Neutrophil 2022

Mexico City, December 3-6

Mexico City, December 3-6, 2022

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Neutrophil 2022

Mexico City, December 3-6

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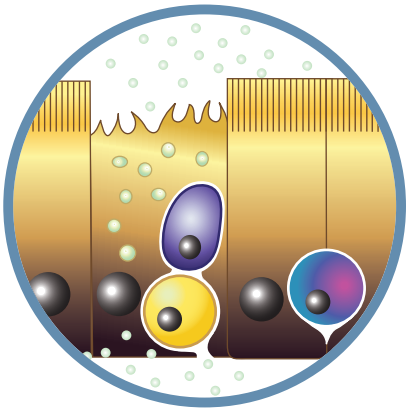

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Neutrophil 2022

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SOCIAL PROGRAM

Welcoming reception and cocktail (December 3, 7:15 pm)

Main courtyard of Palacio de Medicina (old Medicine School of the University of Mexico)
Universidad Nacional Autónoma de México
All registered participants and accompanying guests are invited.
Admission included in registration fees.

Mexican dinner (December 5, 7:30 pm)

Optional. Admission, USD$40/person, paid at registration time. Extra places might be available at registration desk (please inquire).
Location: NH Collection Mexico City Centro Histórico Hotel

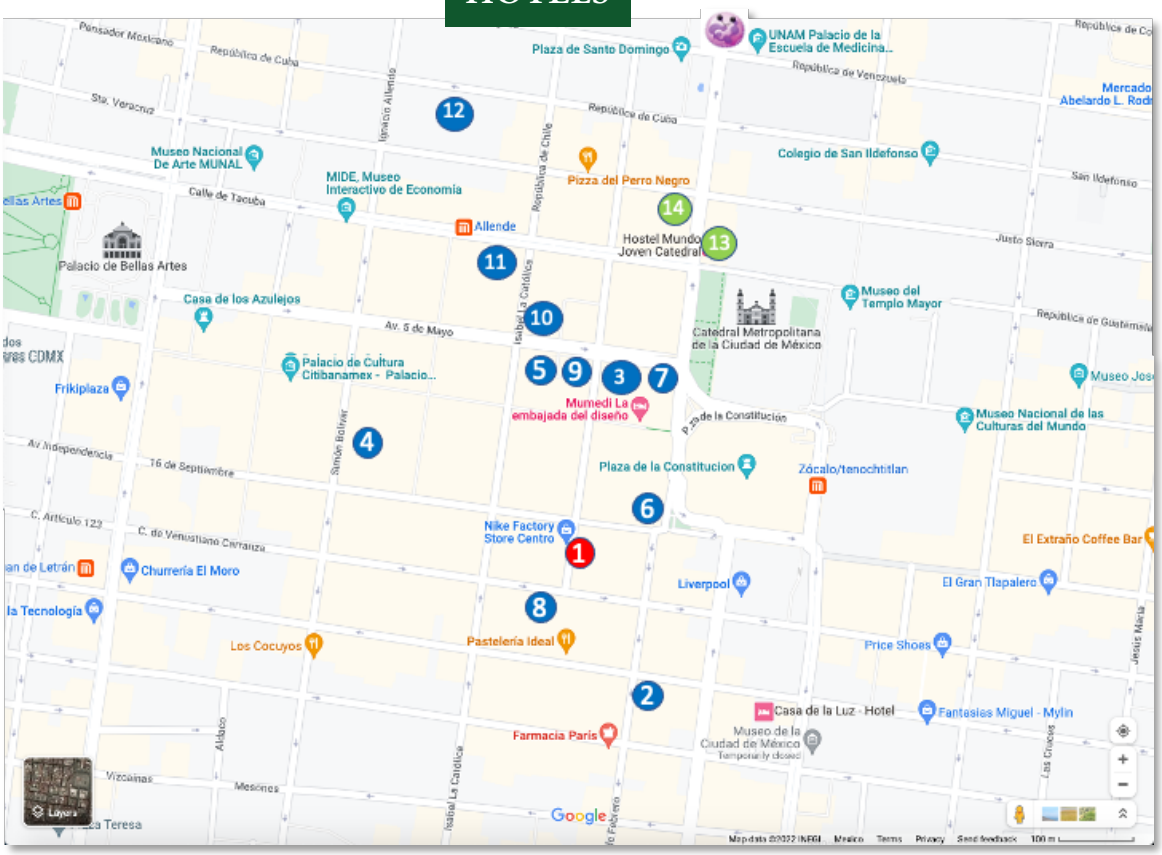
Visit to the pyramids of Teotihuacán (December 7, 8:00 am to 6:00 pm)

Optional organized guided tour to the pyramids, including transportation, entrance, guide and lunch
Registration required, USD $150/person, paid at registration time.
You can add to this optional trip a hot-air balloon ride (about one-hour) over the pyramids, total cost USD $300/person.

Neutrophil 2022

Mexico City, December 3-6

HOTELS



1) Hotel NH Collection Mexico City Centro Histórico (Headquarters hotel)
2) Hampton Inn & Suites Mexico City - Centro Historico
3) Zócalo Central Hotel
4) Histórico Cental
5) Hotel Canadá
6) Gran Hotel Ciudad de México
7) Best Western Hotel Majestic
8) Umbral, Curio Collection by Hilton
9) Hotel MX Zócalo
10) Hotel Rioja
11) Hotel Gillow
12) Hotel Cuba

Youth Hostels

13) Mundo Joven Hostel Catedral
14) Mexico City Hostel

Neutrophil 2022

Mexico City, December 3-6

Good places to get dinner

1) **The Terrace** - Francisco Madero 73; Inside the Best Western Majestic Hotel
2) **Cocina Central** - Plaza de la Constitución 13; Third Floor
3) **Catedral** - Donceles 95; Inside Catedral Hotel
4) **Pirates Burgers** - Isabel la Católica 28
5) **Azul Histórico** - Isabel la Católica 30
6) **Taquería Califa** - Isabel la Católica and 5 de Mayo
7) **Taquería Jalisco de Arandas** - Isabel la Católica 14
8) **El Cuatro 20** - Isabel la Católica 10
9) **La Opera** - Av. 5 de Mayo 10
10) **Café La Pagoda** - Av. 5 de Mayo 10-F
11) **Casa de los Azulejos**
12) **Miralto** - Inside Torre Latinoamericana, Floors 37, 40, and 41
13) **Pizza del Perro Negro** - Donceles 64
14) **El Bajío** - Calle Bolívar 14
15) **La Casa de Toño** - Avenida Madero 20
16) **Café de Tacuba** - Calle Tacuba 28

A) Hotel NH Collection Mexico City Centro Histórico

ANNOUNCEMENT

WORLD CONGRESS ON INFLAMMATION

The World Congress on Inflammation will be held in Québec City, Canada **from July 21-24, 2024.**

The meeting will address some of the latest developments related to inflammation, all set in enchanting Québec City, with its joie de vivre, its warm people and countless culinary delights.

As the cradle of French civilization in North America, Québec City is also a living history lesson with a remarkable mix of architecture, heritage, art, and culture.

We look forward to meeting you
at the Québec City Convention Centre.

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VENUE

Palacio de la Escuela de Medicina
Universidad Nacional Autónoma de México

The meeting is being held at the Palacio de la Escuela de Medicina (the Medical School historic building), located in the heart of Mexico's historic Old City. Part of the facility has been transformed to accommodate modern-day meetings, but most of the premises retain their centuries-old charm. Somewhat less charming is that the compound was once used as the Inquisition headquarters until it was repurposed as the School of Medicine in 1854. It now serves as a flagship for science and culture in the Mexican capital.

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Scientific Program
Auditorio Palacio de la Escuela de Medicina, UNAM

Saturday, December 3

15h00-18h00 On-site registration

18h00 **Introductory remarks**
C Rosales, M Schnoor, PP McDonald

18h15-19h00 **Keynote Lecture**

Dr Arturo Zychlinsky (Max-Planck-Institut für Infektionsbiologie, Berlin)
Neutrophil chromatin and immune defense

19h15 Welcoming Reception

Sunday, December 4

7h30-8h30 Breakfast

Session I: Neutrophil signaling and function

Chairs: Marion Brunck, Marco Cassatella

8h30- 9h00

Maria Fernandes (Université Laval, Québec City, Qc, Canada)
Regulation of neutrophil activation by the inhibitory receptor CLEC12A in rheumatoid arthritis and gout

9h00- 9h30

Marco A. Cassatella (Università di Verona, Italy)
On the characterization of human neutrophil progenitors and PMN-MDSCs

Selected talks from abstracts

9h30- 9h45 **Nina Offermann** (DZNE, Bonn, Germany)
Control of NETosis by proton channels

9h45- 10h00 **Paula Martinez Sanz** (Sanquin Research, Amsterdam, Netherlands)
Humanized MISTRG as a preclinical in vivo model to study human neutrophil-mediated immune processes

10h00-10h30 Coffee break

10h30- 11h00

Mariana J. Kaplan (National Institutes of Health, Bethesda, MD, USA)
Neutrophil dysregulation in systemic autoimmunity

11h00- 11h30

Juliana P. Zuliani (Porto Velho, Brazil)
Neutrophils and Cr-LAAO: Mechanisms and perspectives

Selected talks from abstracts

11h30- 11h45 **Pei Xiong Liew** (Brigham and Women's Hospital**,** Boston, MA USA)
FcγRIIIB engagement on splenic neutrophils promotes their reverse transmigration and anti-tumor immunity

11h45- 12h00 **Matthew Lawrenz** (Univeristy of Louisville, Louisville, KY, USA)
Neutrophils and the black death: Yersinia pestis actively inhibits the synthesis of leukotriene B4 during infection

12h00-13h30 Lunch

12h30-13h00 Technical Talk
Irene Whitney (Honeycomb Biotechnologies, Inc; Boston, MA, USA)
Overcoming obstacles for single-cell RNAseq of neutrophils from patient samples

Session II: Neutrophil trafficking

Chairs: Michael Schnoor, Carole Parent

13h30- 14h00

Carole Parent (U. of Michigan, USA)
The neutrophil nucleus as a signaling hub during chemotaxis

14h00- 14h30

Klaus Ley (Augusta University, GA, USA)
Beta 2 integrin activation in neutrophils

Selected talks from abstracts

14h30- 14h45 **Qing Deng** (Purdue University, West Lafayette, USA)
Engineering Chimeric Antigen Receptor Neutrophils for Targeted Cancer Immunotherapy

14h45- 15h00 **Peter Nigrovic** (Boston Children's Hospital, Boston, MA USA)
Trans-megakaryocyte migration confers enhanced pro-inflammatory capacity upon neutrophils

15h00-15h30 Coffee break

15h30- 16h00

Anna Huttenlocher (U of Wisconsin, USA)
Imaging inflammation and its resolution in zebrafish

16h00- 16h30

Eduardo Vadillo (Instituto Mexicano de Seguro Social, Mexico)
Neutrophil trafficking and function during inflammation and cancer

Selected talks from abstracts

16h30- 16h45 **Daniel Irimia** (Massachusetts General Hospital, Boston, MA, USA)
Transcellular LTB4 biosynthesis orchestrates neutrophil swarming

16h45- 17h00 **Erinke van Grinsven** (University of Oxford, Oxford, UK)
Single-cell RNA sequencing reveals the presence of immature neutrophils in inflamed murine joints

Poster Session I

17h00- 18h30

Authors are requested to be present at their poster from 17h00 to 18h00

Monday, December 5

7h30-8h30 Breakfast

Session III: Mucosal immunology & pathogen interactions

Chairs: Eileen Uribe-Querol, Silvia Uriarte

8h30- 9h00

Charles Parkos (Univ. of Michigan, USA)
Interplay between neutrophils and mucosal epithelia during inflammation and repair

9h00- 9h30

Silvia Uriarte (Univ. of Louisville, USA)
You shall not pass! Unveiling the interplay between neutrophils and newly dominant pathogens at the oral mucosal barrier

Selected talks from abstracts

9h30- 9h45 **Katharine Lodge** (Imperial College London, London, UK)
Hypoxia promotes PI3K and/or HIF1-dependent neutrophil secretion of histotoxic proteins to drive tissue damage in inflammatory lung disease

9h45- 10h00 **Frank Robledo Avila** (Nationwide Children's Hospital, Columbus, OH, USA)
HMGB1 promotes bacterial clearance and limits NETosis

10h00-10h30 Coffee break

10h30- 11h00

Rabindra Tirouvanziam (Emory University; Atlanta, GA, USA)
HDAC11 and MALAT-1 mediate a neutrophil-to-EV-to-neutrophil perpetual cycle of inflammation in human cystic fibrosis airways

Selected talks from abstracts

11h00- 11h15 **Joaquín Cantón Sandoval** (Universidad de Murcia, Murcia, Spain)
Inhibition of nuclear translocation of GAPDH impacts neutrophil migration and ameliorates chronic skin inflammation

11h15- 11h30 **Jennifer Brazil** (University of Michigan, Ann Arbor, MI, USA)
CD11b/CD18 sialylation regulates neutrophil transepithelial migration and inflammatory function in intestine

12h00-13h30 Lunch

Session IV: Neutrophil plasticity and heterogeneity

Chairs: Eduardo Vadillo, and Irina Udalova

13h30- 14h00

Hongbo R. Luo (Harvard University, Boston, USA)
Neutrophil heterogeneity in homeostasis and infection

14h00-14h30

Irina Udalova (University of Oxford, Oxford, UK)
Trancriptional control of neutrophil development and activation

Selected talks from abstracts

14h30- 14h45 **Melissa Ng** (Agency for Science, Technology and Research [A*STAR] Singapore)
Niche-specific reprogramming drives the functional diversification of neutrophils in the tumour environment

14h45- 15h00 **Zvi Fridlender** (Hadassah Medical Center - Hebrew University, Jerusalem, Israel)
Neutrophils drive B-cell recruitment into the tumor and their differentiation to plasma cells

15h00-15h30 Coffee break

Session V: Trainee Session

Chairs: Michael Schnoor, Marina Kardell

Selected talks from abstracts

15h30- 15h45 **Alejandra Lopez Arredondo** (Tecnológico de Monterrey, Monterrey, Mexico)
Conditional immortalization of human CD34^{+} hematopoietic stem cells for neutrophils ex vivo production

15h45- 16h00 **Matteo Napoli** (Biomedical Center LMU, Munich, Germany)
MRP8/14: Fine tuning of Ca^{2+} availability during beta2 integrin activation in neutrophils

16h00- 16h15 **Marina Kardell** (UKM, Münster, Germany)
Role of MYO9b RHOGAP in integrin activity regulation and neutrophil recruitment

16h15- 16h30 **Ruici Lin** (Virginia Tech., Blacksburg, VA, USA)
Protective effects of programmed resolving/immune-enhancing neutrophils in sepsis

Poster Session II

16h30- 18h30

Authors are requested to be present at their poster from 17h00 to 18h00

19h30 **Mexican Dinner**

Tuesday, December 6

7h30-8h30 Breakfast

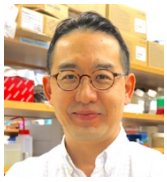
Session VI: Neutrophil in Resolution and Repair

Chairs: Arturo Zychlinsky, Liwu Li

8h30- 9h00

Liwu Li (Virginia Tech., Virginia, USA)
Neutrophil memory and reprogramming dynamics in the treatment of diseases

9h00- 9h30



Minsoo Kim (University of Rochester, Rochester, NY, USA)
Neutrophil functions during initiation, resolution, and memory of viral infection

Selected talks from abstracts

9h30- 9h45 **Gustavo Garcia** (University of California - Davis; Davis, CA, USA)
Neutrophils in circulation of patients with psoriasis are primed for enhanced inflammatory response

9h45- 10h00 **Lucy McGowan** (University of Bristol, Bristol, UK)
Uncovering the role of neutrophils in early fracture repair: friend or foe?

10h00-10h30 Coffee break

10h30- 11h00

Sergio Catz (The Scripps Research Institute, CA, USA)
Molecular mechanisms and translational approaches in the control of neutrophil-mediated inflammation

Session VII: Neutrophils in Health and Disease

Chairs: Carlos Rosales, Zvika Granot

11h00- 11h30

Lai Guan Ng (1. Singapore Immunology Network; Agency for Science, Technology & Research (A*STAR). 2. Shanghai Immune Therapy Institute, Renji Hospital, Shanghai Jiatong University)
Granulopoiesis: a daily balancing act

11h30- 12h00

Zvika Granot (Hadassah-Hebrew University, Israel)
Neutrophil specific targeting as a novel mode of immunotherapy

12h00- 12h30

Oliver Söhnlein (Westfälische Wilhelms-Universität Münster, Germany)
Neutrophils in chronic inflammation – from physiology to therapeutic intervention

12h30 -13h30 **Awards for Trainee Session and Closing Ceremony**

Carlos Rosales, Michael Schnoor, Patrick McDonald

Wednesday, December 7

8h00-18h00 Optional guided tour to visit the **pyramids of Teotihuacán**, including transportation, entrance, guided visit and lunch
Registration required

photo by Carlos Rosales

Travel Award winners from the Histochemical Society

Pei Xiong Liew (Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA)
FcγRIIIB engagement on splenic neutrophils promotes their reverse transmigration and anti-tumor immunity

Lucy McGowan (University of Bristol, Bristol, UK)
Uncovering the role of neutrophils in early fracture repair: friend or foe?

Alejandra Aroca (Spanish National Center for Cardiovascular Research, Madrid, Spain)
Targeting neutrophil aging preserves cardiovascular health

Balázs Enyedi (Semmelweis University, Budapest, Hungary)
"Inflamapping" with GEM-LTB4: Live visualization of LTB4 gradients with a novel fluorescent biosensor

Jonny Coates (William Harvey Research Institute, Queen Mary University of London, UK)
Rapid migration of neutrophils with antigen-presenting cell phenotype into lymphoid organs upon ischemia reperfusion injury

Travel Award winners from Society of Mucosal Immunology

SOCIETY FOR MUCOSAL IMMUNOLOGY

Tommaso Vicanolo (Centro Nacional de Investigaciones Cardiovasculares, Madrid, Spain)
Matrix-producing neutrophils shield the skin

Katherine Lodge (National Heart and Lung Institute, Imperial College London, UK)
Hypoxia promotes PI3K and/or HIF1-dependent neutrophil secretion of histotoxic proteins to drive tissue damage in inflammatory lung disease

Tereza Masonou (University College London, London, United Kingdom)
Investigating neutrophil function during SARS-Cov2 infection in human airway epithelial cells from elderly and children

Lenore Monterroza (Emory University School of Medicine, Atlanta, GA, USA)
Induction of STING/IL-29 signaling in a human lung adenocarcinoma / tumor-infiltrating neutrophil biomimetic model

Travel Award winners from Society of Leukocyte Biology

Lou Martha Wackerbarth (Ludwig-Maximilians-Universität, Munich, Germany)
A20 and the non-canonical NF-κB pathway are key regulators of neutrophil recruitment during fetal ontogeny

Jacqueline Howells (Brown University and Rhode Island Hospital, Providence, RI USA)
Investigating mechanisms of HOXB8-conditional neutrophil progenitor engraftment in the murine host

Omar Rafael Alemán Muñoz (Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Mexico City, Mexico)
Fcγ receptors activate different PKC isoforms in human neutrophils

Yunyun (Anna) Shen (McGill University, Montréal, Canada)
Investigating the impact of neutrophils on natural killer cell activity in breast cancer liver metastasis

Neutrophil 2021
"Zoomed In" 31 May - 1 June, 2021

Young Researcher Award winners from Neutrophil 2021

Dr. **Eman Khatib-Massalha** is a postdoctoral (John Goldman) fellow working in the Mendez-Ferrer group at the Wellcome – MRC Cambridge Stem Cell Institute at the University of Cambridge. Her research focuses on the role of bone marrow niches in haematological malignancies.

Dr. **Alaz Ozcan** is a postdoctoral fellow working in the laboratory of Andres Hidalgo in the Department of Immunobiology at Yale School of Medicine. Her project focusses on chemokine-guided mapping of tissue-specific neutrophil niches.

Dr. **Coraline Radermecker** is a postdoctoral fellow working with Thomas Marichal in the Immunophysiology lab at the GIGA Institute of Liege University, Belgium. Her work relates to investigating how lung marginated neutrophils regulate lung endothelial fate, heterogeneity and stemness.

ABSTRACTS

P1 IN VITRO MODELLING OF NEUTROPHILS AT THE ORAL EPITHELIUM

A.E.L. Ho, N. Boukbir, F. Flores-Borja, E.K. Parkinson, E. Hagi-Pavli

Centre for Oral Immunobiology and Regenerative Medicine, Institute of Dentistry, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, Turner Street, London E1 2AD, United Kingdom.

Objective: To address the mechanisms involved in chronic oral inflammation, we present an in vitro oral epithelial model to study neutrophil transepithelial migration and other immunological processes at the mucosal surface. This is critical for identifying neutrophil- epithelial interactions and signalling during infection and inflammation which results in excessive neutrophil influx, oxidative burst and neutrophil extracellular traps release. Methods: Normal human oral keratinocyte cell line, OKF6/TERT-1 was cultured on transwell filters. To mimic inflammatory conditions that favour neutrophils recruitment, epithelial cells were exposed to relevant stimulants followed by immunoassay tests on collected supernatants. Furthermore, human promyelocytic leukemic HL-60 cells were differentiated into neutrophil-like cells and flow cytometry was used to study expression of key differentiation markers involved in transmigration. Results: Following optimisation of culture conditions, we developed an in vitro model resembling a tight functional oral barrier characterised by high transepithelial electrical resistance and low permeation properties. Moreover, microscopy analyses revealed that OKF6/TERT-1 cells exhibited fluorescence intensity for tight junction proteins. In response to P. gingivalis LPS treatment, cytokines including IL-8, TNF-alpha, GM-CSF, MCP-1 and MIP-1 alpha were released into supernatants. Upon differentiation, HL-60 cells expressed molecules associated with transmigration including CD11b, CD54, neutrophil elastase and myeloperoxidase. Conclusion: We have established a novel in vitro model for studying neutrophil trafficking across the oral epithelial cells. This will help to identify critical innate immune mechanisms that might be associated with epithelial cell damage implicated in oral diseases.

P2 PARALYTIC IMPACT OF ACCELERATION FORCES ON NEUTROPHILS

T. Hundhammer, M. Gruber, S. Wittmann

Department of Anesthesiology, University Hospital Regensburg, Regensburg, Germany.

As intriguing as polymorph nuclear neutrophils (PMNs) are as a subject of research, their short half-life and low threshold for activation account for major influencing factors. Investigation on PMNs usually requires isolated but still as native as possible cells. For studies aiming to detect changes in migration, expression of surface markers or granule contents caused by biological or therapeutic treatments, isolation stress might be detrimental. We hypothesized, that applying even low g-forces significantly affects major PMN functions in an acceleration-dependent manner. Whole blood was centrifuged with different acceleration forces (10g, 20g, 30g, 47g) for 15 minutes. The neutrophil-rich supernatant was analyzed by live cell imaging and flow cytometry. Neutrophil chemotactic migration was examined with 3D-µ-Slide migration chambers (Ibidi GmbH, Germany), where PMN migration was stimulated using an fMLP gradient. Migrating cells were tracked for 22 hours microscopically. For investigation of antigen expression, cells were stained with anti-CD11b, anti-CD62L and anti-66b human antibodies, oxidative burst was detected by Rhodamine 123 and analyzed via flow cytometry. Neutrophils isolated with higher g-forces showed a significant decrease in track length, and expression of surface antigens declined as well as oxidative burst compared to the 1g-control. Our data show that commonly performed neutrophil density isolation significantly affects neutrophil functions, since this usually includes sample centrifugation at more than 500g. Future research on PMNs should avoid isolation methods with centrifugation steps beyond 1g as a partial paralysis may occur. Whether intravital PMN paralysis caused by centrifugational pumps during ECMO is affecting the cells is under ongoing investigation.

P3 MICROBIOTA LICENSES NEUTROPHILS TO RESPOND TO ANTI-CANCER THERAPY

R. Araya1, K. Lam1, A. Huang1,2, Q. Chen1,3, M. Di Modica1,4, A. Lopes1, H. Yang5, H. Liu5, M. Lee5, R. Goldszmid1

1 Laboratory of Integrative Cancer Immunology, NCI-National Institutes of Health, Bethesda, MD, USA. 2 Leidos Biomedical Research, Bethesda, MD, USA. 3 Kelly Government Solutions, Bethesda, MD, USA. 4 Molecular Targeting Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy. 5 Laboratory of Cancer Biology and Genetics, NCI-National Institutes of Health, Bethesda, MD, USA.

The role of neutrophils in tumor development and anti-tumor therapy is context-dependent. Yet, the environmental signals that modulate their function in the tumor microenvironment (TME) remain unclear. The microbiota has recently emerged as a key regulator of the anti-tumor response. Therefore, we hypothesized that microbiota-derived signals modulate neutrophils in the TME. To test this, we implanted tumors in mice with or without microbiota and analyzed cells in the TME transcriptionally, phenotypically, and functionally at the single-cell level. We showed that tumor neutrophils are very distinct from those in other tissues and are heterogenous and dynamic in response to therapy. Moreover, we found that neutrophils are the main source of ROS in the TME and are needed for the response to chemotherapy. In the absence of microbiota, neutrophil dynamics is altered and they are impaired to respond to therapy. Mechanistically, we showed that microbiota-derived peptidoglycan is sufficient to restore the anti-tumor neutrophil function and response to therapy in microbiota-devoid animals. Importantly, we observed that neutrophils are heterogeneous in human cancer and predictive of patient outcomes in a neutrophil subset- and tumor-dependent manner. Collectively, our findings highlight the importance of studying neutrophils in the TME and the contribution of microbiota in licensing these cells for response to cancer therapy.

