuru** von der Forschungsgruppe "Perioperative Patient Safety" (Departement Biomedizin Hebelstrasse) seine Dissertation mit Erfolg beenden. Er befasste sich in seiner Dissertation mit dem Thema: "Study of calcium sparks in skeletal and smooth muscle cells in normal and pathological conditions".

Am 27. Juni 2016 stellte sich **Maria Zimmermann** von der Forschungsgruppe "Cinical Neuroimmunology" (Departement Biomedizin Hebelstrasse) den Fragen des Dissertationskomitees. Der Titel ihrer Dissertation hiess: "B cells and endogenous retroviruses in multiple sclerosis".

Seit dem 11. November 2016 darf sich **Katharina Leonards** von der Forschungsgruppe "Childhood Leukemia" (Departement Biomedizin Hebelstrasse) Frau Dr. nennen. Sie befasste sich in ihrer Doktorarbeit mit dem Thema: "Modelling and targeting epigenetic regulators in acute leukemia".

Auszeichnungen

Dora-Seif-Preis an Markus Heim

Am 31. August 2016 hat **Markus Heim** von der Forschungsgruppe "Hepatology" (Departement Biomedizin Hebelstrasse) gemeinsam mit **Luigi Terraciano**, Institut für Pathologie USB, für die herausragenden Leistungen in der Erforschung, Klassifizierung und Behandlung von Leberkrebs den Dora-Seif-Krebsforschungspreis für "die beste Arbeit, die zur Verbesserung der Frühdiagnostik und Therapie des Krebses beiträgt", erhalten. Insbesondere hebt die Jury die erfolgreiche und international anerkannte interdisziplinäre Zusammenarbeit der beiden Preisträger hervor. Der Preis ist mit 5'000 CHF dotiert.

SLB G. Jeanette Thorbecke Award an Roxanne Tussiwand

Roxanne Tussiwand von der Forschungsgruppe "Immune Regulation" (Departement Biomedizin Mattenstrasse) hat am 49. Treffen der "Society for Leukocyte Biology" im September 2016 den SLB G. Jeanette Thorbecke Award erhalten. Der Preis geht an verdienstvolle junge Nachwuchsforscherinnen.

Das DBM gratuliert ganz herzlich!

Und weil es so schön war, alle Jahre wieder:

Lancet THE LANCET Oncology 2016; 388: 1985–94 IF 44,002

Nasal chondrocyte-based engineered autologous cartilage tissue for repair of articular cartilage defects: an observational first-in-human trial

Marcus Mumme[1,*], Andrea Barbero[1,*], Sylvie Miot[1], Anke Wixmerten[1], Sandra Feliciano[1], Francine Wolf[1], Adelaide M Asnaghi[1], Daniel Baumhoer[2], Oliver Bieri[3], Martin Kretzschmar[3], Geert Pagenstert[1], Martin Haug[1], Dirk J Schaefer[1], Ivan Martin[1], Marcel Jakob[1]

Summary

Background Articular cartilage injuries have poor repair capacity, leading to progressive joint damage, and cannot be restored predictably by either conventional treatments or advanced therapies based on implantation of articular chondrocytes. Compared with articular chondrocytes, chondrocytes derived from the nasal septum have superior and more reproducible capacity to generate hyaline-like cartilage tissues, with the plasticity to adapt to a joint environment. We aimed to assess whether engineered autologous nasal chondrocyte-based cartilage grafts allow safe and functional restoration of knee cartilage defects.

Methods In a first-in-human trial, ten patients with symptomatic, post-traumatic, full-thickness cartilage lesions (2–6 cm^2) on the femoral condyle or trochlea were treated at University Hospital Basel in Switzerland. Chondrocytes isolated from a 6 mm nasal septum biopsy specimen were expanded and cultured onto collagen membranes to engineer cartilage grafts (30x40x2 mm). The engineered tissues were implanted into the femoral defects via mini-arthrotomy and assessed up to 24 months after surgery. Primary outcomes were feasibility and safety of the procedure. Secondary outcomes included self-assessed clinical scores and MRI-based estimation of morphological and compositional quality of the repair tissue. This study is registered with ClinicalTrials.gov, number NCT01605201. The study is ongoing, with an approved extension to 25 patients.

Findings For every patient, it was feasible to manufacture cartilaginous grafts with nasal chondrocytes embedded in an extracellular matrix rich in glycosaminoglycan and type II collagen. Engineered tissues were stable through handling with forceps and could be secured in the injured joints. No adverse reactions were recorded and self-assessed clinical scores for pain, knee function, and quality of life were improved significantly from before surgery to 24 months after surgery. Radiological assessments indicated variable degrees of defect filling and development of repair tissue approaching the composition of native cartilage.

Interpretation Hyaline-like cartilage tissues, engineered from autologous nasal chondrocytes, can be used clinically for repair of articular cartilage defects in the knee. Future studies are warranted to assess efficacy in large controlled trials and to investigate an extension of indications to early degenerative states or to other joints.

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[*] These authors contributed equally to this work.

Cell Stem Cell **Cell Stem Cell** 19, 1–10, November 3, 2016 IF 22,387

Multipotency of Adult Hippocampal NSCs In Vivo Is Restricted by Drosha/NFIB

Chiara Rolando[1,*], Andrea Erni[1,*], Alice Grison[1], Robert Beattie[1], Anna Engler[1], Paul J. Gokhale[2], Marta Milo[2], Thomas Wegleiter[3], Sebastian Jessberger[3], and Verdon Taylor[1]

Summary

Adult neural stem cells (NSCs) are defined by their inherent capacity to self-renew and give rise to neurons, astrocytes, and oligodendrocytes. In vivo, however, hippocampal NSCs do not generate oligodendrocytes for reasons that have remained enigmatic. Here, we report that deletion of Drosha in adult dentate gyrus NSCs activates oligodendrogenesis and reduces neurogenesis at the expense of gliogenesis. We further find that Drosha directly targets NFIB to repress its expression independently of Dicer and microRNAs. Knockdown of NFIB in Drosha-deficient hippocampal NSCs restores neurogenesis, suggesting that the Drosha/NFIB mechanism robustly prevents oligodendrocyte fate acquisition in vivo. Taken together, our findings establish that adult hippocampal NSCs inherently possess multilineage potential but that Drosha functions as a molecular barrier preventing oligodendrogenesis.

Introduction

Somatic stem cells can generate progeny throughout life, but their fates are usually restricted, and they generate specific cell types in their respective tissue. Active adult neural stem cells (NSCs) are present in two regions of the brain: the subventricular zone (SVZ) of the lateral ventricles and the subgranule zone of the hippocampal dentate gyrus (DG). Although both SVZ and DG NSCs are multipotent, they generate specific neuron types. SVZ NSCs become fate restricted during embryonic development and generate multiple interneuron populations from topological locations in the lateral ventricle wall. DG NSCs produce only granule neurons, which contribute to cognition, and loss or dormancy of stem cells during aging can result in psychological disorders and disease. Whereas SVZ NSCs make a significant number of oligodendrocytes, new oligodendrocytes are normally not produced in the adult DG. In vitro, DG NSCs also rarely produce oligodendrocytes, although oligodendrocytic differentiation can be induced by their co-culture with neurons and in vivo by inactivation of the Neurofibromin 1 gene or reprogramming with the transcription factor Ascl1. This suggests an intrinsic and niche-independent fate restriction of DG NSCs that prevents oligodendrocyte formation. How DG NSC potency and particularly oligodendrocytic fate are restricted remains unclear.

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[*] Co-first author

Nature Immunology | nature immunology | Vol. 17, No 10, October 2016 IF 19,381

Foxn1 regulates key target genes essential for T cell development in postnatal thymic epithelial cells

Saulius Žuklys[1,*], Adam Handel[2,*], Saule Zhanybekova[4,*], Fatima Govani[3], Marcel Keller[1,4], Stefano Maio[3], Carlos E Mayer[1], Hong Ying Teh[1], Katrin Hafen[1], Giuseppe Gallone[2], Thomas Barthlott[1], Chris P Ponting[2,4] & Georg A Holländer[1,3]

Thymic epithelial cell differentiation, growth and function depend on the expression of the transcription factor Foxn1; however, its target genes have never been physically identified. Using static and inducible genetic model systems and chromatin studies, we developed a genome-wide map of direct Foxn1 target genes for postnatal thymic epithelia and defined the Foxn1 binding motif. We determined the function of Foxn1 in these cells and found that, in addition to the transcriptional control of genes involved in the attraction and lineage commitment of T cell precursors, Foxn1 regulates the expression of genes involved in antigen processing and thymocyte selection. Thus, critical events in thymic lymphostromal cross-talk and T cell selection are indispensably choreographed by Foxn1.

The thymic microenvironment is unique in its ability to promote the development and selection of naive T cells with a repertoire purged of vital 'self ' specificities, but that are prepared to react to injurious non-self. Thymic epithelial cells (TECs), which can be categorized into separate cortical (cTEC) and medullary (mTEC) lineages, are essential for this competence. cTECs attract blood-borne precursor cells, commit them to a T cell fate and foster their differentiation to express an αβT cell antigen receptor (TCR). Reactivity to major histocompatibility complex (MHC)-peptide complexes presented by TECs authorizes the generation of a bespoke TCR repertoire. Because TCRs are initially generated pseudo-randomly, their specificity is scrutinized during thymocyte development to establish a selected repertoire that is tailored for an individual, whereby cTEC positively select thymocytes that express a TCR with a sufficient affinity for self-antigens. Subsequently, both cTECs and mTECs deplete thymocytes with substantial reactivity to self-antigens, a process that is known as negative selection.