P4 AN APPROACH TO GENERATE N1- AND N2-LIKE HUMAN NEUTROPHILS IN VITRO

M. Ohms, S. Möller and T. Laskay

University of Lübeck, Lübeck, Germany

In tumor models the existence of two neutrophil subsets has been described, the pro-inflammatory N1 neutrophils with anti-tumor activity and the tumor-promoting anti-inflammatory N2 phenotype. In this study we attempted to polarize primary human neutrophils towards a N1 or N2 phenotype in vitro. Pro-inflammatory stimuli were used to polarize N1 cells. In order to polarize neutrophils to an N2 phenotype we attempted to mimic a tumor microenvironment in vitro by culturing neutrophils under hypoxia at low pH (pH 6.7) in the presence of tumor derived metabolites and cytokines, among others lactate, adenosine and TGF-beta. Analysis of cell surface expression of typical N1 and N2 markers as well as cytokine profiles were used to assess the phenotype of in vitro-polarized neutrophils. As expected, N1-polarized neutrophils had a pro-inflammatory phenotype characterized among others by a higher level of ICAM-1 and high secretion of TNF. Neutrophils incubated under a tumor-mimicking in vitro-environment showed high cell surface expression of CXCR2 and secreted high levels of IL-8. N1 cells have been suggested to have a pro-inflammatory whereas N2-cells an anti-inflammatory phenotype. Since pro-inflammatory neutrophils, but not anti-inflammatory neutrophils, exert potent anti-microbial effector functions, the killing of pathogenic microorganisms by the polarized N1- and N2-like cells were investigated. We could show that, as expected, N2-polarized neutrophils exerted a markedly decreased capacity to kill the intracellular pathogen Leishmania donovani. These findings suggest that it is feasible to polarize blood-derived primary human neutrophils towards N1- and N2-like phenotypes in vitro.

P5 PROLONGING NEUTROPHIL STORAGE IN GRANULOCYTE CONCENTRATES USED FOR TRANSFUSION

M-M. Labrecque, CHU de Québec research center, Québec, Canada; A. Murru, CHU de Québec research center, Québec, Canada; G. Paré, CHU de Québec research center, Québec, Canada; J. Acker, Canadian Blood Services, Edmonton, Canada; S. Lesage, Maisonneuve-Rosemont hospital research center, Montreal, Canada; R. Bazin, Héma-Québec, Québec, Canada; M. Girard, Héma-Québec, Québec; M.J. Fernandes, CHU de Québec research center, Québec, Canada.

Transfusion of granulocyte concentrates (GC) is a therapeutic option for neutropenic patients suffering from infections resistant to anti-microbials. A key challenge of GC transfusions is maintaining the viability of neutrophils, the main leukocyte that provides antimicrobial defenses to eradicate infections. Objective: To extend the ex vivo viability and antimicrobial activity of GC neutrophils from 24h to 72h using additives and solutions approved for clinical use. Methods: Neutrophils isolated from healthy donors were resuspended at the same concentration as in GCs in autologous plasma (AP) and supplemented with Plasma-Lyte, SAGM, AS-3 and/or Alburex. Viability, phagocytosis and reactive oxygen species (ROS) were measured by flow cytometry up to 72h of storage at room temperature. Results: All transfusion solutions significantly increased neutrophil viability up to 72h and their functions for 48h compared to neutrophils stored in AP alone. Phagocytosis of opsonized pathogens increased in the presence of more than one additive up to 72h storage compared to AP storage alone. Neutrophils preserved ability to produce ROS only with AS-3. Conclusion: Supplementing AP with Plasma-Lyte and AS-3 significantly prolongs ex vivo neutrophil viability and preserves neutrophil phagocytosis and ROS production for up to 48h of storage. Since extracellular ROS production increased with storage, the addition of anti-oxidants could further prolong neutrophil viability and function at room temperature. Additional functional assays are underway to complete the characterization of this new storage solution. Our findings indicate that clinically-approved solutions can significantly improve the neutrophil storage. Preserving neutrophil function is crucial to optimize the efficacy of GC transfusions. Moreover, increasing GC availability will offer more patients access to this potentially life-saving therapy.

P6 ASSOCIATION OF NEUTROPHIL SUB-POPULATIONS WITH LUNG FUNCTION AND DISEASE ACTIVITY IN CYSTIC FIBROSIS

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Cystic fibrosis (CF) is a recessive genetic incurable disease caused by a mutation in the cystic fibrosis transmembrane regulator (CFTR) gene. Neutrophils, massively recruited into the lung, drive airway inflammation and disease progression characterized by a gradual decline of lung function, the first cause of mortality in CF. Neutrophil sub-populations are known to contribute to disease severity in chronic inflammatory conditions such as lupus or rheumatoid arthritis. These cells characterized by their low density (LDN), have distinct immunophenotype and function. Their presence in circulation in CF adult patient and their contribution to lung function decline and disease activity remain to be described. LDN abundance is higher in CF patient circulation compared with healthy donors (HD) and their presence is related to the ongoing inflammatory process of CF lung disease. LDNs were isolated from 34 CF patients and 35 HD. Their abundance in circulation and immunophenotype were determined by flow cytometry. LDNs characteristics were associated with clinical profiles of CF patients including lung function changes and pulmonary exacerbations (PExs). Circulating LDN proportion was significantly higher in CF compared with HD. LDNs contained mature CD10+CD16+ and immature CD10-CD16- cells. In CF, cell surface expression of CD14 was higher in both mature and immature LDN compared with HD. The proportion of circulating mature LDN in CF was negatively associated with lung function changes measured by FEV1 (%) and FVC (%) and was increased in patients with repeated PExs. Mature circulating LDN association with lung function decline and disease activity suggest these cells indicate a more severe inflammatory process in CF lung. Further functional and prospective analysis is needed to determine their contribution to the antimicrobial response and their potential as predictive indicator of CF lung disease progression.

P7 BILE ACIDS DIRECT NEUTROPHILS TO MAINTAIN INTESTINAL HOMEOSTASIS THROUGH NET FORMATION

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Bile acids (BAs) are generally considered to be potent endocrine hormones that regulate physiological responses such as lipid and bile acids homeostasis. In this study, we found that BAs stimulate neutrophil extracellular traps (NETs) formation through farnesoid X receptor (FXR), bile acids-regulated nuclear receptor. BA-induce NETs provide a barrier in the small intestine against intestinal bacteria. Chenodeoxycholic acid (CDCA), a primary bile acid, significantly enhanced NETs formation and GW4064 and obeticholic acid (OCA), the synthetic agonists for FXR, also enhanced NETs formation, whereas neutrophils isolated from FXR-/- mice did not generate NET formation in response to BAs. Moreover, CDCA enhanced expressions of citrullinated histone 3 (cit-H3) in neutrophils and inhibitors for protein arginine deaminase 4 (PAD4) attenuated CDCA-induced NETs formation. As the increase of intracellular calcium is required for PAD4 activation, we further examined the effects of BAs on intracellular calcium levels in neutrophils. CDCA and FXR agonists significantly enhanced intracellular calcium levels in both human and mouse neutrophils. We found the NETs in the intestinal crypts of small intestines in wild-type mice which were disappeared in the fasted mice. Interestingly, re-feeding restored NETs in the small intestines. FXR-/- mice did not contain intestinal NETs and showed increased the dissemination of bacteria into distant organs such as liver, mesenteric lymph node, and spleen. The dissemination of orally inoculated Salmonella was higher in FXR-/- than wile-type mice. In conclusion, we found that the BAs direct neutrophils to maintain intestinal homeostasis through NETs formation which restricts systemic dissemination of bacteria obtained under nutrient flow.

P8 NOD2-RIPK2 SIGNALING MEDIATES SENSING OF NARNA FROM NEUTROPHIL-EXTRACELLULAR-TRAPS (NETS) IN PRIMARY HUMAN KERATINOCYTES

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The skin is the first line of defense against pathogens like bacteria, fungi and viruses and predominantly composed of keratinocytes. These can act as immune cells that are able to initiate innate immune responses to fight infections by releasing antimicrobial peptides (AMPs) and attracting professional immune cells like polymorphonuclear neutrophils (PMNs) via the release of cytokines such as Interleukin-8. Upon activation these PMNs form neutrophil extracellular traps (NETs) which consist of DNA, AMPs like LL37, and RNA. Both, LL37 and NET-associated RNA (naRNA) can act as a DAMP on immune cells but the responses to NETs in keratinocytes are poorly defined. Here, we show that both N/TERT-1 immortalized and primary normal human epidermal (NHEKs) keratinocytes react with Interleukin-8 release to synthetic RNA. This response depended on NOD2, a cytoplasmic pattern recognition receptor normally associated with peptidoglycan recognition, and its downstream component, RIPK2. Moreover, NETs, whose RNA is sensed via TLR8 in neutrophils and macrophages, also elicited IL-8 release dependent on NOD2-RIPK2, rather than TLR-MyD88, signaling in keratinocytes. Taken together, our data show a novel function of NOD2 as a keratinocyte-specific sensor of RNA and NETs mediating immune stimulation in the skin.

P9 NEUTROPHILS AND MYELOID TGF-B SIGNALING, AN IMPORTANT REGULATORY AXIS IN BOTH CANCER AND STROKE

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Myeloid cells exhibit extensive phenotypic and functional changes under cancer and chronicle inflammation conditions. The underlying molecular and cellular mechanisms remain to be investigated. We discovered that under cancer conditions, myeloid-specific deletion of gene encoding TGF-β receptor II (Tgfbr2, or Tgfbr2Myeko) reprogramed the neutrophils skewing type 1 immune responses, which improved host anti-tumor immunity and inhibited tumor metastasis. Our single cell sequencing analysis further revealed a novel neutrophil subset that is likely responsible for the observed phenotype. Our work differentiates myeloid TGF-β signaling from that in the epithelial compartment. Of tremendous interest, these Tgfbr2Myeko mice developed spontaneous stroke with 100% penetrance. We further discovered that attenuation of TGF-β signaling in myeloid cells resulted in cerebral vascular damage and leakage mediated by inflammatory neutrophils. Our data provide desperately needed insight into inflammation associated stroke etiology.Together, our work demonstrates that myeloid-specific TGFβ signaling is fundamentally important in immune homeostasis for both cancer and stroke conditions. We believe this insight could be utilized for therapeutic intervention of the two most devastating human diseases.

P10 THE GRANULOCYTE COLONY-STIMULATING FACTOR (G-CSF) RECRUITS NEUTROPHILS DURING STREPTOCOCCUS SUIS INFECTION

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Streptococcus suis causes diseases in pigs and it is considered an emerging zoonotic agent. Infections induce an exacerbated inflammation that can lead to septic shock and meningitis. Although neutrophils greatly infiltrate the lesions, actual literature poorly describes their dynamics during S. suis infection. Moreover, very few studies reported the production and role of a key factor in the regulation of neutrophils: the colony-stimulating granulocyte factor (G-CSF). This project aims to characterize the axis G-CSF−neutrophils in the pathogenesis of S. suis infection.In a mouse model of S. suis infection, we first evaluated the recruitment of neutrophils and their activation profile by flow cytometry. We found that the infection provokes a massive neutrophil recruitment from bone marrow to blood and spleen. In both compartments, neutrophils displayed multiple activation markers. In parallel, we observed high levels of G-CSF in the plasma, with peak production coinciding with that of neutrophil recruitment. We then neutralized the effect of G-CSF and highlighted its role in the release of neutrophils from the bone marrow to the blood. However, the neutralization of G-CSF did not dramatically affect the cytokine storm or the bacteremia usually observed in S. suis-infected mice.In conclusion, systemic G-CSF induces the release of neutrophils from the bone marrow to the blood, but its role in inflammation or bacterial clearance seems to be compensated by unknown factors. A better understanding of the role of neutrophils and inflammatory mediators could lead to better strategies for controlling the infection caused by S. suis. Overall, this project fleshes out the knowledge on neutrophil biology in an in vivo model of host-pathogen interaction.

P11 NEUTROPHILS PHENOTYPE AND FUNCTION IN OBESE PATIENTS

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Neutrophils are regarded as the first line of defence against infections mainly because they are the first to arrive at sites of infections. Moreover, neutrophils possess a great repertoire of microbial killing mechanisms. It has been described that circulating blood neutrophils are increased in obesity. Despite having higher numbers of blood neutrophils, people with obesity are more prone to diseases and complications caused by infections. Furthermore, it is not clear whether neutrophils from obese people function adequately against infections. Therefore, this research focuses on studying the microbial killing capacity of blood neutrophils from obese patients. Blood was obtained from obese patients with body max index (BMI) ≥ 30 kg/m2 and from normal weight controls BMI 18.5 – 20 kg/m2. Neutrophils were isolated by density gradient centrifugation. First, neutrophils were characterised by the expression of cell surface markers by multicolour flow cytometry. Neutrophils from obese patients express higher levels of CD66b than neutrophils from normal weight controls. In addition, isolated blood neutrophils were stimulated with PMA to produce reactive oxygen species (ROS). It was found that neutrophils from obese patients had an enhanced ROS production when compared to neutrophils from normal weight controls. It is well known that phagocytosis of pathogens also leads to the formation of ROS that contribute to pathogen clearance. Therefore, the next step is to assess whether neutrophils from obese patients can phagocytise adequately. (Funded by PAPIIT 222120, Mexico)

P12 DIABETES PRIMES NEUTROPHILS FOR NEUTROPHIL EXTRACELLULAR TRAP FORMATION THROUGH TRAINED IMMUNITY

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Neutrophils are primed for neutrophil extracellular trap (NET) formation during diabetes and excessive NET formation from primed neutrophils compromises wound healing in patients with diabetes. Here, we demonstrate that trained immunity mediates diabetes-induced NET priming in neutrophils. Under diabetic conditions, neutrophils exhibit robust metabolic reprogramming comprising enhanced glycolysis via the pentose phosphate pathway and fatty acid oxidation, which result in the accumulation of acetyl-coenzyme A (CoA). ATP-citrate lyase (ACLY)-mediated accumulation of acetyl-CoA and histone acetyltransferases (HATs) further induce the acetylation of lysine residues on histone 3 (AcH3K9, AcH3K14, and AcH3K27) and histone 4 (AcH4K8). The pharmacological inhibition of ACLY and HATs completely inhibited high glucose-induced NET priming. The trained immunity of neutrophils was further confirmed in neutrophils isolated from patients with diabetes. Our findings suggest that trained immunity mediates functional changes in neutrophils in diabetic environments, and targeting neutrophil-trained immunity may be a potential therapeutic target for controlling inflammatory complications of diabetes.

P13 Fc RECEPTORS ACTIVATE DIFFERENT PKC ISOFORMS IN HUMAN NEUTROPHILS

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Receptors for the Fc portion of IgG (FcγR) on neutrophils constitute an important mechanism for recognition of opsonized microorganisms and activation. Human neutrophils express two FcγR, FcγRIIa and FcγRIIIb. Although, signaling mechanisms for each FcγR have been only partially described, it is clear that each FcγR can induce production of reactive oxygen species (ROS), and ERK phosphorylation (pERK). Also, it has been reported that PKC activation is important for ROS production, and in other cell types, PKC activation can induce pERK. Despite this evidence, which PKC isoform can be activated by each FcγR, is still unknow. Thus, we explored which PKC isoforms can be activated by each FcγR in human neutrophils, and whether these PKC isoforms are implicated in ROS production and pERK. Each FcγR was cross-linked by specific monoclonal antibodies, and ROS production or pERK were evaluated in the presence or absence of pharmacological inhibitors for PKC isoforms. FcγRIIa-mediated ROS production was blocked by Gö6976, a PKC α/β inhibitor, but not by LY333531, a PKCβ inhibitor. Rottlerin, a PKCδ inhibitor also blocked FcγRIIa-mediated ROS production. In contrast, FcγRIIIb-mediated ROS production was blocked by Gö6976 and LY333531, but not by Rottlerin. These data suggested that FcγRIIa activates PKCα and δ to mediate ROS production, while FcRIIIB activated PKCα and β isoforms. In addition, Gö6976 did not affect FcγRIIa-mediated pERK, while it did block FcγRIIIb-mediated pERK. Thus, it seems that FcγRIIa did not use PKCα and β to mediate pERK, while FcγRIIIB required PKCα and β isoforms to induce pERK. Together these results shown for the first time, that both FcγRIIa and FcγRIIIb differentially activate PKC isoforms to modulate ROS production and pERK in human neutrophils.

P14 LOW-DENSITY NEUTROPHILS IN OBESITY

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Neutrophils are the most abundant leukocytes in human blood and comprise around 50-70 % of circulating leukocytes. Neutrophils are the first cells to migrate into infection or inflammation sites, in order to eliminate microbial pathogens. The antimicrobial mechanisms of neutrophils include phagocytosis, degranulation, production of reactive oxygen species (ROS), and NETosis. Traditionally, neutrophils are purified from blood by density-gradient centrifugation. In this method, neutrophils appear at the bottom of the tube, while mononuclear cells (MNC) appear above in a lower density layer. However, one possible subtype of neutrophils is co-purified with MNC in the low-density part of the gradient. These neutrophils have been named “low-density neutrophils” (LDN). Althought, LDN have recently been described in healthy conditions, most reports of LDN are associated with cancer and other pathologies such as systemic lupus erythematosus, rheumatoid arthritis, psoriasis, HIV infección, malaria, and tuberculosis. These conditions are characterized by systemic inflammation and in all cases, LDN numbers increase with the severity of the disease. Obesity is a serious condition that also presents chronic systemic inflammation. However, there are not reports on the presence or functionality of LDN in obesity. In order to characterize LDN in obese patients, the phenotype of LDN was determined by multicolor flow cytometry. We found that LDN numbers increase with the severity of obesity and LDN displayed the phenotype CD10+, CD11b+, CD14low, CD15high, CD16bhigh, CD62L+, CD66b+, CXCR4+, CXCR2low. In addition, these LDN had enhanced ROS production. These results suggest that the inflammation environment in obesity could affect numbers of LDN in the bloodstream, and cause these LDN to display an activated phenotype. (Funded by PAPIIT 222120, Mexico)

P15 DISTINCT STIMULUS-DEPENDENT NEUTROPHIL DYNAMICS REVEALED BY REAL-TIME IMAGING OF INTESTINAL MUCOSA AFTER ACUTE INJURY.

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Clinical symptoms in many inflammatory diseases of the intestine are directly related to neutrophil (PMN) migration across colonic mucosa and into the intestinal lumen, yet in vivo studies detailing this process are lacking. Using real-time intravital microscopy and a new distal colon loop model, we report distinct PMN migratory dynamics in response to several models of acute colonic injury. PMN exhibited rapid swarming responses after biopsy-induced intestinal wounds. Similar numbers of PMN infiltrated colonic mucosa after wounding in germ-free mice, suggesting microbiota-independent mechanisms. By contrast, acute mucosal injury secondary to acute colitis induced by treatment of mice with dextran sodium sulfate (DSS) or IL-10 receptor blockade resulted in accumulation of PMN in the mucosa that were largely immotile. Biopsy wounding of colonic mucosa in DSS-treated mice did not result in enhanced PMN swarming however, intraluminal application of LTB4 stimulated an increase in transepithelial migration of PMN. Analyses of PMN that had migrated into the colonic lumen revealed that a majority were recruited directly from the circulation and not from the immotile pool within the mucosa. Decreased PMN motility paralleled increased expression of CXCR4 and apoptosis. Similarly, upregulation of CXCR4 on human PMN was observed in colonic biopsies from people with active ulcerative colitis. This new investigative approach will facilitate elucidating mechanisms regulating PMN migration across intestinal mucosa in vivo and may provide insights into new anti-inflammatory and pro-repair therapies.

P16 HUMAN NEUTROPHILS GENERATE EXTRACELLULAR VESICLES THAT MODULATE THEIR FUNCTIONAL RESPONSES

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Neutrophils produce extracellular vesicles (EVs) termed ectosomes, which were influence the function of other immune cells. Here, we studied neutrophil-derived ectosome (NDE) generation and whether NDEs affect autologous neutrophil responses. We first characterized EV production by neutrophils using flow cytometry, following MISEV 2018 guidelines to facilitate comparisons with other studies. We found that such EVs are principally NDEs, are rapidly released upon neutrophil stimulation with several (but not all) physiological stimuli, and a number of signaling pathways are involved in the induction of this response. When co-incubated with autologous neutrophils, NDE constituents were rapidly incorporated into recipient cells and this triggered and/or modulated neutrophil responses. The pro-survival effect of GM-CSF was reversed; CXCL8 and NET formation were induced in otherwise unstimulated neutrophils; the induction of inflammatory chemokines by TNFalpha was modulated depending on the activation state of the NDE parent cell; and inducible NET generation was attenuated. Our data show that NDE generation modulates neutrophil responses in an autocrine/paracrine manner, and indicate that it probably represents an important aspect of how neutrophils shape their environment and cellular interactions.

P17 STIMULUS-DEPENDENT PI3K-DRIVEN NET FORMATION

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Neutrophil extracellular traps (NETs) can immobilize and kill pathogens, but also contribute to the progression of several inflammatory and auto-immune diseases, as well as cancer. Whence the importance of elucidating the mechanisms underlying NET formation. In this regard, the PI3K signaling pathway has been shown to be crucial; yet little is known about which of its components are involved. Here, we identified the PI3K isoforms and associated signaling partners that are mobilized in response to different classes of physiological NET inducers. Across the various stimuli used, PI3Kalpha and PI3Kgamma isoforms clearly contributed to NET induction, while the participation of other isoforms was stimulus-dependent. Some PI3K isoforms were also found to signal through Akt, the canonical downstream effector of PI3K, while others did not. Downstream of PI3K, Akt and mTOR were used by all stimuli to partially control NET generation. Conversely, the involvement of other kinases depended on the stimulus – both TNFalpha and GM-CSF relied on PDK1, TNFα additionally used S6K, and fMLP signaled through PLCgamma2 instead. We further established that all PI3K isoforms and downstream effectors act belatedly in NET generation, as reported previously for PI3K. Finally, we studied the PI3K-PDK1-Akt signaling hierarchy in human neutrophils and again found stimulus-dependent differences. Our data uncover unsuspected complexity and redundancy in the signaling machinery controlling NET formation through the all-important PI3K pathway. Conserved signaling molecules represent therapeutic targets for pathologies involving NETs and in this regard, the existence of drugs currently used in the clinic or undergoing clinical trials (which target PI3K isoforms, mTOR or Akt), underscores the translational potential of our findings.

P18 THE EFFECT OF CARBON SOURCES ON NEUTROPHIL ACTIVATION

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The main objective of this study was to identify and analyze neutrophil pathways and functions affected by replacing glucose by mannose as the major carbon source with the hopes of identifying a novel mechanism of inflammatory modulation. Neutrophils isolated from 8 donors were divided into two groups, one receiving glucose and the other mannose as the only carbon source. Both groups were activated by PMA or fMLP. ROS production was measured via the NBT test. Cells were then lysed and proteins were digested and desalted in preparation for HPLC and subsequent mass spectrometry analysis. The resulting data was analyzed using Progenesis and Peaks software and a subsequent 2-way ANOVA test to analyze the 2 factors present in this study (carbon source and activation). Differentially abundant proteins were grouped by pathways and GO terms using the software String, Panther and Reactome. Our data did not report significant differences ($p>0.05$) in terms of viability, cell yield and purity, suggesting that mannose does not affect neutrophil density nor vital cell functions. Neutrophils receiving mannose challenged by PMA, showed a significantly weaker ROS production than neutrophils receiving glucose. The exploratory proteomic analysis identified an abundance of proteins involved in degranulation, as well as complement proteins C1s and C1Qb, which to our knowledge haven't yet been described in neutrophils. The distribution of granule proteins between the two groups suggest that mannose had a positive regulatory effect on exocytosis in fMLP-activated cells, resulting in the lower granule protein representation. While a negative regulatory effect by mannose in cells activated by PMA could explain the increased abundance of granule proteins seen in these conditions. This preliminary analysis provided an interesting insight for future follow-up studies.

P19 METHOD MATTERS: THE EFFECT OF PURIFICATION TECHNOLOGY ON NEUTROPHIL PHENOTYPE AND FUNCTION

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Neutrophils are the most abundant leukocytes in human blood and the first cells responding to infection and injury. Due to their limited ex vivo lifespan and the impossibility to cryopreserve or expand them in vitro, neutrophils need to be purified from fresh blood for immediate use in experiments. The aim of this study was to expose the effects of 'classical' density-gradient purification versus the more expensive but faster immunomagnetic isolation on neutrophil phenotype and functionality. We found that in the absence of inflammatory stimuli, density-gradient-derived neutrophils showed increased polarization responses as well as enhanced release of reactive oxygen species (ROS), neutrophil extracellular traps (NETs) and granular proteins compared to cells derived from immunomagnetic isolation. Upon exposure to pro-inflammatory mediators, immunomagnetic isolation-derived neutrophils were significantly more responsive in polarization, ROS production, phagocytosis, NETosis and degranulation assays, in comparison to density-gradient-derived cells. We found no difference in chemotactic response in Multiscreen and under-agarose migration assays, but Boyden assays showed reduced chemotaxis of immunomagnetic isolation-derived neutrophils. Finally, we confirmed that density-gradient purification induces artificial activation of neutrophils, evidenced by differential surface marker expression. Based on these results, we recommend using immunomagnetic separation of neutrophils for studying neutrophil polarization, phagocytosis, ROS production, degranulation and NETosis, whereas for Boyden chemotaxis assays, the density-gradient purification is more suitable.