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* These authors contributed equally to this work.

Blood | blood | 2016;128(6):839–851 IF 11,847

JAK2 exon 12 mutant mice display isolated erythrocytosis and changes in iron metabolism favoring increased erythropoiesis

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Mutations in *JAK2* exon 12 are frequently found in patients with polycythemia vera (PV) that do not carry a *JAK2-V617F* mutation. The majority of these patients display isolated erythrocytosis. We generated a mouse model that expresses *JAK2-N542-E543del*, the most frequent *JAK2 exon 12* mutation found in PV patients. Mice expressing the human *JAK2-N542-E543del (Ex12)* showed a strong increase in red blood cell parameters but normal neutrophil and platelet counts, and reduced overall survival. Erythropoiesis was increased in the bone marrow and spleen, with normal megakaryopoiesis and absence of myelofibrosis in histopathology. Erythroid progenitors and precursors were increased in hematopoietic tissues, but the numbers of megakaryocytic precursors were unchanged. Phosphorylation Stat3 and Erk1/2 proteins were increased, and a trend toward increased phospho-Stat5 and phospho-Stat1 was noted. However, Stat1 knock out in Ex12 mice induced no changes in platelet or red cell parameters, indicating that Stat1 does not play a central role in mediating the effects of Ex12 signaling on megakaryopoiesis or erythropoiesis. *Ex12* mice showed decreased expression of *hepcidin* and increased expression of *transferrin receptor-1* and *erythroferrone,* suggesting that the strong erythroid phenotype in *Ex12* mutant mice is favored by changes in iron metabolism that optimize iron availability to allow maximal production of red cells.

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Loss of *Ezh2* synergizes with *JAK2*-V617F in initiating myeloproliferative neoplasms and promoting myelofibrosis

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Myeloproliferative neoplasm (MPN) patients frequently show co-occurrence of *JAK2*-V617F and mutations in epigenetic regulator genes, including *EZH2*. In this study, we show that *JAK2*-V617F and loss of *Ezh2* in hematopoietic cells contribute synergistically to the development of MPN. The MPN phenotype induced by *JAK2*-V617F was accentuated in *JAK2*-V617F;*Ezh2*$^{-/-}$mice, resulting in very high platelet and neutrophil counts, more advanced myelofibrosis, and reduced survival. These mice also displayed expansion of the stem cell and progenitor cell compartments and a shift of differentiation toward megakaryopoiesis at the expense of erythropoiesis. Single cell limiting dilution transplantation with bone marrow from *JAK2*-V617F;*Ezh2*$^{+/-}$ mice showed inc.reased reconstitution and MPN disease initiation potential compared with *JAK2*-V617F alone. RNA sequencing in *Ezh2*-deficient hematopoietic stem cells (HSCs) and megakaryocytic erythroid progenitors identified highly up-regulated genes, including *Lin28b* and *Hmga2*, and chromatin immunoprecipitation (ChIP)-quantitative PCR (qPCR) analysis of their promoters revealed dec. reased H3K27me3 deposition. Forced expression of *Hmga2* resulted in increased chimerism and platelet counts in recipients of retrovirally transduced HSCs. *JAK2*-V617F-expressing mice treated with an Ezh2 inhibitor showed higher platelet counts than vehicle controls. Our data support the proposed tumor suppressor function of *EZH2* in patients with MPN and call for caution when considering using Ezh2 inhibitors in MPN.

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Biologically and mechanically driven design of an RGD-mimetic macroporous foam for adipose tissue engineering applications

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Abstract

Despite clinical treatments for adipose tissue defects, in particular breast tissue reconstruction, have certain grades of efficacy, many drawbacks are still affecting the long-term survival of new formed fat tissue. To overcome this problem, in the last decades, several scaffolding materials have been investigated in the field of adipose tissue engineering. However, a strategy able to recapitulate a suitable environment for adipose tissue reconstruction and maintenance is still missing. To address this need, we adopted a biologically and mechanically driven design to fabricate an RGD-mimetic poly(amidoamine) oligomer macroporous foam (OPAAF) for adipose tissue reconstruction. The scaffold was designed to fulfil three fundamental criteria: capability to induce cell adhesion and proliferation, support of *in vivo* vascularization and match of native tissue mechanical properties. Poly(amidoamine) oligomers were formed into soft scaffolds with hierarchical porosity through a combined free radical polymerization and foaming reaction. OPAAF is characterized by a high water uptake capacity, progressive degradation kinetics and ideal mechanical properties for adipose tissue reconstruction. OPAAF's ability to support cell adhesion, proliferation and adipogenesis was assessed *in vitro* using epithelial, fibroblast and endothelial cells (MDCK, 3T3L1 and HUVEC respectively). In addition, *in vivo* subcutaneous implantation in murine model highlighted OPAAF potential to support both adipogenesis and vessels infiltration. Overall, the reported results support the use of OPAAF as a scaffold for engineered adipose tissue construct.

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An *RYR1* mutation associated with malignant hyperthermia is also associated with bleeding abnormalities

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Malignant hyperthermia is a potentially fatal hypermetabolic disorder triggered by halogenated anesthetics and the myorelaxant succinylcholine in genetically predisposed individuals. About 50% of susceptible individuals carry dominant, gain-of-function mutations in *RYR1* [which encodes ryanodine receptor type 1 (RyR1)], though they have normal muscle function and no overt clinical symptoms. RyR1 is predominantly found in skeletal muscle but also at lower amounts in immune and smooth muscle cells, suggesting that *RYR1* mutations may have a wider range of effects than previously suspected. Mild bleeding abnormalities have been described in patients with malignant hyperthermia carrying gain-of-function *RYR1* mutations. We sought to determine the frequency and molecular basis for this symptom. We found that some patients with specific *RYR1* mutations had abnormally high bleeding scores, whereas their healthy relatives did not. Knock-in mice with the malignant hyperthermia susceptibility *RYR1* mutation Y522S (MHS $RYR1_{Y522S}$)had longer bleeding times than their wildtype littermates. Primary vascular smooth muscle cells from $RYR1_{Y522S}$ knock-in mice exhibited a higher frequency of subplasmalemmal Ca^{2+} sparks, leading to a more negative resting membrane potential. The bleeding defect of $RYR1_{Y522S}$ mice and of one patient was reversed by treatment with the RYR1 antagonist dantrolene, and Ca^{2+} sparks in primary vascular smooth muscle cells from the MHS $RYR1_{Y522S}$ mice were blocked by ryanodine or dantrolene. Thus, *RYR1* mutations may lead to prolonged bleeding by altering vascular smooth muscle cell function. The reversibility of the bleeding phenotype emphasizes the potential therapeutic value of dantrolene in the treatment of such bleeding disorders.

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LSD Acutely Impairs Fear Recognition and Enhances Emotional Empathy and Sociality

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Lysergic acid diethylamide (LSD) is used recreationally and has been evaluated as an adjunct to psychotherapy to treat anxiety in patients with life-threatening illness. LSD is well-known to induce perceptual alterations, but unknown is whether LSD alters emotional processing in ways that can support psychotherapy. We investigated the acute effects of LSD on emotional processing using the Face Emotion Recognition Task (FERT) and Multifaceted Empathy Test (MET). The effects of LSD on social behavior were tested using the Social Value Orientation (SVO) test. Two similar placebo-controlled, double-blind, random-order, crossover studies were conducted using 100 μg LSD in 24 subjects and 200 μg LSD in 16 subjects. All of the subjects were healthy and mostly hallucinogen-naive 25-to 65-year-old volunteers (20 men, 20 women). LSD produced feelings of happiness, trust, closeness to others, enhanced explicit and implicit emotional empathy on the MET, and impaired the recognition of sad and fearful faces on the FERT. LSD enhanced the participants' desire to be with other people and increased their prosocial behavior on the SVO test. These effects of LSD on emotion processing and sociality may be useful for LSD-assisted psychotherapy.

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Engineered mesenchymal cell-based patches as controlled VEGF delivery systems to induce extrinsic angiogenesis

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Abstract

Therapeutic over-expression of Vascular Endothelial Growth Factor (VEGF) by transduced progenitors is a promising strategy to efficiently induce angiogenesis in ischemic tissues (e.g. limb muscle and myocardium), but tight control over the micro-environmental distribution of the dose is required to avoid induction of angioma-like tumors. Therapeutic VEGF release was achieved by purified transduced adipose mesenchymal stromal cells (ASC) that homogeneously produce specific VEGF levels, inducing only normal angiogenesis after injection in non-ischemic tissues. However, the therapeutic potential of this approach mostly in the cardiac field is limited by the poor cell survival and the restricted area of effect confined to the cell-injection site. The implantation of cells previously organized *in vitro* in 3D engineered tissues could overcome these issues. Here we hypothesized that collagen sponge-based construct (patch), generated by ASC expressing controlled VEGF levels, can function as delivery device to induce angiogenesis in surrounding areas (extrinsic vascularization). A 7-mm-thick acellular collagen scaffold (empty), sutured beneath the patch, provided a controlled and reproducible model to clearly investigate the ongoing angiogenesis in subcutaneous mice pockets. VEGF-expressing ASC significantly increased the capillary in-growth inside both the patch itself and the empty scaffold compared to naïve cells, leading to significantly improved survival of implanted cells.