P20 THE ROLE OF AIRWAY NEUTROPHILS IN PRIMARY CILIARY DYSKINESIA

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Primary ciliary dyskinesia (PCD) is a genetic disorder characterized by recurrent airway infection and inflammation. There is no cure for PCD and to date there are no specific treatments available. Neutrophils are a crucial part of the immune system and are known to be dysfunctional in many inflammatory diseases. In this study, we investigated the phenotype and function of airway neutrophils in PCD, and compared them to blood neutrophils. To this end, paired peripheral blood and spontaneously expectorated sputum samples from patients with PCD ($n = 32$) and a control group of patients with non-PCD, non-cystic fibrosis bronchiectasis ($n = 5$) were collected, whereupon the phenotype and function of the neutrophils were assessed. Sputum neutrophils displayed a highly activated phenotype and were unresponsive to stimuli that would normally induce ROS production and NETosis. In addition, PCD sputum displayed high activity of neutrophil elastase, and impaired the efferocytosis function of healthy donor macrophages. These results suggest that sputum neutrophils are dysfunctional and likely contribute to ongoing inflammation in PCD airways. Further research should focus on anti-inflammatory therapies and stimulation of efferocytosis as a strategy to treat PCD.

P21 EXTRACELLULAR PROTEINS OF MYCOBACTERIUM TUBERCULOSIS STABILIZE HIF-1 ALPHA IN HUMAN NEUTROPHILS

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In has been studied that Mycobacterium tuberculosis (Mtb) protein, Early Secreted Antigenic Target 6-kDa protein (ESAT-6) stabilizes Hypoxia Inducible Factor-1alpha (HIF-1 alpha) in THP-1 macrophages, so the objective of our study was to analyze if extracellular proteins of Mtb could stabilize HIF-1alpha in human neutrophils. We isolated human neutrophils from venous blood of 3 healthy subjects. Mtb H37Rv culture was filtered to obtain extracellular proteins. The proteins were precipitated, desalted and quantified with Bradford, after that we did an SDS-PAGE to observe the bands of the proteins. We cultured neutrophils with no stimulus, CoCl2 and extracellular proteins of Mtb during 4 hours in normoxia. After the culture we did a immunocytochemistry and western blot to identify the presence of HIF-1 alpha. In the SDS-PAGE of the extracellular proteins of Mtb we obtained 4 proteins with the following weights: 62, 49, 38 and 6 kDa. We identified the presence of HIF-1alpha in neutrophils stimulated with CoCl2 and extracellular proteins of Mtb in the immunocytochemistry and western blot. HIF-1alpha was not stabilized in neutrophils that were incubated with no stimulus ($n=3$, $p < 0.001$). In conclusion, extracellular proteins of Mtb stabilize HIF-1alpha in human neutrophils.

P22 CHARACTERIZATION OF THE NEUTROPHIL SUBPOPULATIONS INFILTRATION KINETICS IN THE LUNG OF MICE INFECTED WITH STREPTOCOCCUS PNEUMONIAE

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Streptococcus pneumoniae (S. pneumoniae) is a Gram-positive bacterium and the main cause of bacterial pneumonia worldwide. When S. pneumoniae invades the lower respiratory tract, it is recognized by immune cells, triggering the inflammatory response. Neutrophils have a crucial role in bacterial clearance and classically have been classified as pro-inflammatory cells. However, new studies reported that neutrophils can exert immunomodulatory activity during S. pneumoniae infection and modulate the inflammation in the lungs. Moreover, recent reports have shown that N1 and N2 neutrophil subpopulations are present in infected and uninfected mice. Therefore, the study aim was to evaluate the kinetics of differentiation of neutrophil subpopulations during S. pneumoniae infection. C57BL/6 mice were intranasally inoculated with 3x107 colony forming units (CFU) or PBS and lungs, blood, and spleen were evaluated at 3, 6, 12, 24, and 48 h post-infection (HPI). The results showed that the percentage of neutrophils increased significantly at 6HPI in the lungs and blood. Also, in lungs the N1/N2 neutrophil ratio was approximately 1:10 in uninfected mice, which increased significantly at 12 HPI and became 1:3 at 24 HPI; in contrast, in blood and spleen no changes were observed in this ratio until 12HPI. In conclusion, after infection there was a fast increase in the percentage of neutrophil in the lung and blood since 6 HPI, whereas the N1/N2 neutrophil ratio increased at 12 HPI in lungs. Funding: Agencia Nacional de Investigación y Desarrollo (ANID) - Millennium Science Initiative Program, Millennium Institute on Immunology and Immunotherapy (ICN09_016 / ICN 2021_045; former P09/016-F) and FONDECYT 1211060.

P23 STIM PROTEINS SUSTAIN LOW-AMPLITUDE SPONTANEOUS CA2+ OSCILLATIONS IN MOUSE BONE MARROW NEUTROPHILS

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Neutrophils protect the body from infectious pathogens by performing a wide range of functions relying on cytosolic Ca2+ elevations fuelled by STIM/Orai-mediated store-operated Ca2+-entry (SOCE). To establish the contribution of SOCE in Ca2+ signals driving neutrophil function, we used a Cebpα-driven Cre/Lox approach to generate mice lacking the two STIM isoforms and expressing the ratiometric Ca2+-sensitive probe "Salsa6f" (tdTomato (tdT+)-GCamp6f) in the myeloid lineage. 50% of tdT+ cells flushed from mice bone marrow (BM) were bona fide neutrophils (Ly6G+CD115-F4/80-), a percentage that increased to 87% in tdT+ cells stained with anti-Ly6G, enabling us to obtain >10 million BM neutrophils from a single mouse. In-situ calibration of tdT+Ly6G+ cells yielded a Salsa6f Kd of 160nM, slightly lower than Fura2 (225nM). Salsa6f reported spontaneous Ca2+ oscillations in neutrophils that were more frequent, of higher amplitude, and of reduced duration than with Fura2, validating the probe as a more efficient detector of low-amplitude signals. Fura2-AM loading prolonged the oscillations reported by Salsa6f, indicating that Fura2 distorts the Ca2+ signals by increasing intracellular Ca2+ buffering. The Ca2+ oscillations were abolished by the PLC inhibitor U73122 and absent in neutrophils lacking the Stim1/2 genes, consistent with the involvement of surface receptors coupled to the STIM/Orai pathway. Accordingly, Stim1/2-deficient neutrophils lacked a SOCE component when treated with the SERCA inhibitor thaspigargin. These data show that adherent neutrophils have a high degree of spontaneous Ca2+ activity that can be revealed by genetic Ca2+ indicators. Current experiments aim to establish the functional significance of these low-amplitude Ca2+ signals operated by STIM proteins.

P24 NEUTROPHIL DIVERSIFICATION – NEW OPTIONS IN MALIGNANT MELANOMA

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The cure of cancer patients is still hampered by the resistance of tumor cells against drugs and the immune system. Tumor-associated neutrophil granulocytes (TAN) form an important component of the immunological infiltrate of solid tumors, such as melanoma. Thus, the contribution of TANs to cancer progression and tumor immunity has been a matter of debate for decades. Due to their ability to actively migrate, TAN are recruited to the vicinity and center of melanomas and show enormous functional heterogeneity. These phenomena point to a new understanding of cancer as a highly plastic system, in which cell populations switch into each other depending on the current therapeutic, microenvironmental, and immunological context. Due to the lack of suitable preclinical models to date, the underlying mechanisms of this differential functionality (pro- vs. anti-tumor) are not well elucidated. As part of the Clinical Research Unit 337„PhenoTIme" (https://www.uni-due.de/phenotime/) the generation of novel innovative mouse models with transplanted or spontaneously developing melanomas clearly indicate a reciprocal phenotypic impact between cancer cells and neutrophils in the context of PD-1 targeted immunotherapy in melanoma. Data of tumor-induced proteomic changes in neutrophils in general, but also the impact of the tumor cell phenotype on neutrophil functions open the discussion on the use of neutrophil diversification in cancer therapy.

P25 MHC-II EXPRESSING NEUTROPHILS CIRCULATE IN BLOOD AND MILK DURING MASTITIS AND SHOW HIGH MICROBICIDAL ACTIVITY

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Bovine mastitis is mainly caused by bacterial infection and is responsible for important economic losses as well as alterations of the health and welfare of animals. The increase in the somatic cell count in milk during mastitis is mainly due to the influx of neutrophils which have a crucial role in the elimination of pathogens. For a long time, these first line defenders have been viewed as microbes' killers with limited role in the orchestration of the immune response. However, their role is more complex and we recently characterized an MHC-II expressing neutrophil subset with regulatory capacities in cattle. In this study, we questioned the implication of different neutrophils subsets in the mammary gland immunity during clinical and subclinical mastitis. We describe for the first time that, in blood as in milk, neutrophils are a heterogeneous population and encompass at least two subsets distinguishable by their expression of MHC-II. We observed higher bactericidal capacities of milk MHC-IIpos neutrophils as compared to their classical counterparts, due to a higher production of ROS and phagocytosis ability. MHC-IIpos neutrophils are enriched in milk during a subclinical mastitis as compared to blood. Moreover, we observed a positive and highly significant correlation between MHC-IIpos neutrophils and T lymphocytes present in milk during subclinical mastitis. To conclude, our study opens the way to the discovery of new biomarkers of mastitis inflammation.

P26 METABOLIC SHIFTS DURING IN VITRO HUMAN NEUTROPOIESIS IN DMSO-INDUCED HL-60 CELLS

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Quiescent hematopoietic stem and progenitor cells (HSPC) have low energy requirements and rely on glycolysis. Upon differentiation, shifts in energy sources occur through the translation of multiple enzymes that together activate metabolic pathways for high energy production. HSPC-derived neutrophil progenitors switch their metabolism towards oxidative phosphorylation. While energy sources have been described for resting HSCP and for mature neutrophils, there is currently no report directly identifying energy sources during the successive stages of human neutropoiesis. Here we aimed to unearth a temporal map of the alternating energy sources during the DMSO-mediated differentiation of HL-60 cells towards neutrophil-like cells. We used an oxygraph together with various inhibitors specifically targeting key members of the respiratory chain to measure oxygen consumption at 0, 3, and 8 days of differentiation. We confirmed sequential differentiation by measuring surface marker expression of CD15, CD16b, and CD11b using flow cytometry, and by evaluating morphology on cytospins. In addition, we evaluated activated metabolic pathways measuring carnitine palmitoyltransferase I (CPT1) activity. After 8 days of differentiation, >50% of the culture was composed of CD11b+CD15+ cells. There was an increased activity of CPT1 on day 4 of differentiation followed by a decrease on day 8, suggesting a shift towards mitochondrial oxidation of fatty acids. This work sheds light on the metabolic shifts occurring during human neutropoiesis and confirms the relevance of autophagy and lipid metabolism previously reported.

P27 THE LOCAL MICROENVIRONMENT DRIVES ACTIVATION OF NEUTROPHILS IN HUMAN BRAIN TUMORS

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Malignant brain tumors can be divided in primary gliomas and brain metastasis (BrMs), which are lethal and uncurable cancers. Novel therapeutic approaches focusing on immunotherapies have shown a response in a subset of BrMs, but generally not in gliomas. It is hypothesized that this poor efficacy is due in part to an immunosuppressive tumor microenvironment (TME). We observed an increased abundance of tumor-associated neutrophils (TANs) in aggressive gliomas and BrMs. However, the function of TANs in brain tumors and whether they contribute to an immunosuppressive TME is unknown. We therefore investigated TANs in human glioma and BrM tissue, along with neutrophils from the peripheral blood (PBNs) and performed the first in-depth analysis of their phenotypes and functions.Diverse profiling strategies were used, including sequential immunofluorescence staining, RNA sequencing, and ex vivo functional assays, allowing us to investigate TANs orthogonally. Interestingly, we revealed that brain TANs differ significantly from PBNs phenotypically and transcriptionally. TANs exhibit a distinct pro-inflammatory signature, which is especially enriched in BrMs. Meanwhile, immunosuppressive and pro-angiogenic phenotypes were similar in glioma and BrM TANs. The induction of TAN alterations was driven by soluble inflammatory mediators centered around TNF-alpha, which were predominantly produced by myeloid cells, including tumor-associated macrophages.This study highlights the role of the myeloid niche in sculpting an immunosuppressive and pro-angiogenic TAN phenotype in human brain tumors, which could thus explain the limited response to immunotherapies to date. We identified several potential therapeutic targets that may render the brain TME less immunosuppressive, which will be important to evaluate in future studies.

P28 INVESTIGATING NEUTROPHIL FUNCTION DURING SARS-COV2 INFECTION IN HUMAN AIRWAY EPITHELIAL CELLS FROM ELDERLY AND CHILDREN

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The COVID19 pandemic caused by SARS-CoV2 virus has disproportionally affected the elderly, accounting for around 85% of COVID19 deaths. There is little understanding regarding why COVID19 disease severity increases with age. Neutrophils appear in large numbers in the airways of severe COVID19 patients and it is unknown whether this influx of neutrophils in the airway has a protective or detrimental effect. We aim to understand the role of neutrophils during COVID19 pathology using an experimental infection model of the airway epithelium from children and the elderly. To do this, we utilised an existing scRNAseq dataset of airway neutrophils from COVID19 patients (Yoshida et al., 2021 Nature). We then used a neutrophil transepithelial migration model composed of fully differentiated nasal airway cells grown from healthy elderly and children at air-liquid interface. Once fully differentiated cells were infected with SARS-CoV2 for 24hrs prior to addition of neutrophils to the basolateral (blood) side. We then collected and measured the expression of activation markers and the number of neutrophils that migrate to the apical (air) side of the epithelium. Analysis of scRNAseq data has showed decreased expression of ICAM1, CD44 and ITGAX genes in neutrophils migrated to the COVID+ elderly airway, compared to the COVID+ paediatric airway. Preliminary work with the in vitro model (n=3) shows increased adherence of neutrophils to the infected epithelium across age groups, whilst a more activated neutrophil phenotype (CD11b+) was detected when migrating through the elderly epithelium. Overall, these findings point to an inflammatory neutrophil phenotype influenced by the damaged elderly epithelium and supports the hypothesis that neutrophils are responsible for the severity of disease.

P29 NOX2 NADPH OXIDASE LIMITS NEUTROPHIL DEGRANULATION BY ATTENUATING P38 MAPK ACTIVATION IN THE ORAL MUCOSA

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The leukocyte NADPH oxidase (NOX2) enzyme complex generates reactive oxygen species (ROS) that are essential for microbial killing and the regulation of host inflammatory responses. Here, we show that NOX2 activation is essential for regulating neutrophil recruitment and degranulation in oral mucosal tissues. Neutrophils are recruited to the gingival crevice in high numbers, where they interface with oral biofilm bacteria without inducing damaging inflammation. Our data show that CybbKO mice that lack the Nox2 NADPH oxidase had significantly elevated oral inflammatory burden characterized by soft tissue destruction and alveolar bone recession in murine models of oral inflammation (ligature-induced periodontitis). Severe disease in CybbKO mice was not related to defects in microbial killing but linked to significantly elevated recruitment of neutrophils to oral tissues and their localized activation. Mechanistic studies showed neutrophils from CybbKO mice showed significantly higher degranulation responses as measured by the upregulation of primary (CD63), secondary (CD11b), and tertiary granule markers (CD35) in response to multiple periodontal pathogens. This increase in degranulation correlated with increased p38 MAPK activation in CybbKO neutrophils compared to wild-type (WT) neutrophils. Inhibition of p38 MAPK by SB203580 attenuated hyper degranulation responses in CybbKO neutrophils. Overall, our studies show an important role of NADPH oxidase and derivative reactive oxygen species (ROS) in restraining neutrophil effector function at the oral mucosal barrier. These studies also shed mechanistic insights into the oral complications observed in patients with chronic granulomatous disease (CGD), a severe immunodeficiency caused by genetic defects in NADPH oxidase subunits.

P30 TARGETING NEUTROPHIL AGING PRESERVES CARDIOVASCULAR HEALTH

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Neutrophils are critical drivers of vascular inflammation, as seen during ischemic or vaso-occlusive events. However, targeting neutrophils has not been an option because unspecific inhibition compromises anti-microbial defense. The discovery of a cell-intrinsic clock that controls the circadian properties of neutrophils suggested that this could also drive inflammatory diseases that follow circadian patterns. Because CXCR4 is a key negative regulator of this clock, we examined whether this receptor could be targeted to interfere with neutrophil aging, and to prevent vascular damage. Through flow cytometry, migration assays, single-cell transcriptomics, infection models and intravital microscopy, we found that genetic and pharmacologic CXCR4 activation increased neutrophil migration but critically reduced pathogenic "behaviors" within inflamed vessels, without altering the immune response against pathogens. Using a model of acute myocardial infarction (AMI), we found that cardiac damage displayed diurnal variations in wild-type mice, which were lost in mice with genetically impaired neutrophil aging (Bmal1ΔN and CXCR4WHIM mice). Consistent with our model, administration of a commercial CXCR4 agonist (ATI2341) or custom-designed CXCR4 agonists (P1-3) dramatically improved vascular integrity in a mouse model of sickle cell disease and cardiac health during AMI. Notably, this effect was lost in mice with neutrophil-specific deficiency in CXCR4 (CXCR4ΔN mice), indicating that protection was through specific targeting of neutrophils. Our data demonstrate that the neutrophil clock can be targeted therapeutically to protect from cardiovascular disease.

P31 THE EFFECT OF THE ANTIOXIDANT SYSTEM; VITAMIN E, VITAMIN C, GSH, AND NAC ON NET FORMATION

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During NET formation reactive oxygen species (ROS) are produced to eliminate pathogens but also can cause host damage, hence redox control is important in this regard. The vitamins E, C, and glutathione (GSH) are antioxidants that work in synergy inside the cell and can be found in the bloodstream (30 μM, 100 μM, and 800 μM respectively). The chemical nature, redox potential, and localization of these antioxidants improve the cell's capacity to face ROS-induced damage and maintain the redox balance. The neutrophils have high concentrations of vitamin C (1500 μM) that interacts with endogenous vitamin E and GSH, but during NET formation these concentrations may be insufficient. Previous studies have shown that vitamin E, C, or N-acetyl cysteine (a GSH-precursor) are capable to reduce NETs in vitro, but their combined effect remains unknown. Methods: Neutrophils were pre-loaded with vitamins E, C, GSH, and N-acetyl cysteine (NAC), alone (one antioxidant) or in combination (2, 3, or 4 antioxidants) at different concentrations for 60 minutes. Later NETs were induced by incubating neutrophils with LPS from E. coli for 180 minutes. The NET formation was assessed by fluorometry using Sytox Green, and fluorescent microscopy with DAPI and anti-neutrophil elastase. The total antioxidant capacity (TAC) was evaluated before and after NET induction. The GSH/GSSG ratio, and malondialdehyde (MDA) determination were performed after NET induction. Results: Vitamin E, vitamin C, GSH, and NAC, induce a suppressive effect of NET release that is improved when 2 or 4 antioxidants are added simultaneously. Additionally, preliminary results show an increase in GSH/GSSG ratio and lower MDA levels. These results suggest that antioxidant NET control is through an improvement in the redox balance.

P32 NEUTROPHILS ENCOMPASS A REGULATORY SUBSET SUPPRESSING T CELLS IN APPARENTLY HEALTHY CATTLE AND MICE

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Neutrophils that reside in the bone marrow are swiftly recruited from circulating blood to fight infections. For a long time, these first line defenders were considered as microbe killers. However their role is far more complex as cross talk with T cells or dendritic cells have been described for human or mouse neutrophils. In cattle, these new roles are not documented yet. We identified a new subset of regulatory neutrophils that is present in the mouse bone marrow or circulate in cattle blood under steady state conditions.

These regulatory neutrophils that display MHC-II on the surface are morphologically indistinguishable from classical MHC-IIneg neutrophils. However MHC-IIpos and MHC-IIneg neutrophils display distinct transcriptomic profiles. While MHC-IIneg and MHC-IIpos neutrophils display similar bacterial phagocytosis or killing activity, MHC-IIpos only are able to suppress T cell proliferation under contact-dependent mechanisms. Regulatory neutrophils are highly enriched in lymphoid organs as compared to their MHC-IIneg counterparts and in the mouse they express PDL-1, an immune checkpoint involved in T-cell blockade. Our results emphasize neutrophils as true partners of the adaptive immune response, including in domestic species. They open the way for discovery of new biomarkers and therapeutic interventions to better control cattle diseases.

P33 OBJECTIVE QUANTIFICATION OF NEUTROPHIL MATURATION BY LIVE 3D IMAGING

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Recent studies have identified the heterogeneity in the neutrophil compartment as an important marker of inflammatory disease and cancer. Advanced technologies such as transcriptomics or mass cytometry have been used to characterise neutrophil subsets based on their molecular signatures. In clinical diagnostics, neutrophils have been largely characterised by nuclear morphology using manual counting of 2D colorimetric microscopy images. However, this approach is not quantitative enough, potentially alters the shape of the nucleus and suffers from low resolution and observer bias. Therefore, we are developing a more advanced method to characterize the nuclear morphology of live neutrophils in 3D. Hoxb8 cells, immortalized murine hematopoietic progenitors, can be differentiated into mature neutrophils in vitro, recapitulating the stages of murine neutrophil differentiation. We have engineered Hoxb8 cells to constitutively express GFP in the nuclear membrane with the LentiCRISPR-Cas9 technology. Using the cutting-edge Zeiss Lattice Lightsheet 7 microscope, we have optimised the quantitative and resolute analysis of changes in neutrophil nuclear morphology during their maturation live and in 3D. Moreover, we began to develop a machine-learning algorithm to automatically segment and classify the stages of neutrophil differentiation. We have also generated genetically altered HoxB8 cells with specific knockout of key transcription factors impacting neutrophil differentiation. This novel approach will assist in furthering fundamental neutrophil biology by combining molecular, morphological and behavioural signatures. It may also open new avenues in diagnostics of inflammatory disorders and cancer characterised by abnormal neutrophil subsets.

P34 INVESTIGATING THE IMPACT OF NEUTROPHILS ON NATURAL KILLER CELL ACTIVITY IN BREAST CANCER LIVER METASTASIS

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Breast cancer is the most common cancer diagnosed among women. Four out of five women diagnosed with metastatic breast cancer, where the primary cancer has spread to different organs, die within five years of diagnosis. To metastasize, cancer cells need to escape the detection of the immune system. Interestingly, neutrophils in cancer patients are phenotypically immature compared to healthy individuals. Immature neutrophils have been implicated in promoting the ability of breast cancer cells to metastasize to various sites. Our unpublished data suggest that immature neutrophils can enhance metastasis via natural killer (NK) cell suppression. We hypothesize that immature neutrophils that infiltrate the liver-metastatic microenvironment promote metastasis by suppressing NK-mediated tumor cell killing. RESULTS: Employing a syngeneic mouse model of triple negative breast cancer, we characterized NK cells isolated from liver metastasis bearing mice via flow cytometry. An increase in CD49a+ NK cells and a decrease in CD49b+ NK cells was observed in liver metastasis bearing mice compared healthy mice. We are assessing the direct cytotoxicity of CD49a+ and CD49b+ NK cells on cancer cells and co-culture different neutrophil subsets (immature vs mature) with NK cells to demonstrate the conversion of CD49b+ to CD49a+ NK cells ex-vivo. CONCLUSION: The objective of this project is to gain insights into how immature neutrophils enhance the formation of breast cancer liver metastases. Our results suggest that immature neutrophils induce the conversion of cytotoxic CD49b+ NK cells into pro-inflammatory, helper-like CD49a+ NK cells, which reduces NK-mediated tumor cell killing and increased breast cancer liver metastasis.

P35 NEUTROPHILS AS PROTAGONISTS IN SEVERE MALARIA

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Malaria, caused by the protozoan parasite P. falciparum, annually affects over 200 million people and leads to approximately half a million deaths, mostly in children under the age of five. In the bloodstream, neutrophils co-exist at high density and close proximity to Plasmodium infected red cells (iRBCs). Despite this, little is known about interactions between neutrophils and malaria parasites.

We show that neutrophils have a central role in severe malaria. Activation of neutrophils by cell-free heme induces NETs, which promote malaria immunopathogenesis. Using patient samples and a mouse model, we define two mechanisms of NET-mediated inflammation of the vasculature: activation of emergency granulopoiesis via granulocyte colony-stimulating factor (GCSF) production and induction of the endothelial cytoadhesion receptor intercellular adhesion molecule-1 (ICAM-1). NET components released by circulating DNAse 1 facilitate parasite sequestration on activated endothelial beds and this can be blocked by antibody-mediated GCSF neutralisation. We propose inhibition of NETs as a treatment strategy in severe malaria.

P36 DECIPHERING THE HETEROGENEITY OF HUMAN NEUTROPHILS IN HOMEOSTASIS

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Controversy remains regarding the efficacy of neutrophil transfusions (GTX). While some centers consider them a common life-saving practice for infected neutropenic patients, others do not have such success. Parameters that vary among centers include donor selection, mobilization and collection protocols. Despite their traditional description as a single homogenous population, recent results evidenced functionally distinct neutrophil populations in according to clinical and demographic parameters. Here, we aim to identify neutrophil subsets in health according to gene expression profiles. We collected 25 open-access studies reporting transcriptomes from 165 peripheral blood healthy(control) human neutrophils. In a preliminary analysis of 70 samples from 13 of these independent studies and using non-negative matrix factorization, we identified k=3 as the best cophenetic coefficient suggesting 3 general transcriptional profiles. These clusters underwent gene-set enrichment analysis using the Molecular Signature Database as reference, revealing that a) cluster 1 has upregulated genes for Interferon and cytokine signaling pathways, b) cluster 2 shows upregulation of oxidative phosphorylation genes and c) cluster 3 exhibits a downregulation of genes of the respiratory electron transport and interferon signaling pathways. This pioneering work may eventually permit the subclassification of neutrophil transcriptional profiles according to processing methods and donor demographic data. This may in turn help identifying the best donor profile and processing protocols to improve GTX therapy outcomes in neutropenic patients.

P37 THE ROLE OF NFAT IN HUMAN NEUTROPHILS

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Calcineurin (CN) - Nuclear factor of activated T-cells (NFAT) inhibitors are commonly used immunosuppressants to inhibit adaptive immunity, but they frequently leave patients vulnerable to opportunistic infections. CN-NFAT is not a pathway exclusively used by T-cells but is active also in others, including innate immune cells, where inhibition of CN-NFAT undesirably affects anti-infectious responses. We and others showed the importance of CN-NFAT in monocytes and dendritic cells, while the impact on neutrophils' function after NFAT inhibition remains elusive.

We evaluated the effects of CN-NFAT inhibitors on neutrophil response induced by heat-killed Candida albicans, Aspergillus fumigatus, and other pattern recognition receptors (PRRs) ligands. We performed RNAseq to see global changes in expression and qPCR analysis of selected genes to confirm changes in the expression of important functional and regulatory molecules and used ELISA method for analysis at the protein level. We showed that after PRRs activation, neutrophils initiate the expression of molecules involved in inflammation regulation. Their expression is disturbed in the presence of CN-NFAT inhibitors. After CN NFAT inhibitors treatment, human neutrophils cannot sufficiently produce chemotactic cytokines CLL-2 and CCL-3; an expression of inflammation-mediating molecule COX-2 is also inhibited. Together with the already published adverse effects of CN-NFAT inhibitors on other myeloid cells, these findings can help us explain the increased vulnerability of CN-NFAT inhibitors-treated patients.

P38 *Trypanosoma brucei* BRUCEI-INDUCED AGGREGATED NETS (AGGNETS) DEPEND ON P2X1 AND P2Y6 PURINERGIC RECEPTORS

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T. brucei is an euglenozoan parasite that is able to infect a wide range of hosts, including humans and bovines. In cattle, *T. b. brucei* is one of the pathogenic agents causing animal african trypanosomiasis (AAT) or Nagana disease. Host-infection occurs when tsetse flies transmit infective metacyclic stages via the saliva while taking a blood meal from a host. In the vertebrate host, *T. b. brucei* lives extracellularly in the host blood and multiplies as trypomastigote forms, thus directly interacting with cells of the innate immune system, such as neutrophils. Studies on interactions between the bloodstream trypomastigote form of *T. b. brucei* and bovine neutrophils, shows that *T. b. brucei*-driven early activation of neutrophils is characterized by an increased oxygen consumption rate (OCR) and glycolysis. After 4 h of co-incubation, *T. b. brucei* predominantly induces aggregated NETs (aggNETs). Given that aggNETs were also observed after 18 h of co-incubation, this NET phenotype appeared stable in vitro. NET phenotypes and the volume of *T. b. brucei*-induced aggNETs were determined. Finally, we explored the role of purinergic signalling using chemical inhibition. Current data showed that pre-treatments of bovine neutrophils with the P2X1 inhibitor NF449 and with the P2Y6 inhibitor MRS2578 decreased OCR, ECAR and the formation of *T. b. brucei*-triggered aggNETs, highlighting the role of the purinergic signaling in NET formation triggered by *T. b. brucei*.

P39 THE ROLE OF NEUTROPHILS IN METASTATIC NON-SMALL CELL LUNG CANCER

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Background: Non-small cell lung cancer (NSCLC) accounts for approximately 85% of lung cancers. Neutrophils are a heterogenous population and can interact with T cells. Neutrophils and T cells are found in malignant pleural effusions. The objective of this study is to gain a better understanding of neutrophil interaction with T cells in the NSCLC pleural fluid metastatic niche. Methods: Healthy donor T cells were cultured with and without healthy donor neutrophils in the presence and absence of pleural fluid from NSCLC patients or from pneumonia patients for comparison. T cell proliferation was determined using CellTraceTM CSFE Cell Proliferation Kit and flow cytometry analysis. Results: When T cells were cultured with pleural fluid (NSCLC or pneumonia), there was an increase in the percentage of T cells that divided compared to T cells cultured without pleural fluid. Adding neutrophils into the co-culture model suppressed this pleural fluid up-lift, resulting in fewer T cells entering into proliferation. The T cells that did enter proliferation were able to proliferate to the same degree. This pattern was observed for healthy donor CD8 and CD4 T cells. Conclusion: This data suggests the presence of NSCLC or pneumonia pleural fluid plus neutrophils causes a suppressive signal on T cell proliferation which may impede T cell anti-tumour responses. The focus of future work is to determine possible signalling mechanisms to account for this observed T cell suppression.

P40 AN IN VITRO SYSTEM TO ELUCIDATE THE ROLE OF CD16b IN HUMAN NEUTROPHILS

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Opsonizing-IgG binding of Fc gamma receptors on the surface of neutrophils initiates an immune response that culminates in the elimination of opsonized pathogens. The Fc gamma receptor CD16b is the most abundant Fc receptor on human neutrophil surfaces and its crosslinking leads to activation, prompting ROS production, phagocytosis, and NETs release. The literature has reported human cases lacking CD16b without increased morbidities. In these individuals, it is unclear if the absence of CD16b is balanced by additional Fc receptors, if there is a compensatory mechanism by other immune cells, or if the absence of CD16b is uncompensated without adverse events. Additionally, the exact function of CD16b remains unclear, particularly in the light of the absence of an intracellular domain. Here, we produced a human neutrophilic cell line lacking CD16b, to specifically evaluate the role of CD16b in neutrophil activation by opsonized pathogens. We developed and applied a CRISPR/Cas9 system to knock-out CD16b in human promyelocytic cells HL-60. Single CD16bneg clones were sorted and expanded, and the absence of CD16b was validated by flow cytometry. Control HL-60 cells were differentiated using DMSO over 7 days, yielding a heterogeneous culture composed of neutrophil-like cells as evidenced by cytospins and close to 72% of positivity for neutrophil markers (CD15, CD11b, CD16b, and CD32). Differentiated neutrophil-like cells exhibited phagocytic activity comparable to blood neutrophils. In conclusion, we present a novel cell line to elucidate the function of CD16b in human neutrophils in vitro.

P41 SKAP2 DELETION RESTRICTS BETA-2 INTEGRIN DYNAMICS IN NEUTROPHIL EFFECTOR FUNCTION

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Background: The beta-2 integrin plays a substantial role in neutrophil responses against pathogens, as well as in neutrophil cytotoxicity against antibody-opsonized tumor cells (ADCC). Upon neutrophil activation, the beta-2 integrin induces a tightly modulated signaling cascade that involves Kindlin3, an essential activator of the integrin. However, the signaling pathways and proteins involved in beta-2 integrin affinity conformation have remained elusive. Objective: In this work, we studied the role of Skap2 in the dynamics of beta-2 integrin activation. Methods: We used NB4 cells as a model system to perform an unbiased screening method for potential beta-2 integrin binding partners, namely biotin identification (BioID). Among others, we identified Skap2 as a potential regulator of beta-2 integrin function. Its role in beta-2 integrin regulation was further explored by generating NB4 Skap2 knock out cells and performing adhesion and affinity binding assays using acoustic force spectroscopy. In addition, the consequences of Skap2 deletion were evaluated in assays detecting ROS production, live 3D phagocytosis and ADCC. Results: We found that Skap2 binds to beta-2 integrin in its inactive form. Skap2 is indispensable for beta-2 integrin de-activation and Skap2 deficiency results in impaired beta-2 integrin cluster formation and beta-2 integrin-dependent phagocytosis. Skap2 deficiency significantly reduced ADCC of NB4 cells against different solid tumors, suggesting that Skap2 is an important positive regulator of neutrophil ADCC through beta-2 integrins. Conclusion: These findings reveal that Skap2 is involved in beta-2 integrin de-activation, and provide further understanding of beta-2 integrin regulation in different neutrophil effector functions.

P42 EFFECTS OF THE ALARMIN S100A8/A9 ON THE FORMATION OF PLATELET-NEUTROPHIL COMPLEXES DURING ACUTE INFLAMMATION

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Platelets may modulate immune responses by a direct interaction with leukocytes resulting in their recruitment and activation. During infections phagocytes release the endogenous alarmin S100A8/A9 which acts via binding to toll-like receptor 4. Hitherto, the role of S100A8/A9 in the formation of platelet-neutrophil complexes during acute inflammation is virtually unknown. By using WT, S100A9-/- and TLR4PF4Cre mice, we investigated the impact of S100A8/A9 on the formation of platelet neutrophil complexes. Image Stream analyses of murine whole blood samples revealed that S100A9 deficiency results in significantly increased numbers of platelet-neutrophil complexes upon stimulation. Intravital microscopy of the TNF-α inflamed murine cremaster muscle demonstrated not only increased numbers of platelet-neutrophil complexes in S100A9-/- mice, but increased rolling velocities coinciding with decreased numbers of adherent and transmigrated leukocytes compared to wildtype controls. A murine model of Klebsiella pneumoniae-induced pneumonia further indicated a defect of neutrophil recruitment in S100A9-/- and TLR4PF4Cre mice. These effects were simulated in an in vitro bilayer assay which further demonstrated that platelets primarily remain in the vasculature-like compartment. However, the underlying molecular mechanisms remain to be elucidated. In conclusion, S100A8/A9 shapes the interaction of platelets and neutrophils. These findings will be further investigated with the aim to develop novel therapeutic interventions in patients suffering from acute respiratory distress syndrome.

P43 THE ROLE OF PLATELET-NEUTROPHIL INTERACTIONS DURING YERSINIA PSEUDOTUBERCULOSIS INFECTIONS

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RNAseq analysis of Yersinia pseudotuberculosis (YP)-infected mice showed an enrichment in neutrophil-, platelet- and NET (Neutrophil extracellular traps) transcripts 5 days post infection. YP induces NETs in neutrophils in vitro. Platelets impact neutrophil recruitment and activation. The aim of this study is to investigate the role of platelet-neutrophil interactions and NET formation upon YP infection. To investigate the neutrophil response after exposure to YP, we infected murine bone marrow-derived neutrophils with a wildtype YP strain (YPIII), a virulence-plasmid cured strain (YP12) or single Yop (yersinia outer protein)-deficient mutants, in the presence or absence of platelets. We examined the internalization of bacteria, as well as neutrophil activation and NET formation via flow cytometry.Upon infection with YP12, neutrophils were activated, as measured by increased Mac-1, decreased L-Selectin and PSGL-1 surface expression. Infection with YPIII resulted in reduced activation compared to YP12-infected cells. YP12 was significantly more internalized by neutrophils than YPIII. Loss of YopH in Yersinia restored the neutrophils' capacity to internalize the bacteria. Platelets did not significantly alter internalization, nor expression of Mac-1, L-Selectin or PSGL-1. Neutrophils released NETs in response to YP12, whereas NET formation after infection with YPIII did not differ from uninfected cells.Our results show that YPIII partly disrupts neutrophil activation, phagocytosis and NET formation in vitro and YopH appears to be a critical contributing factor.

P44 CASE OF MISTAKEN IDENTITIES: ATYPICAL EFFEROCYTOSIS OF LIVE NEUTROPHILS SUSTAIN INFLAMMATORY MACROPHAGE BEHAVIOR DURING P. GINGVALIS INFECTION

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ABSTRACT: During an infection, macrophages efferocytose and subsequently degrade apoptotic neutrophils within specialized intracellular compartments called efferosomes. The recognition of apoptosis-associated ligands, such as phosphatidylserine (PS), on dying cells by macrophage efferocytic receptors ensures the specific uptake of apoptotic cells and the exclusion of live cells. Here, we show that the through the production of proteases (gingipains), oral pathogen Porphyromonas gingivalis dysregulates efferocytosis by mediating the uptake of live neutrophils in a PS-independent manner. This 'atypical efferocytosis' of gingipain exposed live neutrophils (gLN) led to dysregulated inflammatory responses characterized by significantly higher expression of pro-inflammatory cytokines and prolonged activation of NF-kB. Interestingly, restoring PS signaling by the addition of PS liposomes in macrophages undergoing atypical efferocytosis was sufficient to dampen inflammatory cytokine production via PPARγ mediated antagonism of NF-kB. Furthermore, we found that while apoptotic neutrophils were cleared by progressive acidification and efferosome maturation, gLNs were retained within efferosomes. This delayed degradation was linked to slower recruitment of Rab5 and Rab7, and delayed lysosomal fusion. gLNs containing efferosomes had significantly lower acidification and proteolysis. Efficient digestion of the engulfed cargo is essential for reprogramming macrophages and the suppression of inflammation. Our data show that macrophages containing gLNs failed to undergo functional and phenotypic shifts towards a pro-resolving state. Thus, we show that P. gingivalis uses a novel mechanism to dysregulate efferocytosis by causing the uptake of live neutrophils in a PS-independent manner. This atypical efferocytosis dysregulates inflammation via delayed proteolytic degradation of ingested cargo and sustained production of inflammatory cytokines.

P45 RESTRAINING NEUTROPHILIC RESPONSES IN THE ORAL MUCOSA – A NEW ROLE FOR THE NADPH OXIDASE

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Congenital defects in neutrophil recruitment and activation are clinically associated with early onset periodontal disease and oral inflammation. Chronic granulomatous disease (CGD) is a life-threatening immunodeficiency caused by inactivating mutations in the leukocyte NADPH oxidase subunit genes. NADPH oxidase generates superoxide, which is rapidly converted to reactive oxygen species (ROS) that play anti-microbial and immunoregulatory roles. CGD patients suffer life-threatening infections and unrelated inflammatory complications, mechanistically linking ROS to the regulation of host inflammatory responses. However, the full extent of oral complications in CGD patients is incompletely understood. CybbKO mice that specifically lack NADPH oxidase activity due to deletion of Nox2/gp91phox subunit exhibited significantly higher mobilization and recruitment of neutrophils into gingival tissues, which correlated with augmented alveolar bone loss in a murine model of periodontitis compared to wild type mice. RNA-seq analysis of gingival tissues showed enrichment of several pro-inflammatory genes, neutrophil granule proteins, and genes associated with osteoclast activity. Strikingly, we found that the lack of Nox2 NADPH oxidase resulted in a failure to activate Nrf2-mediated antioxidant and anti-inflammatory pathways. Restoring Nrf2 function by synthetic agonists or bacterial metabolites was sufficient to block dysregulated neutrophilic responses and alveolar bone loss. Thus, our studies demonstrate that NADPH oxidase-derived ROS are essential for limiting gingival inflammation, in part by redox modulation of Nrf2 mediated pathways and neutrophil responses. Contrary to the established paradigm, our studies demonstrate that low-level generation of oxidants is essential for limiting inflammatory responses within the oral mucosa.

P46 DEVELOPMENT OF THE NOVEL CATC INHIBITOR BI 1291583 FOR THE TREATMENT OF PATIENTS WITH BRONCHIECTASIS

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Bronchiectasis is a heterogeneous respiratory disease characterised by chronic neutrophilic airway inflammation. Uncontrolled neutrophil-derived serine proteases (NSPs), including neutrophil elastase (NE), are implicated in exacerbations and disease progression. As cathepsin C (CatC; dipeptidyl peptidase 1) activates NSPs in bone marrow, CatC inhibition could reduce inflammation in the lung in bronchiectasis. Here we summarise the current development of the novel CatC inhibitor BI 1291583. Preclinical analyses included in vitro CatC binding kinetics and inhibition, cathepsin selectivity, and inhibition of active NE production in cells and lipopolysaccharide-challenged mice. Phase 1 studies in healthy subjects assessed single- and multiple-rising-dose safety, tolerability, pharmacokinetics and pharmacodynamics, relative bioavailability under fasted/fed conditions and effects of itraconazole coadministration. BI 1291583 bound isolated human CatC with an IC 50 of 0.9nM, displayed a >6000-fold selectivity for CatC vs related cathepsins, inhibited active NE production in neutrophil progenitor cells with an IC 50 of 0.7nM, and displayed up to 99% inhibition of active NE production in vivo. BI 1291583 was readily absorbed, safe and well-tolerated in healthy subjects, and dose-dependently inhibited CatC, resulting in up to 86% inhibition of blood NE activity vs placebo. These data informed the design of Airleaf™, an ongoing Phase 2 trial of BI 1291583 (1mg, 2.5mg, 5mg daily vs placebo) in adults with bronchiectasis and a history of ≥2 exacerbations in the past year, or 1 exacerbation and high symptom burden (St. George's Respiratory Questionnaire). The primary outcome is time to first exacerbation. CONCLUSION SBI 1291583 is a fully reversible, highly potent and highly selective inhibitor of CatC that is well tolerated and reduces NE activity in healthy subjects. Airleaf™ will determine the efficacy and safety of BI 1291583 in adults with bronchiectasis.

P47 "INFLAMAPPING" WITH GEM-LTB4 : LIVE VISUALIZATION OF LTB4 GRADIENTS WITH A NOVEL FLUORESCENT BIOSENSOR

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Gradients of chemoattractants orchestrate cellular migration, however, their direct visualization and live measurement is challenging. Here, we report on the development of GEM-LTB4, a genetically encoded fluorescent biosensor for leukotriene B4 (LTB4), a central inflammatory lipid mediator involved in chemotaxis and neutrophil swarming. GEM-LTB4 is structurally based on the G protein-coupled high-affinity LTB4 receptor (BLT1), shows sensitivity in the low nanomolar concentration range, high specificity, and a robust fluorescence increase in response to LTB4. Using this genetically encoded sensor, we monitored the live release of LTB4 from bonemarrow-derived mouse neutrophils and were able to detect LTB4 gradients derived from single neutrophil granulocytes. Furthermore, we created transgenic zebrafish lines expressing GEM-LTB4 in basal and superficial keratinocytes, which enabled the direct measurement and quantification of gradients resulting from exogenously applied LTB4. Finally, these transgenic lines allowed us for the first time to visualize chemoattractant gradients in vivo: with GEM-LTB4, we can detect and map how LTB4 is released from activated neutrophils. The LTB4 sensor presented here is the first in a planned line of GEM-sensors: GPCR-based biosensors for Extracellular Mapping. Our long-term goal with GEM-sensors is to create a toolbox for "Inflamapping", the spatiotemporal visualization of central inflammatory mediators in live tissues. GEM-sensors for IL8 and fMLP are already in development, and further ones are to come.

P48 ESSENTIAL ROLE OF PROSTAGLANDIN E2 FOR THE GENERATION OF UNIQUE CHARACTERISTICS OF LUNG NEUTROPHILS

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Neutrophils are first-line defenders eliminating pathogens with various bactericidal activities without additional transcriptional activation. Accumulating evidence suggests that there are heterogenous neutrophil populations that reside different tissues. Lung resident neutrophils should be tightly regulated to avoid damage to fragile alveolar structures. We show that lung neutrophils (LNs) express distinct surface proteins and genes that definitely distinguish LNs from other neutrophils such as bone marrow and blood neutrophils. LNs show impaired migratory activity toward chemoattractants and produce less amounts of TNF-alpha in response to LPS. We found that transferred labeled bone marrow neutrophils intravenously show similar surface marker expression like endogenous LNs. Stimulation of bone marrow neutrophils with bronchoalveolar lavage fluid elicits LN-associated characteristics. On the crucial molecular cue that maintain LN phenotype, we demonstrate that prostaglandin E2 (PGE2) is a key factor for the generation of LNs with unique immune suppressive characteristics. Inhibition of PKA, a downstream molecule of PGE2-induced signaling, or Tgm2 deficiency diminished anti-apoptotic and anti-inflammatory characteristics and exacerbated LPS-induced acute respiratory distress syndrome with increased production of inflammatory cytokines. Collectively, we show that LNs mediate protective activity of the lungs against pathogenic acute respiratory distress syndrome.

P49 AIRWAY PROTEOMICS IDENTIFIES NEUTROPHIL DERIVED AZUROCIDIN-1 AS A MARKER OF DISEASE SEVERITY AND MICROBIAL DYSBIOSIS IN CHRONIC LUNG DISEASE

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Introduction: Neutrophilic inflammation is a driver of pathophysiology in chronic lung disease. Azuorocidin-1 (AZU1) is a pseudoprotease granule protein implicated in sepsis. This study evaluated the role of AZU1 in inflammatory lung disease. Methods: bronchoalveolar lavage (BAL) was analysed by LC/MS from 43 bronchiectasis (BE) patients and 10 healthy controls. Sputum proteomic studies compared COPD (23), BE (41) and cystic fibrosis (CF;35). Sputum AZU1 was measured by ELISA in 199 BE patients from 3 countries. 20 BE patients were treated with 2-week IV antibiotics to test effects on sputum proteome. 56 BE patients were treated with long-term azithromycin to evaluate the effect of macrolides. Results: AZU1 was significantly higher in BE vs. control BAL ($p=0.01$) and correlated with markers of neutrophilic inflammation. Sputum AZU1 was elevated in CF and BE vs. COPD ($p<0.0001$). AZU1 measured by LC/MS was associated with disease severity. Validation in 199 patients found AZU1 (median 6.5ug/ml IQR 0.73-25.9ug/ml) was associated with BE severity index ($r=0.47,p<0.0001$), quality of life ($r=-0.42,p<0.0001$), FEV1%predicted ($r=-0.48,p<0.0001$) and exacerbations ($p<0.0001$). AZU1 was associated with P. aeruginosa infection by culture and 16S rRNAseq. 2-week antibiotic treatment had no significant effect on sputum AZU1 ($p=0.3$). AZU1 was reduced by -0.47 log units (95% CI -0.907 to -0.039,$p=0.033$) with long-term macrolide in patients with neutrophilic inflammation. Conclusion: AZU1 is associated with disease severity in inflammatory lung disease and may be a novel therapeutic target.

P50 MITOCHONDRIA INDUCED NETS FORMATION IS ENHANCED IN ELDERLY INDIVIDUALS

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In severe COVID-19, the dysregulated inflammatory response includes excessive formation of neutrophil extracellular traps (NETs). The inducing agent is unknown, but our preliminary experiments show that the underlying mechanism is sterile inflammation induced by mitochondria rather than the virus itself. Since age is the main clinical risk factor for poor outcome, we hypothesized that sterile stimuli would induce NETs formation in an age-dependent manner and analyzed neutrophil response to mitochondria as well as investigated the potential mechanism in individuals of different age groups. When compared to healthy adults, neutrophils of elderly individuals (>80 years old) formed 2.7-times more NETs in response to mitochondria and these NETs were 20% more resistant to degradation by DNase I. Plasma of the elderly contained 2.1-times more NETs, was 1.4-times more potent TLR9 activator and induced 4.3-times more NETs when incubated with neutrophils from healthy adults. In a mouse model of acute lung injury, intratracheal mitochondria administration led to 50% lower neutrophil migration but 2-times higher relative NETs formation in the bronchoalveolar lavage of old vs. young mice. We therefore conclude that undegraded residual NETs in plasma of the elderly prime neutrophils via TLR9 signaling, resulting in an increased reactivity towards sterile stimuli, which may exacerbate the ongoing inflammation. Whether this vicious circle is a suitable therapeutic target remains unclear. This work was supported by grants APVV-21-0378, PP-COVID-20-0016 and VEGA 1/0716/20.

P51 DYSREGULATED GLYCOLYSIS IN RHEUMATOID ARTHRITIS NEUTROPHILS

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Background Glycolysis drives pathogenic ROS production and release of neutrophil extracellular traps (NETs), and therefore glucose availability is essential for neutrophil function. The aim of this work was to determine how glucose availability affects apoptosis, ROS and NET production and how this differs between healthy control (HC) and rheumatoid arthritis (RA) neutrophils. Methods Neutrophils were isolated from HC (n=4) and people with RA (n=4) and resuspended in RPMI media with and without glucose (11 mM). Inhibitor of glycolysis (2-DG, 50mM) was added and neutrophils incubated for 15 min before activation with PMA (1ug/ml). ROS was measured for 50 min by luminol-enhanced chemiluminescence and oxygen consumption rate (OCR) was measured by Seahorse XFe96. NETosis was measured by sytox green fluorescence at 4h and apoptosis by flow cytometry after AnnexinV/PI staining at 4h and 24h. RNA was isolated from 0h HC (n=11) and RA (n=53) neutrophils and quantified using RNAseq. Results RNAseq identified genes involved in glucose metabolism as being significantly higher in RA compared to HC neutrophils. Inhibition of hexokinase (2-DG) significantly decreased OCR, ROS and NET production in the presence of glucose ($p<0.05$). The absence of glucose in the media did not decrease ROS and NET production. The number of live/dead neutrophils was not affected by glucose depleted media, however in the absence of glycolysis, the proportion of dead neutrophils that were double annexin V/PI positive was increased. Discussion This study highlights the metabolic plasticity of activated neutrophils and their dependence on glycolysis. ROS and NET production is sustained in deprived glucose environments like synovial fluid, indicating cytosol free glucose acts as a reserve fuel to maintain the activated phenotype.