These data suggest that this strategy confers control (i) on angiogenesis efficacy and safety by means of ASC expressing therapeutic VEGF levels and (ii) over the treated area through the specific localization in an engineered collagen sponge-based patch.

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Noninvasive Contrast-Enhanced Ultrasound Molecular Imaging Detects Myocardial Inflammatory Response in Autoimmune Myocarditis

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Background—Cardiac tests for diagnosing myocarditis lack sensitivity or specificity. We hypothesized that contrastenhanced ultrasound molecular imaging could detect myocardial inflammation and the recruitment of specific cellular subsets of the inflammatory response in murine myocarditis.

Methods and Results—Microbubbles (MB) bearing antibodies targeting lymphocyte CD4 (MB_{CD4}), endothelial P-selectin (MB_{PSel}), or isotype control antibody (MB_{Iso}) and MB with a negative electric charge for targeting of leukocytes (MB_{Lc}) were prepared. Attachment of MB_{CD4} was validated in vitro using murine spleen CD4+ T cells. Twenty-eight mice were studied after the induction of autoimmune myocarditis by immunization with α-myosin-peptide; 20 mice served as controls. Contrast-enhanced ultrasound molecular imaging of the heart was performed. Left ventricular function was assessed by conventional and deformation echocardiography, and myocarditis severity graded on histology. Animals were grouped into no myocarditis, moderate myocarditis, and severe myocarditis. In vitro, attachment of MBCD4 to CD4+ T cells was significantly greater than of MB_{Iso}. Of the left ventricular ejection fraction or strain and strain rate readouts, only longitudinal strain was significantly different from control animals in severe myocarditis. In contrast, contrast-enhanced ultrasound molecular imaging showed increased signals for all targeted MB versus MB_{Iso} both in moderate and severe myocarditis, and MB_{CD4} signal correlated with CD4+ T-lymphocyte infiltration in the myocardium.

Conclusions—Contrast-enhanced ultrasound molecular imaging can detect endothelial inflammation and leukocyte infiltration in myocarditis in the absence of a detectable decline in left ventricular performance by functional imaging. In particular, imaging of CD4+ T cells involved in autoimmune responses could be helpful in diagnosing myocarditis.

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Should viral load thresholds be lowered? Revisiting the WHO definition for virologic failure in patients on antiretroviral therapy in resource-limited settings

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Abstract

The World Health Organization (WHO) guidelines on antiretroviral therapy (ART) define treatment failure as 2 consecutive viral loads (VLs) ≥1000 copies/mL. There is, however, little evidence supporting 1000 copies as an optimal threshold to define treatment failure. Objective of this study was to assess the correlation of the WHO definition with the presence of drug-resistance mutations in patients who present with 2 consecutive unsuppressed VL in a resource-limited setting.

In 10 nurse-led clinics in rural Lesotho children and adults on first-line ART for ≥6 months received a firstroutine VL. Those with plasma VL ≥80 copies/mL were enrolled in a prospective study, receiving enhanced adherence counseling (EAC) and a follow-up VL after 3 months. After a second unsuppressed VL genotypic resistance testing was performed. Viruses with major mutations against ≥2 drugs of the current regime were classified as "resistant".

A total of 1563 adults and 191 children received a first routine VL. Of the 138 adults and 53 children with unsuppressed VL (≥80 copies/mL), 165 (116 adults; 49 children) had a follow-up VL after EAC; 108 (74 adults; 34 children) remained unsuppressed and resistance testing was successful. Ninety of them fulfilled the WHO definition of treatment failure (both VL ≥1000 copies/mL); for another 18 both VL were unsuppressed but with <1000 copies/mL. The positive predictive value (PPV) for the WHO failure definition was 81.1% (73/90) for the presence of resistant virus. Among the 18 with VL levels between 80 and 1000 copies/mL, thereby classified as "non-failures", 17 (94.4%) harbored resistant viruses. Lowering the VL threshold from 1000 copies/mL to 80 copies/mL at both determinations had no negative influenceon the PPV (83.3%; 90/108).

The current WHO-definition misclassifies patients who harbor resistant virus at VL below 1000c/mL as "nonfailing." Lowering the threshold to VL ≥80 copies/mL identifies a significantly higher number of patients with treatment-resistant virus and should be considered.

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Targeting DDR2 in head and neck squamous cell carcinoma with dasatinib

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Squamous cell carcinoma of the head and neck (HNSCC) is the tenth most common tumor entity in men worldwide. Nevertheless therapeutic options are mostly limited to surgery and radio-chemotherapy resulting in 5-year survival rates of around 50%. Therefore new therapeutic options are urgently needed. During the last years, targeting of receptor tyrosine kinases has emerged as a promising strategy that can complement standard therapeutical approaches. Here, we aimed at investigating if the receptor tyrosine kinase DDR2 is a targetable structure in HNSCC. DDR2 expression was assessed on a large HNSCC cohort (554 patients) including primary tumors, lymph node metastases and recurrences and normal mucosa as control. Subsequently, DDR2 was stably overexpressed in two different cell lines (FaDu and HSC-3) using lentiviral technology. Different tumorigenic properties such as proliferation, migration, invasion, adhesion and anchorage independent growth were assessed with and without dasatinib treatment using *in-vitro* cell models and *in-vivo* zebrafish xenografts. DDR2 was overexpressed in all tumor tissues when compared to normal mucosa. DDR2 overexpression led to increased migration, invasion, adhesion and anchorage independent growth whereas proliferation remained unaltered. Upon dasatinib treatment migration, invasion and adhesion could be inhibited *in-vitro* and *in-vivo* whereas proliferation was unchanged. Our data suggest treatment with dasatinib as a promising new therapeutic option for patients suffering from DDR2 overexpressing HNSCC. Since dasatinib is already FDA-approved we propose to test this drug in clinical trials so that patients could directly benefit from this new treatment option.

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Carbonic Anhydrase 8 Expression in Purkinje Cells Is Controlled by PKCγ Activity and Regulates Purkinje Cell Dendritic Growth

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Abstract
Purkinje cell dendritic development is severely compromised after chronic activation of protein kinase C (PKC). In a recent transgenic mouse model of spinocerebellar ataxia 14, the ser361-to-gly (S361G) mutation of the protein kinase C gamma (PKCγ) gene was expressed in Purkinje cells. Purkinje cells from these mutant mice in organotypic slice cultures have the same stunted dendritic tree as Purkinje cells after pharmacological activation of PKC. Because the transgene is exclusively present in Purkinje cells, cerebellar tissue from these mice is an attractive starting material for searching genes which might be interacting with PKCγ in Purkinje cells for inducing the stunted dendritic growth. We have performed a microarray analysis and identified several candidate genes with an increased messenger RNA (mRNA) expression in the PKCγ-S361G transgenic Purkinje cells. Out of these candidates, we have further studied carbonic anhydrase 8 (CA8). We show here that CA8 mRNA and protein expression is strongly induced in PKCγ-S361G transgenic Purkinje cells. Overexpression of CA8 in Purkinje cells in dissociated cultures strongly inhibited Purkinje cell dendritic development and produced a dendritic phenotype similar to PKCγ-S361G. There was no evidence for a direct binding of CA8 to either PKCγ or the type 1 IP3 receptor. Knockdown of CA8 with miRNA did not alter Purkinje cell dendritic development and did not protect Purkinje cells in dissociated cultures from the stunted dendritic growth induced by PKCγ-S361G or by PKC activation. Our results indicate that CA8 is a novel important regulator of Purkinje cell dendritic development and that its expression is controlled by PKCγ activity.

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C-met inhibition blocks bone metastasis development induced by renal cancer stem cells

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Abstract
Cancer stem cells (CSCs) are key players in bone metastasis. In some renal tumors CSCs overexpress the HGF receptor c-MET, speculating that c-MET targeting could lead to bone metastasis inhibition. To address this hypothesis we isolated renal CD105+/CD24−CSCs, expressing c-MET receptor from a primary renal carcinoma. Then, to study their ability to metastasize to bone, we injected renal CSCs in NOD/ SCID mice implanted with a human bone and we tested the effect of a c-MET inhibitor (JNJ-38877605) on bone metastasis development. JNJ-38877605 inhibited the formation of metastases at bone implant site. We showed that JNJ-38877605 inhibited the activation of osteoclasts induced by RCC stem cells and it stimulated osteoblast activity, finally resulting in a reduction of bone turnover consistent with the inhibition of bone metastases. We measured the circulating levels of osteotropic factors induced by RCC stem cells in the sera of mice treated with c-Met inhibitor, showing that IL-11 and CCL20 were reduced in mice treated with JNJ-38877605, strongly supporting the involvement of c-MET in the regulation of this process. To address the clinical relevance of c-MET upregulation during tumor progression, we analysed c-MET in renal cancer patients detecting an increased expression in the bone metastatic lesions by IHC. Then, we dosed CCL20 serum levels resulting significantly increased in patients with bone metastases compared to non-metastatic ones. Collectively, our data highlight the importance of the c-MET pathway in the pathogenesis of bone metastases induced by RCC stem cells in mice and humans.