P52 AN INTERFERON-BETA - FPR2 AXIS PROMOTES THE RESOLUTION OF ACUTE LUNG INFLAMMATION

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Aberrant neutrophil activation underlies acute respiratory distress syndrome. We have shown that interferon-beta (IFN-b) governs the resolution of inflammation by promoting clearance of neutrophils from the lung. However, the underlying mechanisms are incompletely understood, though critical for implementing treatment with IFN-b. In mice, E. coli-induced acute lung injury, is characterized by activation of TLR9, impaired pulmonary bacterial clearance despite massive neutrophil accumulation, suppressed IFN-b production, and aggravated tissue injury. This would delay the resolution of inflammation. Neutralization of endogenous IFN-b impaired killing of E. coli and aggravated tissue injury. Conversely, treatment of mice with IFN-b accelerated clearance of bacteria, attenuated neutrophil accumulation, restored impaired neutrophil phagocytosis, and promoted phagocytosis-induced neutrophil apoptosis and efferocytosis, thereby accelerated resolution of inflammation. Furthermore, IFN-b markedly enhanced lavage fluid levels of 15-epi-LXA4 and RvD1, and selective blockade of ALX/FPR2 partially attenuated IFN-b-driven resolution. Our results identify a critical role for IFN-b in the timely resolution of neutrophil-driven lung inflammation and indicate that this action is largely mediated through generation of pro-resolving lipid mediators that signal through ALX/FPR2. Grant support: Canadian Institutes of Health Research MOP-97742 and MOP-102619.

P53 EFFECTS OF HIGHLY EFFECTIVE MODULATOR TREATMENT (HEMT) ON NEUTROPHIL DYSFUNCTION IN CYSTIC FIBROSIS

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Cystic Fibrosis (CF) is a common genetic disease that causes severe lung damage and leads to life-threatening infections. CF has no cure, but novel therapeutic drugs have emerged targeting defects in the CFTR channel, including the highly effective CFTR modulator therapy elexacaftor/tezacaftor/ivacaftor (ETI). ETI therapy has dramatically improved lung function and life expectancy in eligible patients with drug access, however, bacterial infections persist and the effects of these treatments in restoring phagocytic cell's antimicrobial functions are unclear. Peripheral blood was collected from patients carrying delF508-CFTR mutation and healthy donors (non-CF). Neutrophils were treated with ETI combination and then infected with B. cenocepacia. The cellular expression of CFTR was assessed by microscopy. Furthermore, antimicrobial killing, and the production of Neutrophil Extracellular Traps (NETs) were evaluated. CF and non-CF neutrophils treated in vitro with ETI increased the expression and function of the CFTR channel. The CFTR protein was detected in subcellular compartments colocalizing with lysosomes and plasma membrane markers. CF neutrophils treated with ETI in vitro increased the secretion of NETs and restored their antimicrobial killing capacity against B. cenocepacia. Ex vivo CF neutrophils from patients on clinical ETI treatment showed similar aberrant responses as CF neutrophils from patients without treatment. In summary, although the treatment of CF neutrophils with ETI increased CFTR cellular expression and potentiated the antimicrobial mechanisms of CF neutrophils, ETI in vivo was less effective, suggesting the need for continued research into the effects of HEMT in people with CF.

P54 NEUTROPHIL EXTRACELLULAR TRAPS AND IMMUNOTHROMBOSIS IN COVID-19

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Neutrophil Extracellular Traps (NETs) were initially described as a mechanism for neutrophils to trap and kill bacteria. Since then many more functions of NETs in health and disease have been reported. NETs act as a scaffold for enzymes such as neutrophil elastase (NE) or peptidyl arginine deiminase 4 (PAD4). With the emergence of Coronavirus disease 2019 (COVID-19), it rapidly became clear that NETs are responsible for vascular occlusion, leading to disturbed microcirculation and subsequent organ damage. PAD4 links inflammation and thrombosis by reducing the levels of the metalloprotease ADAMTS13 thereby elevating plasma ultra-large von Willebrand Factor (ul-vWF) levels. We here investigated in more detail the interplay between neutrophils/NETs and other cells/proteins; promoting this occlusion of vessels with a focus on protein citrullination by PAD4. Our results indeed show that the levels of vWF are elevated in the serum of patients with COVID-19 in the intensive care unit; whereas the levels of other coagulation factors such as plasmin, thrombin, and anti-thrombin were comparable. Additionally, analysis of PAD4 treated fibrinogen vs. mock-treated fibrinogen revealed that the citrullination of fibrinogen by PAD4 changes its ultrastructure and renders it less susceptible to fibrinolysis by plasmin. Furthermore, NETs form a composite structure with fibrinogen, further stabilizing it. This stabilization of fibrin by NETs and PAD4 is likely to be responsible for the increased vascular occlusion in COVID-19. In the next step, we want to assess the fibrinolytic activity of other proteases such as NE or Nattokinase and analyze if pre-treatment with DNases fosters the resolution of these vascular occlusions.

P55 NET-BORNE ELASTASE PREVENTS INFLAMMATORY RELAPSE IN INTERCRITICAL GOUT

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Abstract: Gout flares accompanied by urate-lowering therapy (ULT) are prone to be related to the shrinkage of tophi from aggregated neutrophil extracellular traps that had captured monosodium urate crystals in the tissues. A total of 77 patients with gout were included. Serum cell-free DNA levels were compared between patients with various numbers of gout flares. We investigated whether serum cell-free DNA and α1-antitrypsin could be altered after ULT in patients with or without tophi. Serum cell-free DNA correlated to the number of flares in patients with tophaceous gout. ULT induced serum cell-free DNA increase in patients with tophi in both the acute and the intermittent phase. Serum α1-antitrypsin level was higher in acute than that in intermittent gout and an increase of α1-antitrypsin was seen in patients with tophi who received ULT. Chalk-like tophi from mice peritoneal cavities after MSU crystals induced inflammation showed abundant co-expression of IL-1β and IL-6 associated neutrophil extracellular traps. α1-antitrypsin induced the relapse of inflammation during the spontaneous resolution of MSU crystal-induced peritonitis. In the resolution phase of murine tophi, the neutrophil extracellular trap-associated neutrophil elastase degrades the pro-inflammatory cytokines and, thus, ameliorates the inflammation.

P56 RADIOTHERAPEUTIC MODULATION OF NEUTROPHILS IN BREAST CANCER

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Neutrophils are the most abundant type of innate immune cells in humans and form the first line of defense against harmful invaders. Neutrophils are known to contribute to many aspects of tumor progression and metastasis in cancer. The presence of neutrophils or neutrophil-derived mediators in the tumor microenvironment has been associated with poor prognosis in several types of solid tumors. Nevertheless, the effects of both radiotherapy and immunotherapies involve a complex interplay with the immune system. Neutrophils thus become an independent prognostic factor in breast cancer, although data on the modulation of neutrophil responses by radiotherapy are lacking. Recent in vivo data from mass-imaging cytometry and t-distributed neighborhood analyses show high numbers of infiltrating monocytes and neutrophil extracellular traps (NET) -associated factors in untreated breast tumors, which is associated with an immunosuppressive microenvironment. However, irradiated tumors show increased neutrophil infiltration associated with decreased NET production and T-cell infiltration, possibly promoting an immunogenic phenotype of the tumor. In addition, TGF-β and Smad4 signatures were found to be significantly altered by radiotherapy. Accordingly, TGF-β, which is associated with immunosuppressive properties, and Smad4 are highly expressed in untreated tumors compared with irradiated tumors. High TGF-β/Smad activity is controversially discussed but has been described as a poor prognosis for cancer progression. Accordingly, our data provide new insights into the modulation of neutrophils by irradiation and have implications for novel neutrophil-based treatment options via the TGF-β/Smad axis in breast cancer.

P57 LOW-DENSITY NEUTROPHILS IN SLE AND COVID-19.

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Neutrophils are a heterogeneous population with various subsets, differing in their inflammatory and neutrophil extracellular trap (NET) forming potential. Dysregulation of neutrophils and NETs can lead to the development of various inflammatory diseases, such as Systemic Lupus Erythematosus (SLE) and Coronavirus disease 2019 (COVID-19). In COVID-19, aggregated NETs promote vascular congestion and organ damage. Thereby, a specific subpopulation, the low-density neutrophils (LDNs) play a major role due to enhanced NET forming ability and proinflammatory potential. To understand their involvement in disease pathology, we are investigating the presence of LDNs in SLE and COVID-19, but also other diseases, such as sepsis or Felty's syndrome. We detected a significantly higher number of LDNs in patients suffering from COVID-19, compared to normal healthy donors and even SLE. Based on clinical data, we currently determine the correlations between the presence of these cells and various clinical parameters, including overall disease progression, ICU hospitalization, and the need for ventilation. We further analyze the expression of different cell surface molecules, such as CD41a to investigate platelet binging capacity or CD15 as an activation marker, which is reduced expressed in some COVID-19 samples. To better understand the origin of LDNs, we are examining the in vitro generation of these cells with various stimuli. The NET forming potential of both, the LDNs of patients and in vitro generated cells, is analyzed quantitatively and visually. In this way, we are determining the occurrence and behavior of LDNs in different diseases in order to identify potential treatment approaches.

P58 THE ARP2/3 INHIBITORY PROTEIN ARPIN REGULATES ENDOTHELIAL PERMEABILITY AND NEUTROPHIL TRANSMIGRATION.

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The endothelium is a semi-permeable barrier that gets activated during inflammation leading to increased permeability and leukocyte transmigration. Such alterations depend on endothelial actin dynamics and the Arp2/3 complex, which induces the formation of branched actin networks. Arp2/3 is inhibited by proteins such as the recently discovered arpin, which localizes at the lamellipodial edge in fibroblasts where it is thought to finetune Arp2/3 activity and cell migration. However, nothing is known about the role of arpin in endothelium. We found that arpin is expressed in endothelial cells and downregulated after treatment with TNFα and IL-1β. Reduced arpin levels correlated with an increase in actin stress fibers. Stable arpin-depleted HUVEC cells were more permeable compared to control cells. Permeability assays in vivo using arpin knock-out mice confirmed this increase in endothelial permeability. Of note, arpin deficiency generated more contractile actin stress fibers that are known to destabilize endothelial junctions. Surprisingly, inhibiting the Arp2/3 complex in arpin-depleted HUVECs did not rescue the effects on permeability or stress fiber formation. Instead, we observed a partial rescue of hyperpermeability after ROCK1/2 and MLCK inhibition suggesting that arpin acts independently of Arp2/3 inhibition and related to unknown functions of arpin in the regulation of actomyosin contractility. Next, we performed transmigration assays in vitro and observed more neutrophils were able to transmigrate across arpin-depleted endothelial monolayers. Intravital microscopy of inflamed cremaster muscles revealed no differences in neutrophil rolling, but more neutrophils firmly adhered and extravasated in arpin-deficient mice during inflammation compared to WT mice. Our data show that arpin plays important roles in regulating permeability and actomyosin contractility by Arp2/3-independent mechanisms that also impact on neutrophil transendothelial migration.

P59 PAD4 CONTROLS CHEMOATTRACTANT PRODUCTION AND NEUTROPHIL TRAFFICKING IN MALARIA

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In malaria, excessive inflammatory cell trafficking causes organ complications. We identified PAD4, an enzyme highly expressed in myeloid cells (such as neutrophils and macrophages), as a key regulator of inflammation in malaria. In a Plasmodium chabaudi (P. chabaudi) mouse malaria model, PAD4 regulates immunopathology without affecting the number of infected red blood cells. PAD4 promotes neutrophil and CD8+ T cell trafficking by regulating in vivo expression of CXCL1. Similarly, PAD4 regulates CXCL1 and CXCL2 production in response to Plasmodium falciparum (P. falciparum) and TLR ligands in human myeloid cells. Using patient samples, we show that CXCL1 is a biomarker for severe malaria. Therefore, PAD4 controls disease tolerance and represents a potential therapeutic target in malaria.

P60 WITHAFERIN A INTERFERES WITH ACTIN DYNAMICS IN EQUINE NEUTROPHILS

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Withaferin A (WFA) is a plant derivative with in vivo anti-inflammatory properties. Our laboratory recently demonstrated that WFA inhibits in vitro equine neutrophil adhesion, migration, and respiratory burst in response to diverse stimuli, without compromising short-term neutrophil viability. We also documented WFA promotion of apoptosis in primed equine neutrophils after 24 hours, which could hasten resolution of inflammation in clinical patients. The mechanisms underlying WFA inhibition of neutrophil functions and promotion of timely neutrophil apoptosis are unclear and are a focus of our ongoing work. Our early flow cytometry data indicated that WFA upregulates neutrophil surface expression of beta2 integrins under specific stimulation conditions. We followed up on these results by interrogating the impact of WFA on outside-in integrin signaling in neutrophils using manganese stimulation. Importantly, WFA enhanced manganese-induced neutrophil adhesion, in contrast to our previously demonstrated WFA inhibition of adhesion in response to all other stimuli assessed. This WFA augmentation of manganese-stimulated adhesion was dependent upon actin polymerization, as demonstrated by differential effects of latrunculin A or cytochalasin D on adhesion of neutrophils treated with WFA or vehicle control. Using flow cytometry and fluorescence microscopy, we confirmed that WFA interferes with actin polymerization and reorganization in neutrophils. Collectively, these findings provide insight into possible mechanisms for effects of WFA on neutrophil function and apoptosis. Our published data indicates strong potential of WFA as a therapeutic for neutrophil-mediated diseases, and further research is needed to identify WFA target molecules involved in neutrophil integrin signaling and actin dynamics.

P61 *Yersinia pestis* YOP DELIVERY AND BACTERIAL SURVIVAL DURING INTERACTIONS WITH THE NEUTROPHIL-LIKE CELL LINE HL-60

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Yersinia pestis, the causative agent of plague, is a deadly bacterial pathogen that must combat cells of the innate immune system by deployment of a Type III Secretion System (T3SS) for delivery of cytotoxic Yop protein effectors. Neutrophils are one of the first cells encountered in infected tissues. Studies with pathogens and human neutrophils are hampered by the short life-span of neutrophils. The goal of this study was to assess the ability of a neutrophil-like cell line, HL-60, to replicate typical neutrophil/*Y. pestis* interactions. We found *Y. pestis* adhesins such as Ail, plasminogen activator (Pla) and pH 6 antigen (Psa) contribute to Yop delivery to HL-60 cells and the addition of human serum led to a higher dependence on Ail for Yop delivery. Yop delivery via the T3SS was critical for preventing phagocytosis by HL-60 cells. Finally, delivery of Yops via T3SS prevented degranulation of HL-60 neutrophil-like cells as assessed by CD63 exposure using flow cytometry and fluorescence microscopy. In particular, Pla and pH6 antigen play prominent roles in preventing degranulation. The prevention of phagocytosis and/or degranulation by Y. pestis resulted in ~10 to 100-fold increased survival of KIM5 relative to the yopB mutant (a strain unable to deliver Yops or prevent phagocytosis or degranulation) upon interaction with HL-60 cells. These studies demonstrate the potent antimicrobial properties of HL-60 cells for *Y. pestis* strains unable to efficiently deliver Yops and are comparable to studies performed with freshly isolated human neutrophils. Thus, we have a cell line amenable to genetic and transcriptional manipulation for future studies to determine critical cellular pathways that contribute to *Y. pestis* control.

P62 TUNING NEUTROPHILS VIA HIF-ALPHA ISOFORMS TO CONTROL MYCOBACTERIAL INFECTION IN VIVO

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Tuberculosis is an increasing global problem due to the emerging prevalence of multi-drug resistance. Understanding the innate immune response to TB is critical to identify host-derived factors as potential therapeutic targets. We use zebrafish infected with Mycobacterium marinum as an in vivo TB model and identified that host hypoxia signalling modulates the neutrophil response to infection, via hypoxia inducible factors (HIFs). Protective neutrophil nitric oxide production can be increased by HIF-1alpha upregulation or by HIF-2alpha downregulation, enabling neutrophils to control mycobacterial infection more effectively. HIF-2alpha is predicted to be anti-inflammatory however, until now, there have been no transgenic zebrafish models of anti-inflammatory signalling available to determine the mechanisms involved. We have developed an anti-inflammatory zebrafish transgenic reporter line driving GFP under the control of the arginase 2 (arg2) promoter. A proportion of neutrophils upregulated arg2 early after infection challenge, showing a heterogeneous anti-inflammatory response at a time in pathogenesis when pro-inflammatory signals predominate. Immune cell arg2:GFP was modulated by HIF-2alpha, but not HIF-1alpha. Genetic and pharmaceutical inhibition of either arginase or HIF-2alpha was host protective against infection. These data suggest that neutrophils have a heterogeneous response to infections in vivo, with a balance of pro- and anti-inflammatory signals that can be fine-tuned by HIF-alpha isoforms as potential host-derived therapies against hard-to-treat infections like TB.

P63 A20 AND THE NON-CANONICAL NF-kB PATHWAY ARE KEY REGULATORS OF NEUTROPHIL RECRUITMENT DURING FETAL ONTOGENY

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Premature infants are at high risk to develop neonatal sepsis after birth. This has been linked to their functionally limited innate immune response, which is optimally adapted to intrauterine life. We set out to investigate the ontogenetic regulation of neutrophil function during fetal development. Transcriptomic analysis of fetal and adult human neutrophils revealed 128 differentially regulated genes, with a focus on genes being involved in the RelB-regulated non-canonical NFkB signaling pathway. We detected higher levels of p52 and pronounced nuclear RelB localization in fetal neutrophils compared to adult cells, indicating higher activity of non-canonical NFkB signaling. In contrast, we observed down-regulation of the classical NFkB pathway by reduced phosphorylation of IkB upon stimulation. Additionally, we also found an upregulation of the ubiquitin-modifying enzyme A20 (Tnfaip3), negatively regulating canonical NFkB signaling. By generating A20 overexpressing murine Hoxb8 cells, mimicking fetal neutrophils, we were able to show reduced neutrophil adhesion to the inflammatory substrate in a microflow chamber assay. In contrast, mice with a neutrophil specific A20 deletion displayed increased inflammation in an in vivo model of TNF-α-stimulated cremaster muscle venules. Taken together, our results identify A20 and the non-canonical NFkB pathway as key regulators of neutrophil function in the mouse and human fetus. Supported by DFG CRC914 projects A02 (B.W.), A10 (C.S), and B01 (M.S).

P64 INVESTIGATING HUMAN NEUTROPHIL PHENOTYPES THAT ASSOCIATE WITH NEISSERIA GONORRHOEAE

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The human-specific pathogen Neisseria gonorrhoeae (Ngo) causes the sexually transmitted disease gonorrhea, an oppressive public health burden due both to the recent emergence of multidrug-resistant ‘superbug’ strains and the ability of untreated infections to cause inflammation-induced scarring of the maternal reproductive tract and blindness in children born from infected mothers. The hallmark of gonococcal infection is a massive influx of neutrophils that do not seem to clear the infection. The growing awareness that multiple neutrophil phenotypes exist prompted our consideration that these neutrophil populations may differentially respond to Ngo. If true, then we must revisit the potential roles for neutrophils in gonococcal immunity and the immunopathogenesis of infection. The specific objective of this work is to develop a model to study the interaction between Ngo and different neutrophil subsets, to define and characterize the subpopulations of neutrophils that respond to Ngo infection, and to understand the specific response of each neutrophil phenotype to this infection. Using whole blood infection, I performed a high-throughput flow cytometry-based analysis (HTS) of 355 different cluster of differentiation (CD) antigens to reveal those expressed by Ngo-infected neutrophils. After narrowing down our screen, I generated customized HTS plates with our top 20 hits to identify antigens expressed on neutrophils that bound Ngo. I found that expression of CD11b, CD63, CD66c, CD256, CD277 and CD288 identifies neutrophil phenotypes that are more likely to bind Ngo. My ongoing work aims to further understand how these different populations contribute to immunity versus immunopathogenesis against this devastating pathogen.

P65 CORTACTIN LOCALIZATION AND DEGRADATION IN ENDOTHELIAL CELLS DURING INFLAMMATION: IMPLICATIONS FOR NEUTROPHIL TRANSENDOTHELIAL MIGRATION

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During inflammation, the venular endothelium dynamically remodels its actin cytoskeleton to control neutrophil transendothelial migration (TEM). The endothelial actin-binding protein cortactin clusters into ring-like structures surrounding adherent neutrophils to stabilize ICAM-1-mediated neutrophil-endothelial interactions. Moreover, neutrophils can modulate the endothelium through extracellular vesicles. Therefore, we investigated whether endothelial cortactin dynamics are regulated by neutrophils. Confocal microscopy of inflamed cremaster muscles after intrascrotal administration of the pro-inflammatory mediators TNF-alpha, IL-1-beta, CXCL1, and LTB4, revealed that the inflammation caused rapid degradation of cortactin in post-capillary venules. Of note, mice subjected to neutrophil depletion or histamine stimulation maintained endothelial cortactin during inflammation. Co-cultures of human umbilical vein endothelial cells (HUVEC) with different leukocytes confirmed that cortactin degradation is a neutrophil-specific response. Interestingly, a pool of cortactin localized at sites of neutrophil adhesion was protected from degradation. Using pharmacological inhibitors, we found that cortactin degradation was not mediated by endothelial enzymes in response to neutrophil adhesion, but instead occurred due to neutrophil-derived serine proteases (NSP) that are likely delivered into endothelial cells via extracellular vesicles during the extravasation cascade, a process that we are currently investigating. In conclusion, we identified two pools of cortactin that are differentially regulated during neutrophil TEM; one pool is recruited to ICAM-1 clusters to support neutrophil adhesion, whereas the remaining pool is degraded by NSP likely to destabilize endothelial contacts to facilitate neutrophil diapedesis.

P66 INFLUENCE OF SEXUAL DIMORPHISM ON THE CD43 REGULATORY FUNCTION OF NEUTROPHIL EFFECTOR MECHANISMS

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CD43 is a transmembrane glycoprotein expressed by all hematopoietic cells except resting B cells and erythrocytes. In T cells, CD43 participates in multiple functions, such as proliferation, migration, survival, and activation. In neutrophils it has been suggested to contribute to locomotion and migration. This study aimed to evaluate the role of CD43 as a regulatory molecule of neutrophil effector mechanisms in a sex-dependent manner. We compared the production of reactive oxygen species (ROS; flow cytometry) and the formation of extracellular neutrophil traps (NETs; fluorescence microscopy) of bone marrow neutrophils isolated by density gradient centrifugation and a negative selection step of male and female C56BL/6J WT and CD43KO mice. We also evaluated the neutrophils' maturation stage by cytometry and the cell size, nucleus shape, and compaction by light microscopy. As reported by others, our results show that independent of CD43 expression, the sexual dimorphism between male and female neutrophil functionality persists. Interestingly, while male CD43KO neutrophils produced fewer ROS and NETs than those of WT males, female CD43KO neutrophils produced more ROS and NETs than WT female neutrophils. As expected, we observed a higher percentage of immature neutrophils in females WT and CD43KO than in males. Interestingly, our data suggest that CD43 functions like a regulatory molecule with a double role. In males, ROS and NETs production is favored by CD43 expression, and its absence negatively affects both mechanisms. However, in females, CD43 expression lessens ROS and NETs production, and in the absence of CD43, female neutrophils behave like WT male neutrophils and produce higher amounts of ROS and NETS. Overall, these results suggest that CD43 contributes to neutrophil ROS and NETs production in addition to locomotion and migration and that expressing or not CD43 impacts the differences resulting from sexual dimorphism, reverberating on neutrophils' functionality. (Funded by CONACYT and PAPIIT/UNAM, Mexico)

P67 FIBRIN IS A CRITICAL REGULATOR OF NEUTROPHIL EFFECTOR FUNCTION AT THE ORAL MUCOSA

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Objectives: Herein, we focus on the role of fibrin, excessive deposition of which can cause chronic inflammation and severe tissue damage via unknown mechanisms. Fibrin removal, fibrinolysis, is achieved by the proteolytic activity of plasmin. The critical role of defective fibrinolysis becomes evident in patients with type I plasminogen (Plg) deficiency. Indeed, mucosal lesions in humans and mice with Plg deficiency are also characterized by excessive fibrin deposition. We hypothesized that insufficient plasmin-mediated fibrinolysis is a key contributor to oral mucosal immunopathology. Methods: We sought to understand the mechanistic link between mucosal fibrin deposition and immunopathology by using an array of genetically engineered mouse models, including Plg-/-, and Plg-/-Fgg390-396A (mutation in the aMb2 integrin binding site on the fibrin gamma-chain). Results: We demonstrate that (i) Plg-def mice develop spontaneous and severe periodontal bone loss with persistent extravascular fibrin deposits compared with littermate controls, (ii) Plg-def mucosal lesions have a significantly increased neutrophil infiltration, (iii) abrogating the engagement of fibrin to neutrophils through the aMb2 leukocyte integrin receptor was sufficient to prevent Plg def-associated periodontal bone loss, (iv) fibrin-aMb2-neutrophil engagement activated neutrophil effector functions, including the production of reactive oxygen species and neutrophil extracellular traps, and (v) removal of extracellular DNA by DNase I treatment controlled periodontitis in Plg-/- mice. Conclusion: Our work uncovers fibrin as a critical regulator of neutrophil effector functions within the oral mucosal tissue microenvironment and suggests fibrin-neutrophil engagement as a pathogenic instigator and therapeutic target in oral mucosal disease, periodontitis.