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A high-content EMT screen identifies multiple receptor tyrosine kinase inhibitors with activity on TGFβ receptor

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Abstract

An epithelial to mesenchymal transition (EMT) enables epithelial tumor cells to break out of the primary tumor mass and to metastasize. Understanding the molecular mechanisms driving EMT in more detail will provide important tools to interfere with the metastatic process. To identify pharmacological modulators and druggable targets of EMT, we have established a novel multi-parameter, high-content, microscopy-based assay and screened chemical compounds with activities against known targets. Out of 3423 compounds, we have identified 19 drugs that block transforming growth factor beta (TGFβ)-induced EMT in normal murine mammary gland epithelial cells (NMuMG). The active compounds include inhibitors against TGFβ receptors (TGFBR), Rho-associated protein kinases (ROCK), myosin II, SRC kinase and uridine analogues. Among the EMT-repressing compounds, we identified a group of inhibitors targeting multiple receptor tyrosine kinases, and biochemical profiling of these multi-kinase inhibitors reveals TGFBR as a thus far unknown target of their inhibitory spectrum. These findings demonstrate the feasibility of a multi-parameter, high-content microscopy screen to identify modulators and druggable targets of EMT. Moreover, the newly discovered "off-target" effects of several receptor tyrosine kinase inhibitors have important consequences for *in vitro* and *in vivo* studies and might beneficially contribute to the therapeutic effects observed *in vivo*.

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Rapid and efficient magnetization of mesenchymal stem cells by dendrimer-functionalized magnetic nanoparticles

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Aim: Rapid and efficient magnetization of human bone marrow stromal cells (BMSC) through functionalized magnetic nanoparticles (MNP).

Methods: MNP were functionalized with poly(epsilon-lysine) dendrons exposing carboxybetaine residue (CB-MNP) to enhance binding to the cellular glycocalix. BMSC were incubated with CB-MNP or non-functionalized PAA-MNP for 5–30 min in suspension.

Results: CB-MNP functionalization increased the magnetization efficiency by threefold. Remarkably, 66% of cells were magnetized after only 5 min and the maximum efficiency of >80% was reached by 15 min. BMSC viability, proliferation and differentiation were not impaired: actually, adipogenic and osteogenic differentiation were even improved.

Conclusion: Carboxybetaine-dendron functionalization ensured rapid and efficient BMSC magnetization and allowed innovative suspension labeling, with a potential for bypassing adhesion culture of progenitors for regenerative medicine.

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Imperfect Symmetry of Sp1 and Core Promoter Sequences Regulates Early and Late Virus Gene Expression of the Bidirectional BK Polyomavirus Noncoding Control Region

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Abstract

Rearrangements or point mutations in the noncoding control region (NCCR) of BK polyomavirus (BKPyV) have been associated with higher viral loads and more pronounced organ pathology in immunocompromised patients. The respective alterations affect a multitude of transcription factor binding sites (TFBS) but consistently cause increased expression of the early viral gene region (EVGR) at the expense of late viral gene region (LVGR) expression. By mutating TFBS, we identified three phenotypic groups leading to strong, intermediate, or impaired EVGR expression and corresponding BKPyV replication. Unexpectedly, Sp1 TFBS mutants either activated or inhibited EVGR expression when located proximal to the LVGR *(sp1-4)* or the EVGR *(sp1-2)*, respectively. We now demonstrate that the bidirectional balance of EVGR and LVGR expression is dependent on affinity, strand orientation, and the number of Sp1 sites. Swapping the LVGR-proximal high-affinity *SP1-4* with the EVGR-proximal low-affinity *SP1-2* in site strand flipping or inserting an additional *SP1-2* site caused a rearranged NCCR phenotype of increased EVGR expression and faster BKPyV replication. The 5= rapid amplification of cDNA ends revealed an imperfect symmetry between the EVGR- and LVGR-proximal parts of the NCCR, consisting of *TATA* and *TATA*-like elements, initiator elements, and downstream promoter elements. Mutation or deletion of the archetypal LVGR promoter, which is found in activated NCCR variants, abrogated LVGR expression, which could be restored by providing large T antigen (LTag) in *trans*. Thus, whereas Sp1 sites control the initial EVGR-LVGR expression balance, LTag expression can override inactivation of the LVGR promoter and acts as a key driver of LVGR expression independently of the Sp1 sites and core promoter elements.

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Receptor interaction profiles of novel psychoactive tryptamines compared with classic hallucinogens

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Abstract

The present study investigated interactions between the novel psychoactive tryptamines DiPT, 4-OH-DiPT, 4-OH-MET, 5-MeO-AMT, and 5-MeO-MiPT at monoamine receptors and transporters compared with the classic hallucinogens lysergic acid diethylamide (LSD), psilocin, N,N-dimethyltryptamine (DMT), and mescaline. We investigated binding affinities at human monoamine receptors and determined functional serotonin (5-hydroxytryptamine [5-HT]) 5-HT_{2A} and 5-HT_{2B} receptor activation. Binding at and the inhibition of human monoamine uptake transporters and transporter-mediated monoamine release were also determined. All of the novel tryptamines interacted with 5-HT_{2A} receptors and were partial or full 5-HT_{2A} agonists. Binding affinity to the 5-HT_{2A} receptor was lower for all of the tryptamines, including psilocin and DMT, compared with LSD and correlated with the reported psychoactive doses in humans. Several tryptamines, including psilocin, DMT, DiPT, 4-OH-DiPT, and 4-OH-MET, interacted with the serotonin transporter and partially the norepinephrine transporter, similar to 3,4-methylenedioxymethamphetamine but in contrast to LSD and mescaline. LSD but not the tryptamines interacted with adrenergic and dopaminergic receptors. In conclusion, the receptor interaction profiles of the tryptamines predict hallucinogenic effects that are similar to classic serotonergic hallucinogens but also MDMA-like psychoactive properties.

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VEGF receptor-2-specific signaling mediated by VEGF-E induces hemangioma-like lesions in normal and in malignant tissue

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Abstract Viral VEGF-E (ovVEGF-E), a homolog of VEGF-A, was discovered in the genome of Orf virus. Together with VEGF-A, B, C, D, placental growth factor (PlGF) and snake venom VEGF (svVEGF), ovVEGF-E is a member of the VEGF family of potent angiogenesis factors with a bioactivity similar to VEGF-A: it induces proliferation, migration and sprouting of cultured vascular endothelial cells and proliferative lesions in the skin of sheep, goat and man that are characterized by massive capillary proliferation and dilation. These biological functions are mediated exclusively via its interaction with VEGF receptor-2 (VEGFR-2). Here, we have generated transgenic mice specifically expressing ovVEGF-E in β-cells of the endocrine pancreas (Rip1VEGF-E; RVE). RVE mice show an increase in number and size of the islets of Langerhans and a distorted organization of insulin and glucagon-expressing cells. Islet endothelial cells of RVE mice hyper-proliferate and form increased numbers of functional blood vessels. In addition, the formation of disorganized lymphatic vessels and increased immune cell infiltration is observed. Upon crossing RVE single-transgenic mice with Rip1Tag2 (RT2) transgenic mice, a well-studied model of pancreatic β-cell carcinogenesis, double-transgenic mice (RT2;RVE) display hyper-proliferation of endothelial cells resulting in the formation of hemangiomalike lesions. In addition, RT2;RVE mice exhibit activated lymphangiogenesis at the tumor periphery and increased neutrophil and macrophage tumor infiltration and micrometastasis to lymph nodes and lungs. These phenotypes markedly differ from the phenotypes observed with the transgenic expression of the other VEGF family members in β-cells of normal mice and of RT2 mice.

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Identification of Plant-derived Alkaloids with Therapeutic Potential for Myotonic Dystrophy Type I

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Myotonic dystrophy type I (DM1) is a disabling neuromuscular disease with no causal treatment available. This disease is causedbyexpanded CTG trinucleotiderepeats inthe 3' UTR of the dystrophia myotonica protein kinase gene. On the RNAlevel, expanded $(CUG)_n$ repeats form hairpin structures that sequester splicing factors such as muscleblind-like 1 (MBNL1). Lack of available MBNL1 leads to misregulated alternative splicing of many target pre-mRNAs, leading to the multisystemic symptoms in DM1. Many studies aiming to identify small molecules that target the $(CUG)_n$-MBNL1 complex focused on synthetic molecules. In an effort to identify new small molecules that liberate sequestered MBNL1 from $(CUG)_n$ RNA, we focused specifically on small molecules of natural origin. Natural products remain an important source for drugs and play a significant role in providing novel leads and pharmacophores for medicinal chemistry. In a new DM1 mechanism-based biochemical assay,we screened a collection of isolated natural compounds and alibrary of over 2100 extracts from plants and fungal strains. HPLC-based activity profiling in combination with spectroscopic methods were used to identify the active principles in the extracts. The bioactivity of the identified compounds was investigated in a human cell model and in a mouse model of DM1.We identified several alkaloids, including the ß-carboline harmine and the isoquinoline berberine, that ameliorated certain aspects of the DM1 pathology in these models. Alkaloids as a compound class may have potential for drug discovery in other RNA-mediated diseases.

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Role of the JP45-Calsequestrin Complex on Calcium Entry in Slow Twitch Skeletal Muscles

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We exploited a variety of mouse models to assess the roles of JP45-CASQ1 (CASQ, calsequestrin) and JP45-CASQ2 on calcium entry in slow twitch muscles. In flexor digitorum brevis(FDB)fibersisolatedfrom JP45-CASQ1-CASQ2 triple KO mice, calcium transients induced by tetanic stimulation rely on calcium entry via La^{3+}-and nifedipine-sensitive calcium channels. The comparison of excitation-coupled calcium entry (ECCE) between FD-Bfibers from WT, JP45KO, CASQ1KO, CASQ2KO, JP45-CASQ1 double KO, JP45-CASQ2 double KO, and JP45-CASQ1-CASQ2 triple KO shows that ECCE enhancement requires ablation of both CASQs and JP45. Calcium entry activated by ablation of both JP45-CASQ1 and JP45-CASQ2 complexes supports tetanic force development in slow twitch soleus muscles. In addition, we show that CASQs interact with JP45 at Ca^{2+} concentrations similar to those present in the lumen of the sarcoplasmic reticulum at rest, whereas Ca^{2+} concentrations similar to those present in the SR lumen after depolarization-induced calcium release cause the dissociation of JP45 from CASQs. Our results show that the complex JP45-CASQs is a negative regulator of ECCE and that tetanic force development in slow twitch muscles is supported by the dynamic interaction between JP45 and CASQs.

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Generation of a Bone Organ by Human Adipose-Derived Stromal Cells Through Endochondral Ossification

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Abstract

Recapitulation of endochondral ossification (ECO) (i.e., generation of marrow-containing ossicles through a cartilage intermediate) has relevance to develop human organotypic models for bone or hematopoietic cells and to engineer grafts for bone regeneration. Unlike bone marrow-derived stromal cells (also known as bone marrow-derived mesenchymal stromal/stem cells), adipose-derived stromal cells (ASC) have so far failed to form a bone organ by ECO. The goal of the present study was to assess whether priming human ASC to a defined stage of chondrogenesis in vitro allows their autonomous ECO upon ectopic implantation. ASC were cultured either as micromass pellets or into collagen sponges in chondrogenic medium containing transforming growth factor-β3 and bone morphogenetic protein-6 for 4 weeks (early hypertrophic templates) or for two additional weeks in medium supplemented with β-glycerophosphate, L-thyroxin, and interleukin1-β to induce hypertrophic maturation (late hypertrophic templates). Constructs were implanted in vivo and analyzed after 8 weeks. In vitro, ASC deposited cartilaginous matrix positive for glycosaminoglycans, type II collagen, and Indian hedgehog. Hypertrophic maturation induced upregulation of type X collagen, bone sialoprotein, and matrix metalloproteinase13 (MMP13). In vivo, both early and late hypertrophic templates underwent cartilage remodeling, as assessed by MMP13-and tartrate-resistant acid phosphatase-positive staining, and developed bone ossicles, including bone marrow elements, although to variable degrees of efficiency. In situ hybridization for human-specific sequences and staining with a human specific anti-CD146 antibody demonstrated the direct contribution of ASC to bone and stromal tissue formation. In conclusion, despite their debated skeletal progenitor nature, human ASC can generate bone organs through ECO when suitably primed in vitro.

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The MNK-1/eIF4E pathway as a new therapeutic pathway to target inflammation and remodelling in asthma

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Abstract

Therapeutic targets in asthma are reduction of airway inflammation and remodelling, the latter is not affected by available drugs. Here we present data that inhibition of MAPK-activated protein kinase (MNK)-1 reduces inflammation and remodelling. MNK-1 regulates protein expression by controlling mRNA stability, nuclear export and translation through the eukaryotic initiation factor 4E (eIF4E). Airway smooth muscle cells were derived from asthmatic and non-asthmatic donors. Cells were pretreated with CGP57380 (MNK-1 inhibitor) or MNK-1 siRNA, before TNF-α stimulation. Cytokine and protein expression was analysed by ELISA, real time PCR and immunoblotting. Proliferation was monitored by cell counts. TNF-α activated MNK-1 phosphorylation between 15 and 30 min. and subsequently eIF4E between 15 and 60 min. EIF4E activity was inhibited by CGP57380 dose-dependently. Inhibition of MNK-1 by CGP57380 or MNK-1 siRNA significantly reduced TNF-α induced CXCL10 and eotaxin mRNA expression and secretion, but had no effect on IL-8. However, CXCL10 mRNA stability or NF-ϰ B activity were not affected by MNK-1 inhibition. Furthermore, eIF4E was detected in the cytosol and the nucleus, but TNF-α did not affected its export from the nucleus. Cytokine array assessment showed that in addition to eotaxin and CXCL10, asthma relevant GRO α and RANTES were down-regulated by MNK-1 inhibition. In addition, MNK-1 inhibition significantly reduced FCS and PDGF-BB induced cell proliferation. We are the first to report that MNK-1 controls chemokine secretion and proliferation in human airway smooth muscle cells. Therefore we suggest that MNK-1 inhibition may present a new target to limit inflammation and remodelling in asthmatic airways.

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Frontiers in Physiology | frontiers in Physiology | August 2016, Vol. 7, Art. 345 | IF 4,031

Impaired Exercise Performance and Skeletal Muscle Mitochondrial Function in Rats with Secondary Carnitine Deficiency

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Purpose: The effects of carnitine depletion upon exercise performance and skeletal muscle mitochondrial function remain largely unexplored. We therefore investigated the effect of N-trimethyl-hydrazine-3-propionate (THP), a carnitine analog inhibiting carnitine biosynthesis and renal carnitine reabsorption, on physical performance and skeletal muscle mitochondrial function in rats.

Methods: Male Sprague Dawley rats were treated daily with water (control rats; n = 12) or with 20 mg/100 g body weight THP (*n* = 12) via oral gavage for 3 weeks. Following treatment, half of the animals of each group performed an exercise test until exhaustion.

Results: Distance covered and exercise performance were lower in THP-treated compared to control rats. In the oxidative soleus muscle, carnitine depletion caused atrophy (−24%) and impaired function of complex II and IV of the mitochondrial electron transport chain. The free radical leak (ROS production relative to oxygen consumption) was increased and the cellular glutathione pool decreased. Moreover, mRNA expression of markers of mitochondrial biogenesis and mitochondrial DNA were decreased in THP-treated compared to control rats. In comparison, in the glycolytic gastrocnemius muscle, carnitine depletion was associated with impaired function of complex IV and increased free radical leak, whilst muscle weight and cellular glutathione pool were maintained. Markers of mitochondrial proliferation and mitochondrial DNA were unaffected.

Conclusions: Carnitine deficiency is associated with impaired exercise capacity in rats treated with THP. THP-induced carnitine deficiency is associated with impaired function of the electron transport chain in oxidative and glycolytic muscle as well as with atrophy and decreased mitochondrial DNA in oxidative muscle.

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Arthritis Research & Therapy (2016) 18:125 IF 3,979

Notochordal cell conditioned medium (NCCM) regenerates end-stage human osteoarthritic articular chondrocytes and promotes a healthy phenotype

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Abstract

Background: Notochordal cell conditioned medium (NCCM) derived from non-chondrodystrophic dogs has pro-anabolic and anti-catabolic effects upon nucleus pulposus (NP) cells. Here, for the first time, we assessed the ability of NCCM to influence the production of extracellular matrix and inflammatory proteins by healthy and osteoarthritic human chondrocytes within engineered cartilage tissues. We hypothesized that, similar to its action on NP cells, NCCM exerts metabolic and anti-catabolic effects on human articular chondrocytes and has the potential to significantly counteract inflammatory mediators.

Methods: Chondrocytes from nine non-osteoarthritic patients and from six osteoarthritic (OA) donors at the time of total knee arthroplasty were chondro-differentiated in pellets for 2 weeks. Non-OA pellets were exposed for 72 hours to IL-1β/TNF-α and then cultured up to 14 days in 2 % FBS-supplemented NCCM or 2 % FBS-supplemented medium (control (ctr)). OA pellets were cultured in NCCM or ctr medium without pro-inflammatory treatment. Tissues after each culture phase were analyzed biochemically (GAG/DNA), (immuno-) histologically (collagen I, II and GAG) and by Western blotting. Supernatants were analyzed by ELISA.

Results: Response to NCCM was age and disease dependent with healthy chondrocyte pellets (from donors >55 years of age) recovering their glycosaminoglycan (GAG) contents to baseline levels only with NCCM. OA pellets treated with NCCM significantly increased GAG content (1.8-fold) and levels of hyaluronic acid link protein (HAPLN), fibromodulin and SOX-9. The catabolic proteins (matrix metalloproteinase (MMP)-3 and MMP-13) and pro-inflammatory enzyme levels (cyclooxygenase-2 (COX-2)) were markedly reduced and there was significantly reduced secretion of pro-inflammatory chemokines (IL-6 and IL-8).

Conclusions: NCCM restores cartilage matrix production of end-stage human OA chondrocytes towards a healthy phenotype and suppresses the production of inflammatory mediators. Harnessing the necessary and sufficient factors within NCCM that confers chondroprotection and regenerative effects could lead to a minimally invasive agent for treatment of degenerative and inflammatory joint diseases.

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Solvent-facilitated lead disconnection for battery replacement in patients with pacemakers or implantable cardioverter defibrillators

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Aims Battery exchange in pacemaker (PM) or implantable cardioverter defibrillator (ICD) devices may be occasionally problematic because of difficulties in lead disconnection procedures and risk of injuring the fragile leads. This pilot study compares ethanol and dimethyl sulfoxide (DMSO) as solvents to assist removal of leads from PM or ICD device headers in cases of stuck leads or difficulties in untightening device header screws.