P68 PHP-303, A NOVEL NEUTROPHIL ELASTASE INHIBITOR, REDUCES LUNG INJURY IN EXPERIMENTAL ACUTE RESPIRATORY DISTRESS SYNDROME

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The acute respiratory distress syndrome (ARDS) is characterized by dysregulated inflammation leading to acute lung injury (ALI). There are no effective pharmacologic therapies for ARDS. We studied the effects of PHP-303 a potent, selective and reversible inhibitor of neutrophil elastase on oleic acid (OA)-induced ARDS in rats. Rat groups were: control sham or treated with placebo (PLCB) or PHP-303 (3, 10 or 30 mg/kg) for 3 days prior to intravenous OA (150 mg/kg)-induced ALI. Four hours after OA treatment, PHP-303 and PLCB groups were compared. PHP-303 reduced histological lung injury area by 88%, lung edema by 50% and improved lung oxygenation by 38%. Lung neutrophil recruitment, assessed by myeloperoxidase (MPO) immunostaining, was significantly increased after OA injury, and this effect was reduced by 31% by PHP-303. PHP-303 also prevented, almost completely, the recruitment of inflammatory cells in bronchoalveolar lavage fluid (BALF), including a 95% reduction of neutrophil recruitment. In BALF, PHP-303 markedly reduced the levels of the pro-inflammatory cytokines; interleukin-6 and MPO, by 71% and 34% respectively. In summary, pre-treatment with PHP-303 in a rat ARDS model reduces ALI and respiratory failure with coincident reduction of neutrophil recruitment in lung and BALF and inflammatory cytokines in BALF. This study supports the clinical development of PHP-303 for the prevention of ARDS under conditions causing ALI.

P69 A HIGH-THROUGHPUT DEGRADATION ASSAY USING MIMETIC NEUTROPHIL EXTRACELLULAR TRAPS

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Neutrophil extracellular traps (NETs) are comprised of extracellular decondensed chromatin decorated with granule proteins. Interest has grown as research has found NET involvement in a variety of pathologies such as autoimmune, cardiovascular, and pulmonary diseases. Overproduction or ineffective clearance of NETs has routinely been connected to disease severity. There is an unmet need for rigorous and reproducible assays that determine the efficiency of NET clearance. However, in vitro assays based on cell-derived NETs are limited by variability and an inability to manufacture at scale. Therefore, we developed a high-throughput assay using mimetic NETs composed of DNA and histone to address these concerns. The mimetic NETs are created and attached to the bottom of 96-well microplates. The plate is then dried and can be stored for later use. The degradation assay is fluorescence-based and performed with standard plate readers. Readings are taken before and after incubation with the desired biological specimen such as patient serum. We have successfully created mimetic NETs that can be prepared in advance for storage and transport, degrade in DNase within the same time frame as cell-derived NETs, and degrade as expected in healthy serum. The mimetic NETs have a shorter preparation time than cell-derived NETs and uniformly coat microplates. Additionally, mimetic NETs allow for a functional analysis of NET components through systematically controlling the proteins within the synthetic NETs. In conclusion, these mimetic NETs have the potential to create highly reproducible NET degradation assays via a simplified preparation and assay protocol. Findings from this project have the potential to improve knowledge of NET degradation modulators in disease.

P70 EMERGENCY GRANULOPOIESIS DRIVES AN IMMATURE POPULATION OF NEUTROPHILS WITH INCREASED NETOTIC POTENTIAL IN HUMAN CEREBRAL MALARIA

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Background: In 2022 Plasmodium falciparum and its most severe complication, cerebral malaria (CM) caused >500,000 deaths. The pathogenic mechanisms are poorly understood however neutrophils have recently been implicated, with NETs detected in the cerebrovascular in fatal cases. Low-density neutrophils (LDN) are associated with disease severity in P. vivax, as well as in cancer and sepsis that they have increased NETotic ability and endothelial cytotoxicity. Still, the presence and function of LDN and the role of NETs in CM has not been investigated. Hypothesis/aims: We hypothesised that NETs and LDN are elevated in CM cases. NETs may contribute to endothelial injury and LDN may have an increased propensity to form NETs. To investigate this, we performed immunophenotyping, sequencing and functional assays on neutrophils from patients with CM. Methods: Paediatric CM patients and controls were recruited at a specialist unit in Malawi. Plasma was used to measure cytokines and NETs. Purified LDN and NDN were analysed using light microscopy, flow cytometry, ex-vivo formation of NETs and RNA-sequencing. Results: Plasma levels of NETs, IL-6 and IL-1b were increased in CM samples compared with non-CM and healthy controls (HC). Increased LDN were associated with CM compared to non-CM coma and HC. Unlike NDN, the LDN showed elevated transcription of cell cycle and chromatin factors, which is indicative of immaturity. This was confirmed by visualisation of nuclear lobulation. LDN have increased ex-vivo spontaneous NETs formation capacity. Discussion: CM is associated with elevated LDN that represent a highly activated, immature population of neutrophils with an increased proliferation capacity indicating a state of emergency granulopoiesis in the bone marrow. Furthermore, LDN exhibit increased NETotic capacity that is also reflected in the increased detection of NETs in plasma of CM cases and identified in the brain microvasculature of patients dying from CM.

P71 RAPID MIGRATION OF NEUTROPHILS WITH ANTIGEN-PRESENTING CELL PHENOTYPE INTO LYMPHOID ORGANS UPON ISCHEMIA REPERFUSION INJURY

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A hallmark of many cardiovascular diseases including acute myocardial infarction, ischemia reperfusion (IR) injury is a sterile inflammatory disorder associated with both innate and adaptive immune responses. Amongst the first leukocytes responding to IRI are neutrophils that are thought to exert predominantly inflammatory and deleterious innate functions at site of IRI. Here we investigated if neutrophils could also modulate the adaptive immunity post IRI, namely the rapid activation of CD4+ T cells, a mechanism that is not fully understood. Here we used two in vivo IR injury models applied to mouse myocardium and cremaster muscles to analyse the migration pattern and phenotype of neutrophils into the lymphatic and lymphoid system by confocal microscopy and flow cytometry, respectively. We observed a rapid but transient (i.e. form 6 to 4hrs post reperfusion) migration of neutrophils into tissue-associated lymphatic capillaries and draining lymph nodes (dLNs) post IRI. Phenotypically, some dLN-infiltrated neutrophils up-regulated the expression of several markers associated with antigen-presenting cells, including MHC-II, and were observed to interact with T cells in the dLNs. Interestingly, these observations were associated with a rapid activation of CD4+ T cells. Collectively, our data provide the first evidence for the migration of neutrophils into the lymphatic and lymphoid system post IRI. Furthermore, this study highlights a potential new role for neutrophils in shaping the adaptive immune response to upon acute IRI. This may have important implications for clinical outcomes for and treatment of this cardiovascular disease. Work supported by the British Heart Foundation, UK.

P72 MODULAR MIMETIC OF THE POST-CAPILLARY VENULE FOR THE INVESTIGATION OF NEUTROPHIL ALTERATIONS DURING TRANSMIGRATION

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The rapid recruitment of neutrophils to an area of insult or injury is an essential host defense reaction, yet dysregulated neutrophil infiltration is a key driver of pathological inflammation. This dual role complicates the development of targeted biological treatments for neutrophil-mediated diseases. In recent years, analyses of the mechanisms of neutrophil transmigration include a detailed description of the role of the endothelial cell (EC), with a limited study of the mural pericyte (PC) and the basement membrane (BM) in neutrophil trafficking, phenotype, and function. Therefore, here, we present a novel bioengineered venular microsystem that can be used to delineate the contribution of human EC, PC, and BM signals to human neutrophil phenotypic changes during transmigration. We have developed a polyethylene glycol (PEG)-based BM-mimetic scaffold with tunable fiber diameter, density, and thickness using electrospinning, a nanofiber fabrication technique. We have fabricated nanofibrillar substrates that resemble the morphology of the healthy vascular BM (~250um diameter and 1um^2 pore area). Our scaffold also mimics venular tissue by replicating the single-fiber mechanics of the BM (5.2GPa-11.9GPa), a parameter that other systems have overlooked. Via the direct conjugation of peptides and whole proteins to the electrospun fibers, we can render the scaffold bioactive and culture EC and PC independently or as a bilayer. The tunable chemical composition of the fibers also enables the study of neutrophil interactions with different ECM components. Using a PDMS-based platform and real-time microscopy, we can also visualize each step of the neutrophil recruitment process and observe neutrophil interactions with each component of the vascular wall. Ultimately, the high tunability of this venular model can replicate healthy and pathological vascular states while observing these events in real-time.

P73 CORRELATION BETWEEN NEUTROPHIL EXTRACELLULAR TRAPS EXPRESSION AND PRIMARY GRAFT DYSFUNCTION FOLLOWING HUMAN LUNG TRANSPLANT

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Primary graft dysfunction (PGD) is the major complication of lung transplantation within the peri-operative period. PGD is characterized by alveolar epithelial and vascular endothelial damage, lung edema, inflammation and hypoxemia. Up to 30% of recipients develop the most severe form of PGD (Grade 3; PGD3). Animal studies suggest that neutrophils contribute to inflammation through the release of neutrophil extracellular traps (NETs) and vascular occlusion. The main objective was to correlate NETs formation (NETosis) in PGD3 vs non-PGD3 recipients. Clinical data and blood samples were collected from donors and recipients pre-, intra- and postoperatively (up to 72hrs). Inflammatory inducers of NETs' release (interleukins [IL-6, IL-8], and components (myeloperoxidase [MPO], MPO-DNA complexes and cell-free DNA [cfDNA]) were quantified by ELISA. By histology and immunohistochemistry on lung biopsies from donor grafts, we observed various degrees of vascular occlusion and neutrophils undergoing NETosis. Also, in recipients intra- and postoperatively, circulating inflammatory (IL-6, IL-8) and NETosis biomarkers (MPO-DNA, MPO, cfDNA) were up to 4-fold higher in PGD3 recipients vs non-PGD3 ($p = 0.041$ to 0.001). In summary, perioperative elevation of NETosis biomarkers is associated with PGD3 following human lung transplantation, these biomarkers might serve to identify recipients at risk of PGD3 and initiate preventive therapies.

P74 LUNGS FROM COVID-19 DECEASED PATIENTS ARE CHARACTERIZED BY NEUTROPHIL EXTRACELLULAR TRAPS RELEASE AND UPREGULATION OF NERVE DEVELOPMENT TRANSCRIPTIONAL PROGRAMS

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The COVID-19 pandemic emerged in China in 2019 and rapidly spread across the world. The causative agent is the Severe Acute Respiratory Coronavirus 2 and clinical manifestations of the disease range from asymptomatic to severe forms, characterized by acute respiratory distress syndrome (ARDS) and thrombotic events leading to patient deaths. While dysregulated immune and tissue-damaging responses are thought to contribute to disease pathophysiology, much remains to be discovered about COVID-19 immunopathology. Neutrophil Extracellular Traps (NETs) have previously been implicated in tissue damage and thrombi formation. Hence, we first investigated whether NETs were released in the lungs of severe COVID-19 patients. We uniquely detected NETs by confocal microscopy in lung autopsy material from COVID-19 deceased patients. NETs were located in the four anatomical compartments of the lung: the bronchi, the alveoli, the interstitium and the blood vessels. Second, to objectively identify processes implicated in the severe form of the disease, we performed in situ RNA sequencing on the same lung material from severe COVID-19 patients. We found 615 differentially regulated transcripts between control and COVID-19, among which 395 were upregulated in severe COVID-19 patients. Surprisingly, COVID-19 lung contained many transcripts enriched in processes associated with nerve signaling and development. Using confocal microscopy, we observed that such transcripts were not found in neutrophils but rather in cells in close vicinity of NETs-rich areas. In conclusion, we found that severe Covid-19 is associated with NET release and processes implicated in nerve development, the significance of which remains to be investigated.

P75 THE LUNG MARGINATED NEUTROPHIL – ENDOTHELIAL CELL AXIS REGULATES LUNG CELLULAR TRAFFICKING AND ENDOTHELIUM HOMEOSTASIS

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The lung exerts vital functions that are sustained by both structural and immune components. The lung microenvironment has indeed been shown to shape the functional identity of immune cells to ensure appropriate responses that maintain its immunophysiological functions under homeostasis and pathogenic conditions. In line with this, one intriguing feature of the lung is the presence, at steady-state, of a substantial pool of neutrophils located in the microvasculature, called marginated neutrophils (MarNeu). Lung MarNeu display a unique transcriptomic profile as compared to other neutrophils across the whole body, which is mostly associated with vascular growth and repair. Here, we used two different approaches of acute MarNeu targeting in wild-type C57BL/6 mice at steady-state and found that targeting MarNeu has a substantial long-term impact on cellular trafficking and endothelial cell (EC) homeostasis. Indeed, two weeks after MarNeu depletion, we found that, even though the MarNeu pool was restored, EC permeability and ability to mediate leukocyte transendothelial migration were strikingly impaired. We are currently employing single cell and bulk RNA-sequencing approaches, in vivo imaging and EC-related assays to further investigate the MarNeu-EC axis and the long-term control of EC stemness, identity and functions by MarNeu.

P76 INDUCTION OF STING/IL-29 SIGNALING IN A HUMAN LUNG ADENOCARCINOMA / TUMOR-INFILTRATING NEUTROPHIL BIOMIMETIC MODEL

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Objective: LKB1-mutant lung adenocarcinoma (LUAD), is an intractable form of lung cancer. LKB1-mutant LUAD is poor in T-cells and does not respond to checkpoint inhibitor therapy, and instead is enriched in tumor-infiltrating neutrophils (TINs). STING is a key innate sensor mediating anti-tumor responses that is inhibited in LKB1-mutant LUAD. However, the status of STING signaling and if it can be induced in TINs to antagonize tumors is unknown. Here, we developed a novel human LKB1-mutant LUAD/TIN model to tackle this question.Methods:Based on prior work from our lab (Dobosh, STAR Protoc. 2021), we grew the H1944 LKB1-mutant LUAD line at air-liquid interface on Alvetex scaffolds (Reprocell), followed by the transmigration of human blood neutrophils. The effect of the STING agonist MSA-2 was assessed in the model by a 20-plex immune mediator assay (Mesoscale).Results: We successfully manufactured a LKB1-mutant LUAD/TIN model from H1944 cells and human blood neutrophils. MSA-2 treatment resulted in significant increases in the STING-regulated mediators IL-6, IP-10 and MIP-1beta. Most strikingly, MSA-2 treatment induced IL-29 (type III interferon) levels by 27-fold, while neither type I nor type II interferons were induced.Conclusions:The model showcased here mimics LKB1-mutant LUAD/TIN interplay and shows responsiveness to STING agonist treatment through induction of type III (but not type I and II) interferon and other immune mediators. Since IL-29 receptor expression is limited to epithelial and myeloid cells (including neutrophils) and IL-29 is known to mediate immunomodulatory and anti-tumor responses, future work will address if and how the large IL-29 increase observed upon MSA-2 treatment affects LUAD proliferation and TIN activation in this context.

P77 BIOMECHANICAL PHENOTYPE OF CIRCULATING NEUTROPHILS IS ALTERED IN ANCA ASSOCIATED VASCULITIS

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Background: Neutrophils are central to the pathogenesis of ANCA associated vasculitis (AAV); they mediate vascular injury and are the target of the autoantibodies. Pathological activation of neutrophils can lead to decreased cell deformability, reducing their ability to traffic through microvasculature and potentially increase the capacity for endothelial damage and vascular inflammation. Objectives: To investigate the morpho-rheological characteristics of immune cells in (AAV) using Real Time-Deformability Cytometry (RT-DC), a novel technique able to identify biophysical properties of individual cells, such as size, deformability, and elasticity using only 50µl of whole blood. Methods: Whole blood from healthy volunteers (HV), patients with active AAV, or patients with AAV in remission was analysed using RT-DC. Isolated neutrophils from HV were stimulated with MPO-ANCA IgG or control IgG and analysed using RT-DC Results: Neutrophils from patients with active AAV were significantly stiffer than patients in remission or HV, displaying decreased deformation and increased elasticity. In patients with active AAV, there was strong inverse correlation between neutrophil deformation and disease activity as measured by BVAS score. Isolated neutrophils stimulated with MPO-ANCA IgG demonstrated a similar phenotype of decreased deformability compared to unstimulated cells or those stimulated with control IgG. Conclusion: The phenotype of increased cell stiffness seen in active AAV may lead to increased neutrophil retention in pulmonary and renal microvasculature, increasing the potential for neutrophil-endothelial cell interactions and microvascular damage. Morphorheological parameters can be rapidly measured using a small volume of whole blood meaning RT-DC may be a useful technique to aid identification of disease activity in AAV.

P78 NEUTROPHILS CONTRIBUTE TO AN EARLY ACTIVATION OF PRO-INFLAMMATORY CD4+ T-HELPER CELLS IN RESPONSE TO ISCHEMIA/REPERFUSION INJURY IN VIVO

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Ischemia/reperfusion injury (IRI) is a sterile inflammatory disorder and clinical complication of many cardiovascular disorders such as stroke, organ transplantation or myocardial infarction. Neutrophils are the main drivers of the pathophysiology of IRI during the acute phase of the disease due to their innate immune functions. Interestingly, both pro-inflammatory and regulatory T-cell subpopulations also contribute to the pathogenic and reparative processes of IRI, respectively. However, the dynamics and exact cellular and molecular mechanisms of this response is unclear. Here we investigated the contribution of neutrophils to the early activation of CD4+ T-cells in IRI. Using a mouse model of IRI applied to the cremaster muscles, we noted a rapid but transient activation of T-helper cells in the draining lymph nodes (LNs) 24hrs post-reperfusion. This response coincided with the presence of a subpopulation of MHC-II high/ICAM-1 high neutrophils interacting with T-cells in the draining lymph nodes (LNs) and preceding the entry of other professional antigen presenting cells. Specifically, qPCR analyses on purified CD4+ T-cells from LNs demonstrated the gene upregulation for key regulatory transcription factors, glycolytic enzymes, surface markers and cytokines characteristics of the activation of CD4+ T-cells with pro-inflammatory activities (i.e. Th1/Th17 subpopulations). Interestingly however, this response was totally abolished in animals subjected to neutrophil depletion. Overall, this study indicates the contribution of neutrophils in the activation of pro-inflammatory T-cells during the early phase of the disease. Work supported by the British Heart Foundation & WHRF, UK.

P79 *Mycobacterium tuberculosis* INFECTION PROGRESSION IS ASSOCIATED WITH DIFFERENTIAL NEUTROPHIL MATURATION IN MACAQUE MODEL OF TUBERCULOSIS

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Neutrophils (PMN) are important effectors during *Mycobacterium tuberculosis* infection (TB), having protective and detrimental role depending on disease stage. To better understand the role of PMN in TB pathophysiology, a clear disease definition, tissue exploration and early analysis of the immune responses are necessary to precise their contribution in TB outcome. In this study, the objective was to characterize neutrophil phenotypes dynamic and their link with T lymphoid responses in cynomolgus macaque model of TB. Six cynomolgus macaques were infected with 25CFU of MTB intrabronchially, and followed longitudinally for up to 30 weeks. We analyzed immune cells from blood and bronchoalveolar lavage (BAL) using mass cytometry associated with unsupervised analysis. Animals were separated in two groups according to clinical symptoms, PET-CT imaging and bacteriology: fast progressors (FP) and slow progressors (SP). As early as 2 weeks after exposure, changes of neutrophil phenotypes were observed in both groups. Compared to SP, FP demonstrated elevated levels of immature neutrophils and pre-neutrophils in blood at week 14 and 18. This was associated with CD4+ and CD8+ T cells decrease in blood from FP, showing activated and effector memory (TEM) phenotypes. Overall, our data indicates that the progression of the TB is associated with elevated immature and pre-neutrophils, decreased CD4 and CD8 T cells with TEM phenotypes in the blood. Neutrophil immaturity could be associated with immunomodulatory properties impairing TB response. Ongoing analysis on blood transcriptomic signature along with cytokine environment and PMN/T cells interaction assays will be helpful to define the immune mechanism underlying the disease evolution.

P80 TRANSCRIPTIONAL LANDSCAPE OF CLOSTRIDIOIDES DIFFICILE INFECTION INDUCED NEUTROPHILIA

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Clostridioides difficile infection (CDI) is the #1 nosocomial infection in the US. Neutrophils are the first cells recruited to colonic tissue after CDI. Although neutrophils are clearly heterogeneous cells, the exact phenotypic/functional nature of CDI-induced neutrophils remains undefined. Here, we took an unbiased transcriptomics approach to examine neutrophil heterogeneity in the bone marrow, blood, and colon of uninfected and C. difficile-infected WT mice. Further, we determined the effect of a common genetic SNP in the gene for leptin receptor (LEPR) in regulating CDI-induced neutrophilia. Single-cell RNA sequencing was performed using 10x genomics platform and compared to published sequences using Seurat 4.0 in R. Our data reveal that compared to uninfected mice, CDI induces expression of genes key to neutrophil activation, chemotaxis, extravasation, phagocytosis, and cell death. Comparisons between WT and LEPR mutant mice reveal that during acute CDI, pre- and immature neutrophils of mutant mice had significantly more transcripts of genes involved in formation of azurophil and specific granules, secretory vesicles, and ROS production. Mature neutrophils of LEPR mutant mice also had more transcripts of genes involved in degranulation and extravasation. We have previously shown that both mice and humans with the LEPR mutant SNP have worse CDI outcomes. Our scRNAseq analyses suggest that the mechanisms of worse CDI in hosts with LEPR mutation is driven by SNP-associated neutrophil heterogeneity, whereby increased neutrophil migration, extravasation, and degranulation in LEPR mutant hosts augments intestinal epithelial barrier damage, resulting in worse CDI. In the future, this LEPR SNP has the potential to be used as a personalized medicine approach to risk-stratify CDI patients.

P81 NEUTROPHIL RESPONSE TO INFECTION: DIRECTED VIA MULTIPLE SIGNAL SOURCES

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With increasing health crises, there is a need to better understand the immune system and develop improved ways of treating disease. Neutrophils are the body's initial defense against pathogens. Their response is dictated via signals with immune and vasculature cells. I am investigating how vascular signaling modulates neutrophil responses to bacteria. To do this, I am using a microfluidic device containing relevant features and architectures of the infectious microenvironment including a model blood vessel. It consists of an endothelial cell lined lumen with ports for adding neutrophils in it and pathogen outside of it. This initiates a neutrophil response allowing for investigating signaling within the system and the phenotypical response of neutrophils attacking the pathogen. This system allows for investigation of cell-cell interactions in a controlled but physiologically relevant environment. Past work with this device has probed the role of endothelial cells, the primary cell of the vasculature. It was found endothelial cells release signaling molecules that play a vital role in enhancing the neutrophil response to Pseudomonas aeruginosa. I am investigating how neutrophil responses vary with respect to migration and effector mechanism use, including reactive oxygen species (ROS) production and phagocytosis, when targeting different bacteria such as P. aeruginosa, Salmonella enterica, Listeria monocytogenes and Staphylococcus aureus. I have found increased production of ROS in neutrophils responding to P. aeruginosa and S. enterica than other bacteria. Research of signaling interactions driving neutrophil responses will yield insights helping identify targets of interest for future clinical applications. These results will improve treatment options for current and future health crises.

P82 OLFACTOMEDIN-4 EXPRESSING NEUTROPHILS EXAGGERATE CLOSTRIDIODES DIFFICILE TOXIN-INDUCED EPITHELIAL INJURY

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Neutrophils are the first immune cells that are recruited to colonic mucosa after Clostridioides difficile infection (CDI). These cells have a significant role in CDI pathogenesis, wherein both too many and too few neutrophils are associated with worse disease outcomes. Olfactomedin 4 (OLFM4) is a glycoprotein that is expressed in a subset of human and mouse neutrophils. Higher number of OLFM4+ neutrophils are associated with worse outcomes in patients with bacterial and viral infections, suggesting that they can be a driver of disease severity. However, the underlying biological mechanism(s) by which OLFM4+ neutrophils exacerbate disease are unknown. Here, we utilized single-cell neutrophil RNA-seq, in vitro neutrophil-epithelial co-cultures, and in vivo murine models (both wildtype, WT and OLFM4 deficient, OLFM4-/- mice) to examine the role of OLFM4+ neutrophils in CDI pathogenesis. Our data reveal that: (i) CDI increases the number of OLFM4+ neutrophils, and (ii) OLFM4+ neutrophils exacerbate intestinal epithelial damage caused by C. difficile toxins in vitro. During acute CDI in vivo, OLFM4-/- mice had: (iii) higher expression of type 2 cytokines (IL-4 and IL-5) and significantly more blood and tissue eosinophils, along with (iv) faster resolution of diarrhea and better survival, despite having the same number of pathogens and C. difficile toxin titers, compared to WT mice. Since type 2 immunity has been shown to be protective in CDI, our data in conjunction with published literature suggest that OLFM4 exaggerates epithelial injury, dampens CDI-induced Type 2 immune responses, and worsens clinical disease by reducing eosinophil-driven protective gut responses.