Methods and results Of the total number (527) of our patients requiring battery replacement due to end-of-life (EOL) warnings, conventional exchange was not possible in 34 (6.5%) due to embedding of the lead within blood-derived material. Of these, 30 (17 with PM, 13 with ICD) consented to the study and were randomly assigned to a primary attempt at lead disconnection by ethanol ($n = 17$) or by DMSO ($n = 13$). If the primary attempt failed, a secondary attempt at lead disconnection was undertaken using the alternate solvent. Ethanol was a superior solvent compared with DMSO, yielding successful disconnection at primary attempt in 88.2% (15/17) vs. 23.1% (3/13) of cases. In 8 patients in whom the primary DMSO-attempted disconnection failed, a secondary attempt with ethanol yielded success in 6 (75%) cases. Use of either ethanol or DMSO in lead disconnection was not associated with any adverse events or effects.

Conclusion Ethanol has utility as a simple and inexpensive modality for lead disconnection from ICD or PM headers.

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HIV Medicine (2016), 17, 615–622 IF 3,341

Correlating HIV tropism with immunological response under combination antiretroviral therapy

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Objectives
A significant percentage of patients infected with HIV-1 experience only suboptimal CD4 cell recovery while treated with combination therapy (cART). It is still unclear whether viral properties such as cell tropism play a major role in this incomplete immune response. This study therefore intended to follow the tropism evolution of the HIV-1 envelope during periods of suppressive cART.

Methods
Viruses from two distinct patient groups, one with good and another one with poor CD4 recovery after 5 years of suppressive cART, were genotypically analysed for viral tropism at baseline and at the end of the study period.

Results
Patients with CCR5-tropic CC-motif chemokine receptor 5 viruses at baseline tended to maintain this tropism to the study end. Patients who had a CXCR4-tropic CXC-motif chemokine receptor 4 virus at baseline were overrepresented in the poor CD4 recovery group. Overall, however, the majority of patients presented with CCR5-tropic viruses at follow-up.

Conclusions
Our data lend support to the hypothesis that tropism determination can be used as a parameter for disease progression even if analysed long before the establishment of a poorer immune response. Moreover, the lasting predominating CCR5-tropism during periods of full viral control suggests the involvement of cellular mechanisms that preferentially reduce CXCR4-tropic viruses during cART.

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DNA Repair 44 (2016) 92–102 IF 3,111

Active DNA demethylation by DNA repair: Facts and uncertainties

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Abstract
Pathways that control and modulate DNA methylation patterning in mammalian cells were poorly understood for a long time, although their importance in establishing and maintaining cell type-specific gene expression was well recognized. The discovery of proteins capable of converting 5-methylcytosine (5mC) to putative substrates for DNA repair introduced a novel and exciting conceptual framework for the investigation and ultimate discovery of molecular mechanisms of DNA demethylation. Against the prevailing notion that DNA methylation is a static epigenetic mark, it turned out to be dynamic and distinct mechanisms appear to have evolved to effect global and locus-specific DNA demethylation. There is compelling evidence that DNA repair, in particular base excision repair, contributes significantly to the turnover of 5mC in cells. By actively demethylating DNA, DNA repair supports the developmental establishment as well as the maintenance of DNA methylation landscapes and gene expression patterns. Yet, while the biochemical pathways are relatively well-established and reviewed, the biological context, function and regulation of DNA repair-mediated active DNA demethylation remains uncertain. In this review, we will thus summarize and critically discuss the evidence that associates active DNA demethylation by DNA repair with specific functional contexts including the DNA methylation erasure in the early embryo, the control of pluripotency and cellular differentiation, the maintenance of cell identity, and the nuclear reprogramming.

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Ann Hematol Springer (2016) 95:1627–1636 IF 3,022

Anti-thymocyte globulin-induced hyperbilirubinemia in patients with myelofibrosis undergoing allogeneic hematopoietic cell transplantation

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Abstract Allogeneic hematopoietic cell transplantation (allo-HCT) remains the only curative treatment option for myelofibrosis (MF) despite the emergence of novel targeted therapies. To reduce graft rejection and graft-versus-host disease (GvHD), current allo-HCT protocols often include in vivo T lymphocyte depletion using polyclonal anti-thymocyte globulin (ATG). Shortly after ATG administration, an immediate inflammatory response with fever, chills, and laboratory alterations such as cytopenias, elevation of serum C-reactive protein, bilirubin, and transaminases can develop. Here, we explore whether MF patients, who commonly exhibit extramedullary hematopoiesis in the liver, might be particularly susceptible to ATG-induced liver toxicity. To test this hypothesis, we analyzed 130 control and 94 MF patients from three transplant centers treated with or without ATG during the allo-HCT conditioning regimen. Indeed, hyperbilirubinemia was found in nearly every MF patient treated with ATG (MF-ATG 54/60 = 90 %) as compared to non-ATG treated MF (MF-noATG 15/34 = 44.1 %, $p < 0.001$) and respectively ATG-treated non-MF patients of the control group (control-ATG, 43/77 = 56 %, $p < 0.001$). In contrast, transaminases were only inconsistently elevated. Hyperbilirubinemia was in most cases self-limiting and not predictive of increased incidence of non-relapse mortality, hepatic sinusoidal obstruction syndrome (SOS) or liver GvHD. In sum, awareness of this stereotypic bilirubin elevation in MF patients treated with ATG provides a relatively benign explanation for hyperbilirubinemia occurring in these patients during the early transplant. However, attention to drug levels of biliary excreted drugs is warranted, since altered bile flow may influence their clearance and enhance toxicity (e.g., busulfan, antifungal agents).

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Expression of Leukemia-Associated Nup98 Fusion Proteins Generates an Aberrant Nuclear Envelope Phenotype

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Abstract
Chromosomal translocations involving the nucleoporin *NUP98* have been described in several hematopoietic malignancies, in particular acute myeloid leukemia (AML). In the resulting chimeric proteins, Nup98's N-terminal region is fused to the C-terminal region of about 30 different partners, including homeodomain (HD) transcription factors. While transcriptional targets of distinct Nup98 chimeras related to immortalization are relatively well described, little is known about other potential cellular effects of these fusion proteins. By comparing the sub-nuclear localization of a large number of Nup98 fusions with HD and non-HD partners throughout the cell cycle we found that while all Nup98 chimeras were nuclear during interphase, only Nup98-HD fusion proteins exhibited a characteristic speckled appearance. During mitosis, only Nup98-HD fusions were concentrated on chromosomes. Despite the difference in localization, all tested Nup98 chimera provoked morphological alterations in the nuclear envelope (NE), in particular affecting the nuclear lamina and the lamina-associated polypeptide 2α (LAP2α). Importantly, such aberrations were not only observed in transiently transfected HeLa cells but also in mouse bone marrow cells immortalized by Nup98 fusions and in cells derived from leukemia patients harboring Nup98 fusions. Our findings unravel Nup98 fusion-associated NE alterations that may contribute to leukemogenesis.

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Behavioural Brain Research | Behavioural Brain Research | 317 (2017) 393–400 | IF 3,002

Behavioural endophenotypes in mice lacking the auxiliary $GABA_B$ receptor subunit KCTD16

Flurin Cathomas[a,b], Hannes Sigrist[a], Luca Schmid[a], Erich Seifritz[b,d], Martin Gassmann[c], Bernhard Bettler[c], Christopher R. Pryce[a,d]

Abstract

Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the brain and is implicated in the pathophysiology of a number of neuropsychiatric disorders. The $GABA_B$ receptors are G-protein coupled receptors consisting of principle subunits and auxiliary potassium channel tetramerization domain (KCTD) subunits. The KCTD subunits 8, 12, 12b and 16 are cytosolic proteins that determine the kinetics of the $GABA_B$ receptor response. Previously, we demonstrated that *Kctd12* null mutant mice (*Kctd12*$^{-/-}$) exhibit increased auditory fear learning and that *Kctd12*$^{+/-}$ mice show altered circadian activity, as well as increased intrinsic excitability in hippocampal pyramidal neurons. KCTD16 has been demonstrated to influence neuronal excitability by regulating $GABA_B$ receptor-mediated gating of postsynaptic ion channels. In the present study we investigated for behavioural endophenotypes in *Kctd16*$^{-/-}$ and *Kctd16*$^{+/-}$ mice. Compared with wild-type (WT) littermates, auditory and contextual fear conditioning were normal in both *Kctd16*$^{-/-}$ and *Kctd16*$^{+/-}$ mice. When fear memory was tested on the following day, *Kctd16*$^{-/-}$ mice exhibited less extinction of auditory fear memory relative to WT and *Kctd16*$^{+/-}$ mice, as well as more contextual fear memory relative to WT and, in particular, *Kctd16*$^{+/-}$ mice. Relative to WT, both *Kctd16*$^{+/-}$ and *Kctd16*$^{-/-}$ mice exhibited normal circadian activity. This study adds to the evidence that auxillary KCTD subunits of $GABA_B$ receptors contribute to the regulation of behaviours that could constitute endophenotypes for hyper-reactivity to aversive stimuli in neuropsychiatric disorders.

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Natalizumab-induced POU2AF1/Spi-B upregulation A possible route for PML development

Maria Meira[1], PhD; Claudia Sievers[1], PhD; Francine Hoffmann[1], BSc; Aiden Haghikia[2], MD; Maria Rasenack[1], MD; Bernhard F. Décard[1], MD; Jens Kuhle[1], MD; Tobias Derfuss[1], MD; Ludwig Kappos[1], MD; Raija L.P. Lindberg[1], PhD

Abstract

Objectives: To assess messenger RNA (mRNA) expression of POU2AF1 and Spi-B and their potential regulatory microRNAs (miRNAs) in natalizumab-treated patients with multiple sclerosis and in therapy-associated progressive multifocal leukoencephalopathy (PML).