P83 SINGLE-CELL RNA SEQUENCING UNCOVERS THE HETEROGENOUS NATURE OF EMBRYONIC NEUTROPHILS

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Neutrophils play a crucial role as first line defenders to tissue damage and infections in the adult immune system. Like most other innate immune cells, neutrophils are known to emerge before birth, first appearing in circulation at embryonic day 12.5. However, while other myeloid cell populations are well studied and known for their involvement during embryogenesis, less attention has been paid to neutrophils. Our project has focussed for the first time on this largely uncharacterised population and its role during prenatal development. Here, we performed in depth single cell RNA sequencing of the early immune compartments in late gestation and after birth and used in silico phenotyping to characterize the heterogenous nature of neutrophils on their journey from progenitor to mature immune cell. Integration of published transcriptomics data of adult neutrophils allowed us to compare the cellular signatures and uncover changes to haematopoiesis over the course of development. Next, we performed multiplexed ex vivo flow cytometry to establish detailed kinetics with respect to embryogenetic development as well as circadian rhythm. Tissue clearing-aided 3D imaging enabled us to study cellular location and distribution in situ. We surveyed the functional defensive potential of these early cells using a variety of in vitro assays. Collectively, this project provides a first glimpse into the complex and kinetically evolving landscape of neutrophils during embryonic development.

P84 HUMAN NEUTROPHIL KINETICS: A CALL TO REVISIT OLD EVIDENCE

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Introduction: The half-life of human neutrophils is still controversial with estimates ranging from 7-9 hours to 3.75 days. This debate should be settled to understand neutrophil production in the bone marrow, and the potential and limitations of emergency neutropoiesis following infection or trauma. Furthermore, cellular lifespan greatly influences the potential effect(s) neutrophils have on the adaptive immune response. Objective: We posit that blood neutrophils are in exchange with different tissues, but particularly the bone marrow as it contains the largest pool of mature neutrophils. Furthermore, we tested the hypothesis that the oldest neutrophils are the first to die following a so-called conveyor belt model. Old data were revisited. Results: the reanalysis is in favor of a conveyer belt model with neutrophils having a fixed life span in homeostasis. The 'conveyor' accelerates under conditions of high demand which was mimicked by a systemic LPS challenge in healthy volunteers. This situation leads to redistribution of neutrophils from tissue to blood leading to blood neutrophilia. It is important to emphasize that the post-mitotic transfer time in the human bone marrow is at least 4-5 days making neutropoiesis a relatively slow process. The location of the main clearance of neutrophils in homeostasis is still under debate, albeit the bone marrow is a likely candidate. Death of neutrophils under infectious conditions is mainly taking place at the infected tissue locations. Conclusion: These guiding principles shed new light on our interpretation of existing neutrophil lifespan data and offer recommendations for future research.

P85 CD18 DEFICIENCY IN ZEBRAFISH: A NEW MODEL ORGANISM TO STUDY LAD TYPE I

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Leukocyte adhesion deficiency (LAD) type I is an immune disease associated with neutrophilia, impaired neutrophil recruitment to sites of inflammation and impaired wound healing. This disease is caused by mutations in the CD18 gene ITGB2. As part of the beta2 integrin family (CD11/CD18), CD18 is crucial for neutrophil function in the mammalian system. The zebrafish (Danio rerio) is a widely accepted model to study neutrophil biology. However, the role of CD18 in zebrafish neutrophils is still incompletely understood. In this study, we generated CD18-deficient zebrafish using CRISPR/Cas9 to analyse the role of CD18 in zebrafish neutrophils in response to inflammatory conditions. We analysed neutrophil recruitment to i) sites of sterile injury after tail fin transection, and ii) sites of infection after microinjection of E. coli into the otic vesicle in CD18 wild-type (WT) and knock-out (KO) zebrafish larvae. Further, the wound healing capacity of these zebrafish larvae was characterized. In CD18 KO zebrafish larvae 5 days post fertilization the number of recruited neutrophils to a sterile injury was reduced by 33% compared to CD18 WT zebrafish larvae 6 h after tail fin transection. This was accompanied by a significantly increased number of neutrophils observed in circulation. At this timepoint, neutrophils are mainly recruited from circulation, indicating that CD18-deficient neutrophils could not effectively extravasate from the vasculature. Similarly, neutrophil recruitment to the otic vesicle was reduced in CD18 KO zebrafish larvae as compared to CD18 WT zebrafish larvae 6 h after E. coli injection into the otic vesicle. However, assessing the wound healing capacity of CD18 KO zebrafish larvae did not yield conclusive results. Altogether, our data indicate that CD18 plays a similar role in zebrafish neutrophil biology as in mammals, and reveal the zebrafish as a valid model to study LAD type I.

P86 RECRUITMENT OF CD62LLOW NEUTROPHILS IN ACUTE INFLAMMATION CORRELATES WITH A PRE-EXISTING REACTIVENESS AND WITH PLASMA CYTOKINE LEVELS

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Background The pool of circulating neutrophils during homeostasis changes remarkably during acute inflammation. Additional subsets are rapidly recruited: CD16low neutrophils are thought to be recruited from the bone marrow, while the origin of CD62Llow neutrophils is largely unknown. The stimulus for and if there is individual variability in subset recruitment during acute inflammation is also unknown. Objectives To determine if the recruitment of neutrophil subsets during acute inflammation corresponds with circulating cytokines. To determine if subset recruitment is correlated to pre-existing neutrophil reactiveness. Methods Volunteers were given a LPS endotoxin bolus followed by 3 hours continuous infusion. Blood was taken before and after 3 hours of administration. Samples were directly measured on an automatic flow cytometer. In addition, baseline blood was left for 3 hours in the blood tube to measure neutrophil reactiveness. Plasma cytokines were measured over time. Results CD62Llow neutrophil recruitment to the circulation correlated with the amount of circulating cytokines like TNFα, IL6 and IL8, and negatively correlated with IL10. More reactive donors for activation at baseline also had higher amounts of circulating cytokines and more recruitment of CD62Llow cells upon LPS challenge. Conclusions Especially the recruitment of the CD62Llow subset during LPS challenge correlated with the amount of circulating cytokines. It is unknown if this relationship is causal. Interestingly, pre-existing sensitivity in individuals correlated with the excessiveness of the response to LPS challenge. This is an important finding, since it might be used to predict the immune response in different acute inflammatory situations.

P87 NEUTROPHIL EXTRACELLULAR TRAPS TRIGGER AN ENHANCED PRO-INFLAMMATORY RESPONSE IN MACROPHAGE SUBPOPULATIONS IN RHEUMATOID ARTHRITIS PATIENTS VIA THE AHR PATHWAY

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Objective. Activated neutrophils (PMN) form neutrophil extracellular traps (NET). Increased NET formation has been reported in rheumatoid arthritis (RA). Moreover, macrophages (MAC) play a key role in RA pathogenesis. We have previously shown that NET are pro-inflammatory on resting non-polarized MAC, whereas NET partly inhibit the response of LPS-stimulated MAC. We now aimed to characterize inflammatory properties of NET on polarized MAC sub-populations in RA versus healthy donors (HD) and to determine the mechanisms/pathways involved. Methods. Blood PMN/monocytes were purified from HD/RA patients. Monocytes were differentiated into non-polarized (M0), anti-inflammatory (M2a, M2c) or pro-inflammatory (M1) MAC. NET were induced in vitro by stimulating PMN with PMA. Mouse (wild-type, TLR9-KO) bone marrow was used to differentiate MAC and to prepare NET. MAC were cultured with NET in the presence/absence of LPS or an aryl hydrocarbon receptor (AhR) antagonist. Cell purity/phenotype/activation were estimated by flow cytometry/ELISA. The pathway triggered was estimated by bulk RNA-seq. Gene expression was confirmed by qRT-PCR. Results. All resting MAC subpopulations were activated by NET, leading to a pro-inflammatory response. The pro-inflammatory cytokine IL-8 was secreted, whereas secretion of the immunomodulatory IL-10 was minimal. Even M2 MAC were activated toward this pro-inflammatory profile, with a stronger response in RA patients. In response to LPS, NET inhibit IL-6 secretion in HD MAC whereas RA MAC were resistant to this anti-inflammatory activity of NET. In mouse MAC, TLR9 is not involved in NET recognition. RNA-sequencing suggested involvement of the AhR pathway in MAC in response to NET via CYP1A1 induction. Triggering of the AhR pathway was confirmed using AhR antagonists. Conclusion. Pro-inflammatory activities of NET clearly dominate anti-inflammatory activities in MAC, particularly in RA patients, suggesting a pathogenic role of NET via AhR signaling.

P88 HDAC11 INHIBITION MODULATES THE PROTEIN CARGO OF EXTRACELLULAR VESICLES FROM NEUTROPHILS RECRUITED TO CYSTIC FIBROSIS LUNGS

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Objective: Histone deacetylase 11 (HDAC11) is a rapidly acting defatty-acylase catalyzing demyristoylation and depalmitoylation of target substrates. The lysine defatty-acylase activity of HDAC11 has been shown to regulate protein translocation to late endosomes/lysosomes, impacting both anti-viral responses and phagocytosis. In chronic cystic fibrosis (CF) lung disease, neutrophils are the predominant luminal subset, displaying a distinct phenotype characterized by hyperexocytosis, active repression of bacterial killing and a high secretion of extracellular vesicles (EVs). Methods: We aimed to characterize CF lung neutrophil-derived EVs using a multi-omic approach focused on their protein content (by mass spectrometry) and the transcriptome of naïve neutrophils exposed to those EVs (by RNAseq). Results: The dominant proteomic signature of CF lung neutrophil-derived EVs featured secretory granule proteins and antioxidant enzymes. Inhibition of HDAC11 by the defatty-acylase activity inhibitor SIS-17 led to reduced granule protein and antioxidant enzyme contents of these EVs. Furthermore, CF lung neutrophil-derived EVs promoted a massive down-regulation of mRNA content in receiving naïve neutrophils. In comparison, EVs from SIS17-treated CF lung neutrophils led to transcriptional upregulation of antioxidant genes controlled by Nrf2 through the delivery of PARK7. Conclusions: Our data suggest that CF lung-recruited neutrophils modify the phenotype of newly transmigrated neutrophils (and possibly of other cells in that microenvironment, like epithelial cells and macrophages) by delivering EV-borne effectors and transcriptional regulators, driving chronic disease.

P89 LOW DENSITY NEUTROPHILS ARE NEW INFLAMMATORY PLAYERS IN HEART FAILURE: ROLE OF NEUTROPHIL EXTRACELLULAR TRAPS (NETs)

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Circulating neutrophils regroup two subtypes: high- and low-density neutrophils (HDNs and LDNs). LDNs count represents about 2% of total neutrophil under physiological conditions, but is increased in multiple pathologies and have a higher capacity to release pro-inflammatory cytokines and neutrophil extracellular traps (NETs). Heart failure either with reduced (HFrEF) or preserved ejection fraction (HFpEF) is characterized by low-grade chronic inflammation. We wanted to determine if LDNs count is increased in HF patients and if their inflammatory properties are enhanced vs HDNs. HDNs and LDNs were isolated from human blood by density gradient and purified by cell sorting. NETs formation (NETosis) was quantified by confocal microscopy. Circulating inflammatory and NETosis biomarkers were measured by ELISA. Neutrophil adhesion onto endothelial cells (ECs) was assessed by optical microscopy. HDNs and LDNs counts were increased up to 39% and 2740% respectively in hospitalized acute decompensated (ADHFpEF) patients vs healthy individuals. In all HF patients, the correlations between LDNs counts and circulating inflammatory (CRP, IL-6 and -8, NT-proBNP, and NETosis components (MPO, MPO-DNA complex and citrullinated histone 3; H3Cit) were all significant. LDNs expressed more MPO, H3Cit and NETs and were more pro-adhesive onto ECs. In summary, increasing circulating LDNs and NETosis components could be considered as new biomarkers of inflammation severity in HF patients.

P90 *Porphyhromonas gingivalis* LYSATE INDUCES NETOSIS ON PERIPHERAL BLOOD POLYMORPHONUCLEAR CELLS OF HEALTHY PATIENTS.

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Neutrophils or polymorphonuclear cells (PMN) are innate immune cells whose main functions are phagocytosis, degranulation, formation of reactive oxygen species (ROS) and release of extracellular DNA traps (NET). NETosis is a process of cell death resulting from the release of nuclear chromatin and bactericidal proteins from neutrophils in the presence of pathogens or certain molecules. The objective of this work was to analyze the in vitro formation of NET in PMN cells from peripheral blood of healthy patients in the presence of different concentrations (10E7,10E8 and 10E9) of lysate of *Porphyromonas gingivalis* (ATCC 33227) and to compare them with different controls (unstimulated cells, and stimulated with phorbol myristate acetate [PMA] and dental alginate) at different incubation times (15, 30 and 60 minutes). Once the informed consent was signed, blood samples were obtained and PMN cells were separated by density gradient. 10E6 PMN were incubated by triplicate in boxes of 24 wells per condition. Results were analyzed by fluorescence microscopy for DNA detection with DAPI and cellular labeling of H2DCFDA (2',7'dichlorodihydrofluorescein diacetate; 1mg/ml), an indicator for ROS formation, was analyzed by flow cytometry. Results in the control group and with alginate neither showed cellular morphological changes nor induced NETosis; while incubation with PMA and *P. gingivalis* lysate promoted NETosis; and it was dose-dependent in the group stimulated with *P.gingivalis*. Therefore, we conclude the total lysate of *P.gingivalis* is a bacterial stimulus of NETosis in peripheral blood PMN cells incubated in vitro, and the activation mechanism in the formation of these traps is mediated by ROS.

P91 TEMPORAL PHOSPHOPROTEOMIC ANALYSIS OF NEUTROPHILS DURING BACTERIAL INFECTION REVEALS FUNCTIONS RELATED TO TRANSCRIPTIONAL AND TRANSLATIONAL DYNAMICS

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Neutrophil dysfunction has been previously shown to associate with adverse outcomes. These include heightened susceptibility to nosocomial infection, of which Staphylococcus aureus is a common causative organism. Therefore, understanding pathways by which neutrophils interact with this pathogen and what happens when neutrophils rendered dysfunctional will offer novel insights into the pathobiology of infection and offer the prospect of new non-antibiotic therapies. A time-course study of neutrophil phosphoproteomic response to heat-killed S. aureus was conducted for such objectives. Bayesian Hierarchical Clustering was performed to group proteins based on their phosphorylation status and what functions they enrich for. Based on phosphoproteomic signature, autophagy, phagocytosis, and degranulation are enriched among phosphorylated proteins, while mRNA processing is the consensus term among dephosphorylated proteins. A big portion of dephosphorylated proteins are RNA-binding proteins including HNRNP and RS proteins, which can be activated by either phosphorylation or dephosphorylation. Complementary proteomic and RNAseq data analysis show that neutrophils are transcriptionally active as early as 15-30 minutes and start producing necessary proteins in 60-120 minutes after encountering bacteria, with CXCL8 and members of AP1 transcription factors (c-FOS, JUNB, FOSB, FOSL1) demonstrate the most dramatic change. In conclusion, bacterial stimulation disrupts autophagy and transcriptional machinery in neutrophils. For further investigation, ribosome profiling, among others, will be utilised to investigate how neutrophils navigate transcriptional and translational dynamics during bacterial encounter.

P92 A BISPECIFIC MONOCLONAL ANTIBODY TARGETING PSL AND PCRV ENHANCES BRONCHIECTASIS PATIENT NEUTROPHIL-MEDIATED KILLING OF PSEUDOMONAS AERUGINOSA

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Introduction: Impaired neutrophil killing of P. aeruginosa (PA) is a feature of bronchiectasis, a chronic neutrophilic inflammatory lung disease. We tested whether a bispecific monoclonal antibody (Mab0001) targeting Psl exopolysaccharide and T3SS component PcrV could enhance neutrophil clearance of PA in bronchiectasis. Methods: Sputum PA isolates (n=100 patients) underwent WGS for target evaluation. Bronchiectasis patient (n=9-11; Dundee,UK), matched control (n=5), and healthy control (n=5) blood neutrophils were incubated with PA alone, with Mab0001 (0.2-200nM), or isotype control; phagocytosis (GFP-PA) was quantified at 15 min or PA survival quantified at 2h. Endogenous anti-Psl and PcrV titres in patient sera (n=31) were measured by ELISA; competition with Mab0001 was tested by spiking serum (n=3-5) into the above assay and epithelial cytotoxicity assays. Results: Psl and PcrV were well conserved targets in infected patients; 72/100 isolates had a full Psl operon and 99/100 expressed PcrV. All patients had endogenous anti-PcrV antibodies, but only 3/31 had anti-Psl titres; no evidence of antibody competition with Mab0001 was found. Mab0001 increased bronchiectasis patient neutrophil-mediated killing of PA by 34.6±8.1% and phagocytosis (%positive cells) by 26.5±13% (mean±SD; 200nM), similar to effects in healthy (killing:+36±8.6%, phagocytosis:+22±10%) and matched controls (killing:+30.1±7.6%). Conclusion: Mab0001 enhanced neutrophil clearance of P. aeruginosa in vitro and is a promising anti-Pseudomonal therapy. A planned clinical trial will evaluate Mab0001 activity in vivo.

P93 FUNCTIONAL DEVELOPMENT OF NEUTROPHILS IN THE HUMAN BONE MARROW

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Background The functional development of neutrophil subsets in the human bone marrow is largely unknown. It is important to know when different antimicrobial functionalities develop in the neutrophil subsets because the different subsets are released from the bone marrow into the circulation during inflammatory conditions. Their purpose there is still unknown. Objectives To characterize the functional development of the following neutrophil subsets in the human bone marrow: promyelocytes, myelocytes, metamyelocytes, banded neutrophils, mature neutrophils and hypersegmented neutrophils. Methods We used flow cytometric analysis and cell sorting to sort the six subsets from bone marrow samples that we obtained from volunteers. The six subsets were tested for their bacterial phagocytosis- and killing capacity, calcium influx response and migration capacity to different stimuli. Results Phagocytosis capacity developed abruptly between the metamyelocyte (median 22.6%) and banded stage (median 68.7%). The bacterial killing capacity of cells that phagocytosed bacteria developed more gradually and reached an optimum in the banded subset. The calcium influx response developed gradually for every stimulus and generally reached an optimum in the mature subset. Migration towards a stimulus was first seen in the banded subset and reached an optimum in the mature subset. Conclusions The different anti-microbial functions were overall optimally developed in the banded and mature subsets. The antimicrobial function of younger neutrophil subsets (promyelocytes, myelocytes and metamyelocytes) is suboptimal, as is the case for more activated hypersegmented neutrophils. It is likely that these subsets have a different function other than a primarily antimicrobial function when they are released into the circulation during inflammatory conditions.

P94 IN VIVO DEUTERIUM LABELING OF NEUTROPHIL PRECURSORS IN HUMAN BONE MARROW IDENTIFIES A LINEAR CONVEYOR BELT STRUCTURE OF NEUTROPHIL MATURATION

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The circulatory lifespan of the human neutrophil is still fiercely debated, with estimates ranging from 6 hours to 5.4 days. The gap in knowledge on neutrophil progenitor proliferation and differentiation in the bone marrow precluded adequate analysis of the kinetics of circulatory neutrophils. This is the first study to measure the kinetics of the different neutrophil progenitors during granulopoiesis in healthy human bone marrow, both before and after experimental endotoxemia. 6,6-2H-glucose pulse-chase labeling was applied in 12 healthy volunteers, and at 1 to 9 days after the labeling, blood and bone marrow samples were simultaneously obtained. For 8 volunteers the labeling procedure was repeated and at 3 to 6 days after the second labeling experimental endotoxemia was induced by i.v. injection of 2 ng/kg LPS. A second blood and bone marrow sampling was performed 4h after the induction of experimental endotoxemia. The different progenitor stadia of the neutrophil were sorted, their DNA was isolated and 2H-label enrichment in the DNA was determined by GC-MS. This data provides invaluable new insights, clarifying that neutrophil differentiation in the bone marrow follows a first-in is first out principle/ linear conveyor belt structure. Furthermore, in contrast to the current dogma, myelocytes are not dividing and are thus part of the PMP. During acute inflammation evoked by LPS challenge we find banded and mature neutrophilia in the circulation together with shorter transit times through banded and mature neutrophil pools of the bone marrow. This accelerates the output of mature cells at the expense of the size of the mature bone marrow pool.

P95 INVESTIGATING MECHANISMS OF HOXB8-CONDITIONAL NEUTROPHIL PROGENITOR ENGRAFTMENT IN THE MURINE HOST

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Neutropenia or neutrophil dysfunction are associated with increased susceptibility to severe bacterial and fungal infections. Recently, we characterized murine neutrophil progenitor cell lines (NPs) that are conditionally-immortalized via HoxB8 expression and are uniquely capable of engrafting in the naïve murine host. We propose that NPs may serve as a therapeutic adjunct for reducing infection resulting from neutropenia or neutrophil dysfunction. To achieve this, it is first important to understand the mechanisms of NP engraftment in the hematopoietic niche. We have observed that NPs home and/or engraft via a VLA4-independent, beta1 integrin-dependent mechanism. Further, we found that engrafted NPs proliferate and differentiate into mature neutrophils that are mobilized to the periphery via canonical CXCR2 signaling. Here, we describe studies to determine the impact of cytoreductive conditioning of host niche space via antibody-mediated depletion of Ly6G-expressing cells or busulfan-mediated HSPC ablation on NP engraftment. To further evaluate the potential translational utility of NPs, we also probe candidate integrin alpha subunits and signaling receptors to determine their role in NP homing and engraftment. These studies provide essential insight into the fundamental biology of NP engraftment in addition to their therapeutic potential.

P96 naRNA IS A CANONICAL NEUTROPHIL EXTRACELLULAR TRAP (NET) COMPONENT AND NOVEL INFLAMMATION-AMPLIFYING COMPOSITE DAMP

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Neutrophil extracellular traps (NETs), composed of DNA, histones, and antimicrobial proteins, have emerged as a key feature of cellular innate immunity. Whilst DNA has been in focus as a primary structural component of NETs, we here characterize NET-associated RNA (naRNA), as a new canonical, abundant, and largely unexplored NET component. naRNA decorated all types of NETs in complex with the antimicrobial peptide LL37. In fact, naRNA was pre-associated with LL37 intracellularly as a ‘composite’ danger-associated molecular pattern (DAMP) prior to neutrophil activation. Externalized, naRNA propagated NET formation in naïve PMN, dependent on TLR8 in humans and Tlr13 in mice, in vitro and in vivo. naRNA-TLR8/Tlr13 signaling significantly contributed to the highly sensitive pro-inflammatory response of both tissue cells, and other immune cell types. Those responses could be blocked by inhibition and genetic ablation of RNA receptors or RNase treatment. Importantly, naRNA strongly drove skin inflammation in vivo, whereas genetic ablation of RNA sensing drastically ameliorated skin inflammation in the imiquimod psoriasis model. Our data highlight naRNA as a novel composite DAMP signaling and amplifying neutrophil activation. Moreover, naRNA emerges as the likely driver of inflammation in conditions previously linked to NETs and extracellular RNA, suggesting blockade of TLR-mediated RNA sensing as potential new intervention target.

P97 ENGINEERING CHIMERIC ANTIGEN RECEPTOR NEUTROPHILS FOR TARGETED CANCER IMMUNOTHERAPY

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Neutrophils, the most abundant white blood cells in circulation, are closely related to cancer development and progression. Primary neutrophils from healthy donors present potent cytotoxicity against human cancer cell lines through direct contact and reactive oxygen species (ROS) generation. However, due to their short half-life and resistance to genetic modification, neutrophils have not yet been engineered with widely used chimeric antigen receptors (CARs) to enhance their anti-tumor cytotoxicity for targeted immunotherapy. Here, we genetically engineered human pluripotent stem cells (hPSCs) with different synthetic CARs and successfully differentiated them into functional neutrophils by implementing a novel chemically-defined differentiation platform. Neutrophils expressing the chlorotoxin (CLTX)-T-CAR presented specific cytotoxicity against glioblastoma (GBM) cells in monolayer and 3D cultures. In a GBM xenograft mouse model, systematically-administered CLTX-T-CAR neutrophils displayed enhanced anti-tumor activity and prolonged animal survival compared with peripheral blood neutrophils, hPSC-neutrophils, and CLTX-NK-CAR natural killer (NK) cells. We collectively established a new platform for producing CAR-neutrophils, paving the way for myeloid cell-based therapeutic strategies that would complement and boost current cancer treatment approaches.