Methods: Expression of POU2AF1/Spi-B was analyzed by using real-time reverse transcription PCR assays on isolated B/$CD8^+$ T lymphocytes and peripheral blood mononuclear cells (PBMCs) from cohorts of untreated and natalizumab-treated patients with and without PML. Longitudinal expression analysis was performed on $CD4^+$, $CD8^+$ T and B cells from 14 patients who interrupted natalizumab therapy for 8 weeks. The miRNA profiling was conducted in PBMCs from 5 untreated and 5 natalizumab-treated patients using low-density arrays followed by validation with single miRNAs assays in untreated and natalizumab-treated patients.

Results: POU2AF1 and Spi-B mRNAs were upregulated in B and CD81 T cells from natalizumabtreated patients, which was validated in PBMCs from different cohorts of natalizumab-treated patients with and without PML, with a noteworthy higher expression of Spi-B in patients with PML. In contrast, downregulation of POU2AF1/Spi-B expression was measured in B and CD81 T cells after natalizumab discontinuation. Seventeen differentially expressed miRNAs including miR-10b, a regulator of POU2AF1 mRNA, were identified in long-term natalizumab-treated patients compared with untreated ones.

Conclusions: Upregulation of POU2AF1 and Spi-B, known transactivators of the JC virus, the causative agent for PML, and its association with occurrence of PML in natalizumab-treated patients, corroborates POU2AF1/Spi-B as potential biomarkers for PML risk, which merits further evaluation.

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Interferon-driven deletion of antiviral B cells at the onset of chronic infection

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Inadequate antibody responses and perturbed B cell compartments represent hallmarks of persistent microbial infections, but the mechanisms whereby persisting pathogens suppress humoral immunity remain poorly defined. Using adoptive transfer experiments in the context of a chronic lymphocytic choriomeningitis virus infection of mice, we have documented rapid depletion of virus-specific B cells that coincided with the early type I interferon (IFN-I) response to infection. We found that the loss of activated B cells was driven by IFN-I signaling to several cell types including dendritic cells, T cells, and myeloid cells. This process was independent of B cell–intrinsic IFN-I sensing and resulted from biased differentiation of naïve B cells into short-lived antibodysecreting cells. The ability to generate robust B cell responses was restored upon IFN-I receptor blockade or, partially, when experimentally depleting myeloid cells or the IFN-I–induced cytokines interleukin-10 and tumor necrosis factor–α. We have termed this IFN-I–driven depletion of B cells "Bcell decimation." Strategies to counter Bcell decimation shouldthus help us better leverage humoral immunity in the combat against persistent microbial diseases.

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REVIEWS

Immunity | Immunity | 45, August 16, 2016

Complement-Mediated Regulation of Metabolism and Basic Cellular Processes

Christoph Hess[1,*], and Claudia Kemper[2,3]

Complement is well appreciated as a critical arm of innate immunity. It is required for the removal of invading pathogens and works by directly destroying them through the activation of innate and adaptive immune cells. However, complement activation and function is not confined to the extracellular space but also occurs within cells. Recent work indicates that complement activation regulates key metabolic pathways and thus can impact fundamental cellular processes, such as survival, proliferation, and autophagy. Newly identified functions of complement include a key role in shaping metabolic reprogramming, which underlies T cell effector differentiation, and a role as a nexus for interactions with other effector systems, in particular the inflammasome and Notch transcription-factor networks. This review focuses on the contributions of complement to basic processes of the cell, in particular the integration of complement with cellular metabolism and the potential implications in infection and other disease settings.

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Herzlich willkommen, allerseits!

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Herzlich willkommen, allerseits!

Ava und Zélie Develioglu
Geboren am 05.10.2016

Christmas in Slovakia

Vianoce (Christmas in Slovak) is the biggest family event of the year. It is driven very much by Catholic tradition and despite the globalization of this wonderful feast, Christmas in Slovakia is still very traditional, especially in rural areas.

While there is quite some overlap with the neighbouring countries going back to the times Slovakia was part of Austrian-Hungarian empire in the 19th century, some traditions are nevertheless, I believe, special to our country and I will guide you through them in the next few paragraphs.

Advent time

Everything starts four weeks before Christmas, when Advent begins. During this time we prepare an advent wreath with 4 candles and every Sunday one of these is lit. The last one is lit on the last Sunday before Christmas Eve.

This is also a time, when Christmas markets open their doors for visitors to enjoy the Christmas mood, meet with friends, eat good food and drink mulled wine or punch. The largest and most beautiful one is localized on the Main square in our capital, Bratislava (Fig.1).

One of the traditional food available is a sort of potato pancakes called *"lokše"*, which we usually enjoy with roasted goose or with a duck liver filling. The other specialty is a pork or chicken *"cigánska"*, a fried piece of meat served in a toasted bun with sautéed onions and mustard.

The most important day for kids in the pre-Christmas period is the 6th of December, the day of St. Nicholas. On the night of the 5th of December, children clean their shoes and put them in the windows, for St. Nicholas to bring them sweets. Usually, little things are given, the custom was always: chocolate, candy and fruits and to the bad children a piece of coal, an onion or a potato.

The rest of the Advent is spent organizing the presents and baking lots of different sort of Christmas cookies, the most famous one being *"medovníčky"*, a special kind of gingerbread (Fig.2), the recipe for which you can find at the end of the article. In every family it is quite normal to bake for days and make lots of cookies with different shapes, fillings and decoration, and also very traditional ones with poppy seeds or nut fillings. All of this puts quite a heavy burden on the female members of every family :).

Christmas preparations

Then, Christmas arrives. Christmas Eve, on Decem-

Fig. 1

Fig. 2

ber 24th, is the most important day in the Christmas period for us. It is called ***"Štedrý deň"*** (generous day) and it has a reason – we get our presents on this day!

In Slovakia, children are told that gifts are brought by *"Ježiško"* (Baby Jesus or Christkind) to whom they write letters with their Christmas wishes.
During the day, we eat very little, usually only a soup made from dried mushrooms as the story is that if you fast all day then you will see the golden piglet in the evening :o). The real reason is that the Christmas Eve dinner consists of many different courses and you should have lots of space ready for it :).

Usually on this day we also put up the tree and decorate it. This tradition comes from the times when the tree would be brought from the forest. In those days you want it as fresh and green as possible for Christmas day, so you did not put it up very early. The traditional tree decorations are made from wood and dried corn leaves called *"šúpolie"* (Fig.3).

Christmas Eve
First, we put gifts under the tree without the children seeing (when I was a kid, we used to go with my brother and grandfather for loooong walks, so others could arrange the gifts) and then when everything is ready, the oldest man of the family rings the bell, meaning Ježiško has already delivered the gifts. The whole family then assembles around the Christmas tree and sings Holy Night, Silent Night (*Tichá noc, svätá noc* in Slovak). The whole family then moves to the Christmas table and there follows the longest 2-hour-period in a child's life as they are generally not allowed to open the gifts before the dinner is finished.

Christmas dinner
We usually include a few rituals into the Christmas dinner:

Walnuts - these are thrown into every corner of the house right after dinner to ensure abundance of food for the following year.

An extra plate - It is traditional to lay down one more place at the table, so if a beggar should come calling you can invite him to join.

An apple cut in the middle – if the cut revealed a star-like shape, it means happiness and health. This apple is then served to everyone at the table – this symbolizes the unity of the family.

Fig. 3

Fig. 4

Wafers – these are very thin, like a communion wafer, but as big as a dessert plate. We each put some honey, bits of walnuts on our wafter before stick another on top. The people at the table should then take a bite from each other's' wafers – this will ensure that they will love each other for the next year.

The first real course is soup – it is usually a soured cabbage soup called *"kapustnica"* with sour cream, dried mushrooms, chorizo-like sausage and smoked ham. We eat it with bread, which symbolizes plenitude for next year.

Then the main course is fish. This is a fresh water local carp. In Slovakia they are sold alive on the streets from big tanks and pools during the week before Christmas. To the great discomfort of adults and great enjoyment of children many people keep theirs alive at home in the bath till Christmas Eve in order to have it really fresh and to get rid of the possible muddy taste (Fig.4). We also used to have a carp in our bath at least for two days and I would spend hours in that bathroom with my brother, name the fish and talk to it.

For the dinner, the fish meat is cut into horse-shoe shapes for good luck (everything has a mystery in Slovakia). We coat it in flour, egg and breadcrumbs and then fry it. We eat potato salad with the fish. Depending on the region this can be either a mayonnaise salad with carrots, peas and more, or a simple vinegar potato salad with onions.

Finally, after the dinner, we all assemble around Christmas tree again and finally are allowed to open all of the Christmas gifts!

Later on, we go to church for midnight mass. This is a very special mass, with a unique atmosphere, since the lights are turned-off and the whole church is usually only lit by candlelight.

Christmas Day and Boxing Day

Since we usually return home quite late from midnight mass, we get to bed around 2-3am. That's why on the next day everybody sleeps in and we have a very late rich breakfast, sort of brunch style :). Kids are allowed to stay in their pyjamas and watch fairy tales. On this day families stay together; children play with new toys. We then have an early dinner – usually pork escalope.

On December 26th, Boxing Day, we usually visit our relatives and friends.

Jakub Zmajkovic

Medovníčky recipe

Ingredients

- ½ cup (125 g) butter
- ½ cup + 1 Tbsp (180 g) honey
- 1¾ cup (220 g) icing sugar
- 3 eggs
- 1½ tsp baking soda
- 3 tsp medovniky spice mix
- (cinnamon, cloves, cardamom, anise, allspice, coriander, nutmeg)
- 5 cups (600 g) plain flour
- 1 egg

Instructions

1 Heat the butter, honey, and sugar in a small pot until just melted, stirring constantly.

2 While warm, but not too hot (so you don't cook the eggs), add the eggs and mix.

3 In a bowl mix the dry ingredients and pour in the warm egg mixture. Mix the dough until incorporated. The dough will be somewhat sticky.

4 Cover and cool in a cold place (the fridge) for a few hours or overnight.

5 Roll out the dough about 4mm (1/64 inch) thick, using only enough flour to keep the dough from sticking. Cut out with desired cookie cutters. Transfer to a greased or lined cookie sheet.

6 Beat an egg and with a pastry brush brush the cookies with the egg.

7 Bake at 180C (355F) for about 10 min, or until golden.

8 When cool, store in an airtight container for a few days (or practically forever).

Leave plain or decorate with your favourite icing (I mix egg whites with icing sugar to make mine). Classic medovníky are often decorated only in white, with lots of curls and dots.

"What Is Hanukkah?"

Hanukkah (also written Chanukah) is a Jewish holiday celebrated for eight days and nights, and symbolizes the victory of the Maccabees on the Seleucid Empire during second Temple (Holy Temple) period. The Hanukkah celebration start on the eve of the 25th of the Jewish month "Kislev" according to the Hebrew calendar, and this year it is celebrated over the same days as Christmas and the New Year, from the 25th of December to January 1st. Hanukkah is the Festival of Lights, celebrating the triumph of light over darkness, of purity over adulteration, and of spirituality over materiality.

Hanukkah story

More than twenty-one centuries ago, the Holy Land was ruled by the Seleucids (Syrian-Greeks), who sought to forcefully Hellenize the people of Israel. Antiochus IV, the leader of the Seleucids was in control of the region. He began to oppress the Jews severely, placing a Hellenistic priest in the Temple, massacring Jews, prohibiting the practice of the Jewish religion, and desecrating the Temple by requiring the sacrifice of a non-kosher animal on the altar. Two groups opposed Antiochus: a basically nationalistic group led by Mattathias the Hasmonean and his son Judah Maccabee, and a religious traditionalist group known as the Chasidim, the forerunners of the Pharisees. Both groups joined forces in a revolt against both the assimilation of the Hellenistic Jews and oppression by the Seleucid Greek government. Their leading quote was "Let us fight unto death in defense of our souls and our Temple!", and indeed against all odds, and follow a series of difficult battles the war was won. Judah and the Maccabees drove the Greeks away from the land, reclaimed the Holy Temple in Jerusalem and rededicated it to the service of God. On the twenty-fifth of the month of Kislev, in the year 3622, Judah and the Maccabees built a new altar for the service of God. Since the golden Temple's menorah (a seven branched candelabrum) had been stolen by the Greeks, the Maccabees made one of cheaper metal, but when they sought to light it, they found only a single pot of olive oil that had miraculously escaped contamination by the Greeks, but it was only sufficient to light for one day.

By a miracle of God, the menorah continued to burn for eight days, till new oil was made available. This miracle proved God's protection of the Jews and, in memory of this, our sages appointed these eight days and nights for annual thanksgiving and for lighting candles.

Many people define the major Jewish holidays as those that feature traditional holiday meals, Kiddush (blessing of the wine), holiday candle-lighting, etc. Only biblical holidays such as Rosh Hashanah (Jewish New Year), Yom Kippur, Sukkot, Passover and Shavuot fit these criteria.

Hanukkah was instituted some two centuries after the Bible was completed and canonized. Nevertheless, it is traditionally celebrated in a major and very public fashion. The requirement to position the menorah, also referred to as Hanukkiah, at the window or in front of the door symbolizes the desire to

give the Hanukkah miracle a high-profile, and lightning of the candles gives the holiday its religious observance. The candles are arranged in the Hanukkiah, which holds nine candles, so that there is one for each night, plus a Shamash (servant) which is positioned at a different height. On the first night, one candle is placed at the far right. The Shamash candle is lit and three blessings are recited. After reciting the blessings, the first candle is then lit using the Shamash candle, and the Shamash candle is placed in its holder. Each night, another candle is added from right to left (like the Hebrew language), and on Shabbat, Hanukkah candles are lit before the Shabbat candles. On the eighth night, all nine candles (the 8 Hanukkah candles and the Shamash) are lit.

Hanukkah customs

Hanukkah customs include eating foods fried in oil, such as Latkes and Sufganiot (see recipes below), because of the significance of oil to the holiday.

Playing with the dreidel is traditional at this time. The dreidel or Sevivon is a spinning top on which are inscribed the Hebrew letters *Nun, Gimmel, Hei* and *Pe*, an acronym for *Nes Gadol Hayah Poh*, "a great miracle happened here", referring to the miracle of oil. Outside Israel, the fourth letter on the dreidel is usually Shin, for the Hebrew word Sham ("there" [i.e. in the Holy Land]). The letters also stand for the Yiddish words **n**it (nothing), **g**anz (all), **h**alb (half) and **s**htell (put), which are the rules of the dreidel game. One variation of playing the dreidel is that everyone puts in one coin. A person spins the dreidel, if it lands on *Nun* nothing happens, if it lands on *Gimel* you get the all pot, if it lands on *Hei* you get half of the pot, and if it lands on Shin you put one in. when the pot is empty everybody puts one in and play continues until one person has everything, after which the pot is redivided so that everyone can play all over again.

Another unifying custom of the holiday is the singing of Hanukkah songs after the candle lightning. A large number of songs have been written on Hanukkah themes, some of the best known are "*Ma'oz Tzur"* (Rock of Ages), *"Hanukkiah Li Yesh*" ("I Have a Hanukkah Menorah"), *"Kad Katan*" ("A Small pot"), *"S'vivon Sov Sov Sov*" ("Dreidel, Spin and Spin") and more. Another custom refers to the giving of Hanukkah gelt (Yiddish for "money") known as *"Dmei Chanukah*" in Israel, which is a gift of a small amount of money given to the children. All of those customs represent the spirit and the symbolization of the holiday.

Hanukkah recipes

Sufganiyot—jelly doughnuts recipe:

Ingredients:

* 4½ tsp. dry yeast
* ¼ cup warm water
* 1½ cups of slightly warm milk
* ¾ cup sugar
* 1 tsp. salt
* 2 eggs
* 6 tbsp. margarine, melted
* 6 cups flour
* Oil for frying
* Confectioners' (icing) sugar for dusting
* Cranberry or strawberry jam for filling.

Directions:

1. Place yeast, warm water and 1 tsp. sugar in bowl. Let sit for 10 minutes until bubbled.
2. Mix yeast mixture, sugar, margarine, eggs, salt, and milk with 2 cups of flour on a low speed.

3. Slowly add in the rest of the flour until dough is no longer sticky.
4. Knead for 5 minutes, then cover the bowl with a damp cloth and let rise approximately 1 hour, until dough has doubled in size.
5. Roll out the dough approximately ½ inch thick, cut circles, and let rise 30 minutes.
6. Heat oil in a frying pan or pot to around 370F (185C). Drop in a few doughnuts at a time. Flip each doughnut so each side browns.
7. Remove from oil and drain on a paper towel.
8. Doughnut assembly: use a sharp knife or pointed spatula to poke a small hole in the side of each doughnut. Fill a Ziploc bag with ½ cup of the jam, and cut a small hole at the corner. Stick the Ziploc corner into the hole and squirt out approximately 1 tsp. jam into each doughnut. Using a fine-mesh strainer, sprinkle confectioners' (icing) sugar over the top of each doughnut.

Eat and enjoy!

Hanukkah Potato Latkes recipe

Ingredients:

* 4½ tsp. dry yeast
* ½ an onion
* 2 tbsp. oil
* 3 tsp. salt
* 1.5 lbs. waxy potatoes
* 2 eggs
* ¼ cup flour
* Oil for frying

Directions:

1. Dice the onion and sauté it in 2 tbsp. oil and 1 tsp. salt until golden.
2. Grate the potatoes (by hand or in a food processor). Immediately transfer the grated potato to a bowl of cold water.
3. Place the eggs, flour, fried onion and 2 tsp. salt in a separate bowl. Drain the grated potato well, add it to the rest of the ingredients and mix immediately.
4. Heat 2-4 tbsp. of oil in a frying pan, over medium heat.
5. For uniform latkes, use a 1/4 or 1/8 cup measuring cup. Scoop the batter and gently drop it into the oil. Press down gently with the back of the measuring cup to flatten. Fry 2-3 minutes until golden, then flip the latkes and fry 1-2 minutes on the second side. Repeat until all the mixture has been fried.
6. Possible vegetable alternatives: zucchini (courgette), sweet potato, and carrot.

To sum up, Hanukkah is, in my point of view, a family uniting holiday where everyone gets together, lighting candles, celebrating, singing and eating sweats, and if you look around and listen closely you might even see some Hanukkah lights and hear the voice of the singers.

Wish you all a Happy Hanukkah!

Moran Elbaz

DBM General Assembly

Thursday, December 15, 2016
16:00h
Small Lecture Hall, ZLF

DBM Christmas Party

Thursday,
December 15
17:30h
Centro

Nach Hause kommen, das ist es,
was das Kind von Bethlehem allen schenken will,
die weinen, wachen und wandern auf dieser Erde.

(Friedrich von Bodelschwingh, 1831–1910)