P98 NEUTROPHILS FUEL THE DEVELOPMENT OF NAFLD THROUGH LIPID DELIVERY TO HEPATOCYTES FROM ADIPOSE TISSUE

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Non-alcoholic fatty liver disease (NAFLD) is a chronic liver disease in which hepatic fat is accumulated without alcohol intake. Although neutrophils, the most abundant innate immune cells, are involved in the inflammatory process of NAFLD, the contribution of neutrophil for fat accumulation in the liver during NAFLD has not been fully understood yet. In this study, we found that neutrophils deliver lipids from adipocytes into liver, contributing the pathogenesis of NAFLD. Neutrophil exposed to free fatty acids exhibited the increased numbers of lipid droplets, and the inhibition of fatty acid uptake using inhibitors of CD36 and Fabp4 (fatty acid binding protein 4) completely inhibited fatty acid uptake in neutrophils and neutrophils co-cultured with adipocytes also exhibited increased numbers of lipid droplets, suggesting lipid-laden capacity of neutrophils. We further cultured these lipid-laden neutrophils with hepatocytes and found that neutrophils deliver ingested lipids into hepatocytes. We further confirmed the existence of lipid-laden neutrophils using a murine model of NAFLD. Peripheral neutrophils isolated from mice fed with high fat diet (HFD) exhibited the increased numbers of lipid droplets than neutrophils isolated from mice fed with normal chow. Moreover, liver-infiltrating neutrophils isolated from NAFLD mice also exhibited the increased numbers of lipid droplets. Finally, we examined the existence of lipid-laden neutrophils in patients with NAFLD. Peripheral neutrophils isolated from patients with NAFLD exhibited the increased numbers of lipid droplets than neutrophils isolated from healthy subjects, suggesting the existence of lipid-laden neutrophils during the pathogenesis of NAFLD. Our findings suggest the possible role of neutrophils as a lipid-delivering vehicle during the pathogenesis of NAFLD.

P99 NEUTROPHIL EXTRACELLULAR TRAPS TRIGGER OSTEOCLASTOGENESIS AND INFLAMMATORY BONE DESTRUCTION

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Neutrophil infiltration is a hallmark in diseases of inflammatory bone destruction such as RA and Periodontitis. However, the mechanisms by which neutrophils participate in pathogenesis and tissue destruction remain unclear. Neutrophil extracellular trap (NET) formation has been previously associated with inflammation and autoimmunity. Yet, the direct role of NETs in tissue destruction have not been defined. Through complementary, single cell sequencing, proteomic and imaging approaches, we demonstrate that NETs trigger osteoclastogenesis in human monocytes, via a mechanism that is independent of RANKL activation but relies on neutrophil elastase and histone signaling through Toll-like 4 receptor. Of note, protein carbamylation in NETs, a post-translational modification that triggers specific adaptive immunity previously associated with erosive bone disease, potentiated osteoclastogenesis. In vivo, in an animal model of inflammatory bone loss, we confirm that NETs mediate osteclastogenesis and bone destruction. Removal of NETs through DNase 1 treatment as well as inhibition of NET formation with genetic models (PAD4-/- and NE-/-), prevent osteoclastogenesis and bone destruction. Furthermore, NETs are documented in disease lesions of inflammatory bone loss, while carbamylated NET complexes are significantly elevated in serum and lesions of subjects with inflammatory bone loss, in association with disease severity. These observations support important local and systemic roles for NETs in the development of inflammatory bone diseases.

P100 MATRIX-PRODUCING NEUTROPHILS SHIELD THE SKIN

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New phenotypic and functional tissue-dependent states have been recently attributed to neutrophils and have shifted our view of these cells from purely immune to homeostatic. This change has in turn raised the possibility that neutrophils have evolved complementary strategies for organismal defense, e.g. at barrier tissues that line the external environment. While examining the functional diversification of neutrophils, we discovered a population of neutrophils residing in barrier tissues (lung, intestine and skin) that expressed mRNAs encoding proteins needed to build extracellular matrix. By comparing control with neutrophil-depleted mice, we discovered a pivotal role for the maintenance of matrix composition, structure as well as mechanical stiffness in the skin. We find that this matrix-producing program relies on TGFb signalling, and selective loss of the TGFb receptor (TGFbDN mice) in neutrophils recapitulated the matrix and mechanical alterations seen in neutropenic mice. Finally, we investigated the contribution of matrix-producing neutrophils in barrier function. Neutropenic or TGFbDN mice featured increased permeability of the skin and, in the context of skin wounds, neutrophils created a matrix-rich ring around the wound that shielded the organism from the entry of external microorganisms and small molecules. We conclude that, beyond their canonical antimicrobial functions, neutrophils protect the host from external aggressors by physically reinforcing barriers. Because this program is already active in the steady state, we propose that barrier sites harbour innate immune cells pre-emptively poised to prevent, rather than fight, pathogenic attacks.

P101 ACQUISITION OF PROANGIOGENIC NEUTROPHILS VIA SITE-SPECIFIC ACTIVATION OF THE CCL20–CCR6 AXIS BY TNF-α IS REQUIRED FOR ISCHEMIC MUSCLE REVASCULARISATION

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Signaling via the inflammatory cytokine TNF-α has been implicated in post-ischemic new vessel formation. Certainly, mechanisms of TNF-α-driven inflammatory cascades promoting vessel formation within ischemic muscle tissues have not been characterized yet. Post-ischemic muscle tissue revascularization in hindlimbs of mice was monitored upon permanent femoral artery ligation by Laser Doppler imaging and light sheet fluorescence microscopy. TNF-α-mediated activation and contribution of immune-inflammatory pathways to new vessel formation were studied in in vitro and in vivo experiments using flow cytometric, biochemical and molecular biological tools. TNF-α-mediated signaling via TNFR1 but not TNFR2 was found to be required for post-ischemic muscle tissue revascularization. Systemic and local immunoprofiling, bone marrow transplantation studies and transcriptome analysis identified bone marrow derived neutrophils (nph) as critical cellular components for the initiation of new vessel formation. Mechanistically, we show that TNF-α-TNFR1-dependent production of the C-C motif chemokine ligand CCL20 by vascular cells and cell surface translocation of the corresponding CCR6 receptor on nph determines directed migration of proangiogenic nph to sites of ischemia. In a murine model of diabetes featuring insufficient post-ischemic angiogenesis, we observed reduced expression levels of CCL20 and numbers of proangiogenic nph, whereas sequential administration of rCCL20 combined with the statin family member Fluvastatin improved ischemic muscle tissue revascularization. Our results demonstrate that the site-specific activation of the CCL20-CCR6 axis by TNF-α is required for the acquisition of proangiogenic nph and thus may offer immunotherapeutic potential to promote ischemic peripheral muscle tissue revascularization.

P102 NEUTROPHILS ROLL FASTER; AND ADHERE AND TRANSMIGRATE LESS IN RESPONSE TO CXCL1 DIMERS COMPARED TO MONOMERS IN VENULES OF THE INFLAMED CREMASTER MUSCLE

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In mice, the chemokine CXCL1, also known as keratinocyte-derived chemokine (KC), is one of the main chemokines that induces recruitment of peripheral blood neutrophils to sites of injury or infection. KC mediates recruitment by activating the CXCR2 receptor and binding tissue glycosaminoglycans (GAGs) that regulate receptor interactions. KC reversibly exists as monomers and dimers, and in peritonitis models, neutrophil recruitment is induced by monomers at low concentrations, and by dimers at higher concentrations. Here, we explored the impact of chemokine dimerization on neutrophil recruitment dynamics of the extravasation cascade. We analyzed neutrophil extravasation by intravital microscopy in venules of the cremaster muscle perfused with recombinant WT CXCL1 or CXCL1 dimers. KC dimer compared to WT KC induced weak interactions of neutrophils with the vascular endothelium as manifested by faster rolling and reduced firm adhesion, which, in turn, resulted in a significant decrease of transmigration. In addition, arrest assays after injection of KC dimer via the carotid artery showed that neutrophils do not adhere properly as compared to WT KC. These data suggest that KC dimer is less efficient in inducing neutrophil recruitment, potentially due to incomplete B2-integrins activation, which is currently under investigation. We suggest that shifting the balance of chemokine monomers and dimers in vivo might be a novel strategy to avoid excessive neutrophil recruitment in neutrophil-inflicted inflammatory diseases such as colitis and sepsis.

P103 EFFECT OF DIPEPTIDYL PEPTIDASE-1 (DPP1) INHIBITION ON NEUTROPHIL SERINE PROTEASES, PROTEOME AND FUNCTIONAL RESPONSES: RESULTS FROM THE STOP-COVID19 TRIAL

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Introduction: DPP1 is an activator of neutrophil serine proteases implicated in severe COVID19. We investigated once-daily dosing of Brensocatib 25mg—an oral competitive inhibitor of DPP1—for 28 days in hospitalized COVID19 patients. A prespecified substudy of the trial evaluated effects of DPP1 inhibition on neutrophil inflammatory markers, proteome and function. Methods: The substudy was performed at 2 UK sites. Blood samples were obtained at days 1, 8, 15 and 29. Analyses included peripheral blood neutrophil LC/MS and functional testing, CyTOF, mRNAseq and immunoassays for neutrophil-associated markers. Results: 152 patients were enrolled (Brensocatib n=75; Placebo n=77; Jun20-Jan21). Plasma elastase activity was significantly reduced from day 8 in the Brensocatib arm ($p<0.0001$), with increased neutrophil surface expression of protease-cleavable C5aR1 by day 29 ($p<0.05$). LC/MS showed significant reductions in neutrophil cathepsin G and the pseudoenzyme azurocidin-1 (AZU1) (FDR<0.01) at day 29. In serum, AZU1 levels, but not total elastase or proteinase 3 protein, were significantly reduced ($p<0.0001$). There were no differences in endogenous or ex-vivo-generated neutrophil extracellular traps, neutrophil phagocytosis, circulating immune cell proportions or gene expression. Conclusion: Brensocatib treatment reduced neutrophil serine protease activity and had significant effects on AZU1, an underrecognized potential DPP1 target.

P104 *S. aureus* ALPHA-TOXIN DISRUPTS NEUTROPHIL CALCIUM SIGNALING AND EARLY PATTERNING OF NEUTROPHIL RESPONSE TO INFECTION

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Calcium signals initiated via store-operated calcium entry (SOCE) are essential for neutrophil activation. Given the importance of SOCE in immune cell function, this pathway poses an opportunity for pathogens to manipulate as a mechanism of immune evasion. The *S. aureus* alpha-toxin (Hla) is a pore-forming toxin that contributes to disease pathogenesis. While high concentrations of Hla induce cell death, neutrophils are relatively resistant to lysis, suggesting that the biologic action of Hla on neutrophils is not solely conferred by lytic susceptibility. Using in vivo 2-photon microscopy, we observed that in the first 3 hours after infection, hla- *S. aureus* induced neutrophil migration towards a discreet wound where neutrophils formed dense clusters at the wound edge. In contrast, neutrophil localization and clustering was markedly impaired by WT *S. aureus*. In vitro, neutrophil chemotaxis and clustering is disrupted by recombinant Hla or WT *S. aureus*. Hla forms a narrow, ion selective pore, therefore we hypothesized that Hla impairs neutrophil function through dysregulation of calcium or other ion fluxes. Sublytic Hla did not induce calcium influx, suggesting that the pore is not permeable to calcium. However, Hla caused rapid membrane depolarization. Depolarization decreases the electrogenic driving force for calcium and indeed, Hla pre-treatment suppressed SOCE and calcium-dependent LTB4 production, a key mediator of neutrophil clustering. Our data suggest that Hla disrupts the early patterning of the neutrophil response to infection, at least in part through impairment of neutrophil calcium signaling. Given the rapid doubling time of S. aureus we propose that this early mis-localization of neutrophils enables establishment of infection.

P105 NEUTROPHIL EXTRACELLULAR TRAP RELEASE RESULTS IN LOW-DENSITY NEUTROPHILS IN ALCOHOLIC HEPATITIS THAT CONTRIBUTE TO LIVER DAMAGE

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Massive inflammation and liver failure are main contributors to high mortality in alcohol-associated hepatitis (AH) and neutrophils in the liver and circulation correlate with poor clinical outcomes. We hypothesized that neutrophils contribute to liver damage and inflammation in AH. Neutrophils extracellular traps (NETs) significantly increased in neutrophils isolated from AH patients compared to controls. We discovered a unique LDNs population in AH patients and alcohol-fed mice. Transcriptome analysis of LDNs and high-density neutrophils (HDNs) from AH patients revealed that LDNs are functionally exhausted while HDNs are activated. AH HDNs have increased ROS and produce higher ROS upon LPS stimulation than control HDNs whereas AH LDNs fail to respond to LPS. LDNs are generated from HDNs after alcohol-induced NET release in vitro and this LDN subset has decreased phagocytosis. Moreover, LDNs had reduced homing and clearance by macrophage efferocytosis, and dysfunctional neutrophils remained in circulation and liver. Depletion of both HDNs and LDNs in vivo prevented alcohol-induced NET production and liver damage in mice; G-CSF treatment also ameliorated alcohol-induced liver injury. CSF polarized toward M2-like phenotype and increased hepatocyte proliferation in hepatocytes. Finally, G-CSF increased G-CSF receptor expression and reduced levels of phosphorylated β-catenin in hepatocytes. In summary, neutrophils contribute to liver damage through increased NET formation which increases defective LDNs in AH. Alcohol induces activated HDNs and defective LDNs. G-CSF treatment ameliorates alcohol-induced liver injury and promotes hepatocyte proliferation. Here, we provide mechanistic insights for therapeutic interventions in AH.

P106 NEUTROPHIL INTEGRIN ALPHA9 PROMOTES DEEP VEIN THROMBOSIS

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The integrin alpha9 (ITGA9) is highly expressed on neutrophils, upregulated upon neutrophil activation and transmigration, and is known to stabilize neutrophil adhesion to the activated endothelium. However, the role of neutrophil ITGA9 in the pathogenesis of deep vein thrombosis (DVT) is not known. In the current study, we evaluated the mechanistic role of neutrophil ITGA9 using a mouse model of DVT (inferior vena cava, IVC-stenosis). We observed that neutrophil ITGA9 levels were significantly increased following DVT and that was concomitant with the increase in several neutrophil activation and inflammatory markers. Next, we observed significantly reduced thrombosis incidence and thrombus weight on day 2 following IVC stenosis in neutrophil specific ITGA9 deficient mice as compared to littermate control mice. Neutrophil specific ITAG9 deficiency also resulted into reduced neutrophil content and citrullinated histone H3-positive cells in IVC thrombus. Next, we performed deep proteomic profiling using Tandem mass tag (TMT)-based mass spectrometry (MS) in neutrophils isolated ITAG9 deficient and control mice (6-hr post IVC stenosis). After comparing controls and ITGA9 deficient neutrophils, we found total 179 differentially expressed proteins with several key functions such as apoptosis, cell cycle regulation, chaperones, and kinases. Detailed bioinformatic analyses of the neutrophil proteome revealed reduced expression of proteins associated with glycolysis, inflammasome activation and neutrophil extracellular traps formation (NETosis) in neutrophils isolated ITAG9 deficient mice as compared to control mice. In conclusion, we revealed a previously unknown role of ITGA9 as a critical regulator of neutrophil hyperactivation in the pathogenesis of DVT.

P107 POINT-OF-CARE, AUTOMATED FLOW CYTOMETRY HOLDS SIGNIFICANT CLINICAL AND INVESTIGATIONAL VALUE

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Introduction: The neutrophil compartment responds quickly to infectious (MAMPs) and inflammatory cues (DAMP's and cyto-/chemokines) found in damaged and inflamed tissues. This fast response makes neutrophils challenging to study as they become activated in a blood collection tube within 30 minutes, altering results significantly. This ex-vivo activation becomes even more pronounced due to the long work-up times of traditional flow analyses. Objective: to test whether fast, automated flow cytometry can limit ex vivo neutrophil activation and can be used for improved diagnosis and monitoring of inflammatory conditions. Methods: Several patient and healthy control cohorts were analyzed point-of-care (PoC) directly (within 30 min) after venipuncture: 1) 86 patients at the emergency department (ED) with suspected infection, 2) 34 trail runners before, directly after and 24 hours after extreme exercise, 3) 47 ex-COVID-19 patients 2 years after the initial disease. Cohort 1 was analyzed by a device located at the ED. Cohort 2 and 3 were analyzed by a mobile, automated flow cytometry field laboratory in a van. Results: 1) PoC, automated flow cytometry offers very good diagnostic value for bacterial (AUC 0.86) and viral (AUC 0.93) infections. 2) exercise induced neutrophilia is associated with banded and hyper segmented subsets, which resolved within 24 hours, 3) ex-COVID-19 patients still show activated neutrophils 2 years after initial disease. Conclusion: These results show that fully automated PoC flow cytometry can be applied in fast clinical decision making. Furthermore, the mobile, automated flow cytometry field laboratory provides innovative data that will lead to better understanding of short and long term innate immune responses to pathogenic and normal physiological stimuli.

P108 SEVERE MALARIA MICE MODEL DEVELOPS NEUTROPHIL EXTRACELLULAR TRAPS

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Malaria accounts for approximately 450 000 world-wide deaths each year, such fatal cases are related to an exacerbated inflammatory response that causes severe organ failure. Infection of C57BL/6 mice with Plasmodium berghei ANKA (PbA) is a lethal experimental model that recapitulates a wide range of physiological events that take place during severe malaria in humans. Since it has been proposed that the formation of neutrophil extracellular traps (NETs) contributes to organ and tissue damage in patients that die from this parasitic infection, we aimed to asses if infection with PbA induced the formation of NETs in vivo and in vitro, as well as the possible inducers of this phenomena. Therefore, C57BL/6 mice were inoculated with PbA parasitized red blood cells (pRBC), on the seventh day after infection an increase in peripheral blood neutrophils was observed in addition to the presence of NET-like structures in blood smears of PbA infected animals. Moreover, in vitro assays demonstrated that the interaction between healthy mice bone marrow neutrophils and pRBC or PbA infected animal serum triggered the formation of NETs (composed by nucleic acid, myeloperoxidase, and elastase). Thus, this animal model can be used to study NETs participation in severe malaria, and serve to evaluate potential therapeutic interventions related to this cellular mechanism.

P109 PHOSPHOPROTEOMIC ANALYSIS OF NOX2-DEPENDENT AND NOX2-INDEPENDENT NETOSIS

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Neutrophil extracellular traps (NETs) are web-like structures comprised of chromatin and antimicrobial proteins, such as neutrophil elastase and myeloperoxidase. They are released through an active, pro-inflammatory form of cell death, termed NETosis. NETs trap pathogens, exposing them to high concentrations of antimicrobial proteins, and prevent their dissemination in the host. However, dysregulated NETosis exacerbates and contributes to both infectious (e.g malaria, COVID-19) and sterile (e.g cancer, lupus) disease. Despite their role in immunity and inflammation, the mechanisms by which NETs are made remain poorly understood. Many stimuli require NADPH oxidase (NOX2) derived ROS to induce NETosis, whereas others are NOX2-indepedent. However, all NETosis stimuli lead to the same characteristic features: nuclear delobulation, chromatin decompaction, plasma membrane rupture and chromatin release. Furthermore, both pathways are dependent on kinase activity. To investigate the signalling pathways involved in NETosis, we conducted tandem mass tag phosphoproteomics on human neutrophils stimulated with either PMA (NOX-dependent stimulus) or calcium ionophore (NOX-independent stimulus). We identify common and differentially regulated pathways between these two stimuli. This work will help elucidate novel NETosis regulators, which could be targeted to treat inflammatory disease.

P110 MAST CELL-DERIVED IL-17A FACILITATES NEUTROPHIL DIAPEDESIS BY MEDIATING NEUTROPHIL BREACHING OF THE PERICYTE LAYER IN VIVO

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Fundamental to the establishment of a rapid immune response following infections and injury, neutrophil diapedesis is driven by complex interactions between leukocytes and the different components of blood vessel walls. Here, we identified perivascular mast cells (MCs) as key regulators of neutrophil behaviour within the sub-endothelial space of inflamed venules. Using confocal intravital microscopy, we observed directed abluminal motility of neutrophils along pericyte processes towards perivascular MCs and establishment of neutrophil extravasation hotspots. Conversely, MC-deficiency or pharmacological or genetic blockade of IL-17A, suppressed neutrophil sub-endothelial migration and breaching of the pericyte layer. Mechanistically, identifying MCs as a significant cellular source of IL-17A, we found that MC-derived IL-17A regulated the enrichment of key effector molecules ICAM-1 and CXCL1 in pericytes close to MCs. Collectively, we identified a novel MC-IL-17A-pericyte axis as modulator of the final steps of neutrophil diapedesis, with clinical implications for controlling inflammatory disorders driven by neutrophils and/or MCs. Work supported by the British Heart Foundation, UK.

P111 EX VIVO CULTURED BLOOD CLOTS DEVELOP A PMN-MDSC SIGNATURE AND SHED SOLUBLE LECTIN-LIKE OXIDIZED LOW-DENSITY LIPOPROTEIN RECEPTOR-1 (SLOX-1)

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The objective was to identify common innate immune responses to thrombosis in diverse healthy donors. Whole blood from 21 consenting donors (12M, 9F, ABO, Lea/Leb/null, Fya/Fyb/null) was coagulated at 37°C and cultured for 4 hours (cultured clot system). Negative controls (fresh blood (FB), fresh clots, cultured heparinized blood) and positive controls (LPS-spiked clots) were included. RNA extracted from blood samples was analyzed by RNA sequencing (n=10, edgeR) with RT-PCR validation (n=5, matched-pairs t-test). Plasma and serum sLox-1 was measured by ELISA (n=21). Clot tissue sections were immunostained for Lox-1 and CD15. Cultured clots upregulated 1412 genes and downregulated 1410 genes vs FB. OLR1, which encodes Lox-1, had a 7.47 log2-fold increase in cultured clots vs FB (p.adj = 4.2e-16), which was validated by RT-PCR (p = 0.03). Biomarkers of PMN-MDSCs (IL8, VEGFA, IL1A, CXCL2; p.adj = 5e-18) were upregulated with OLR1 in cultured clots. sLox-1 levels were negligible in plasma of most donors and copiously shed into cultured clot serum (median 775 pg/mL). Lox-1 was specifically expressed by CD15+ polymorphonuclear cells, platelets, and neutrophil-platelet aggregates suggesting a scavenger role. Lox-1+ PMN-MDSCs are immunosuppressive and emerge in hypoxic environments such as severe COVID-19 and cancer. In COVID-19 blood samples, elevated Lox-1+ PMN-MDSC levels were associated with reduced L-arginine and activated platelets. sLox-1 was previously detected in aspirated coronary thrombi, but the cell source was not identified. Our data suggest that thrombi can elicit in situ differentiation of PMN-MDSCs that shed sLox-1. Consequences of sLox-1 shedding from PMN-MDSCs during thrombosis remains to be determined.

P112 EFFECT OF AGE AND SEX ON CALCIUM SIGNALING IN HUMAN NEUTROPHILSM.

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There is sex and age variability in the immune response to infections and vaccination. The calcium ion (Ca2+) regulates different cellular functions. We analyzed the consequences of sex and aging on Ca2+ mobilization, TRPM2 and CRAC channels expression in human neutrophils, by flow cytometry, inmunofluorescence and qRT-PCR. Blood percentages of granulocytes, mature and precursors neutrophils were equivalent between young and older adults. However, TRPM2 and ORAI1 mRNA expression were lower in neutrophils of older adults, mainly in females. Interestingly, Ca2+ entry in response to CXCL8, C5a, fMLP, LPS or thapsigargin was not decreased. A stronger Ca2+ transient followed by an identical Ca2+ influx to CXCL8 was observed in older adult females. In addition, the Ca2+ response to LPS was delayed and prolonged in neutrophils of older adult males. There were no differences in the expression of CXCR2, CD88, FPLR1, and TLR4. In contrast, neutrophil chemotaxis towards CXCL8, C5a, or fMLP was lower in older adults of both sexes. ORAI1 and TRPM2 channels were detected intracellularly. TRPM2 was mainly in late endosomes in young adults and in lysosomes in older adults in two distinct cell populations: TRPM2low and TRPM2high. In summary, besides Orai1 other ORAI isoforms may build heteromeric CRAC channels to mediate SOCE in human neutrophils and other non-SOCE mechanism may also contribute to Ca2+ entry in these cells. Defective neutrophil chemotaxis in aging is not mediated by alterations in Ca2+ signals; nevertheless, the low TRPM2 and ORAI1 expression in females may affect other functions.

P113 UNTANGLING THE NETs- BIOLOGICAL TRIGGERS OF NET FORMATION

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One way for neutrophils to trap, neutralize, and kill invading pathogens is through the release of Neutrophil Extracellular Traps (NETs). NETs are web-like structures composed of a neutrophil's DNA decorated with cytosolic and granular anti-microbial proteins. NETs have been mainly studied in response to the chemical stimulus PMA, which activates the NADPH oxidase complex, and triggers production of reactive oxygen species (ROS). While essential for PMA-induced NETosis, ROS is not required for NET formation in response to ionophores or Candida albicans. Numerous stimuli have been described to induce NETs, including cytokines, lipid mediators, bacterial products, live pathogens, and DAMPs. Importantly, for many biological stimuli it is often difficult to judge and understand their relevance as NET inducers, since conclusions vary greatly between studies and appear to depend on the experimental setup. In my PhD project, I first aimed to define a set of biologically relevant stimuli that robustly induces NET formation. To this aim, I examined NET release and ROS production by human neutrophils from healthy donors to compare biologically relevant and classically NET inducers in a standardized in vitro assay. My results demonstrate that i) comparably few biological relevant stimuli consistently trigger NET formation and ii) that NET formation occurs in a stimulus- (and sometimes donor-) specific manner, resulting in distinct kinetics and extents of NET release. Together my data support the concept that blood neutrophils form a heterogeneous population with distinct predispositions to release NETs in response to activation signals.

P114 NEUTROPHIL REDOX SIGNALLING REGULATES PROTECTIVE GD T CELL RESPONSE TO INFLUENZA A VIRUS INFECTION.

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Acute viral lung disease caused by infection with respiratory viruses such as Influenza A Virus (IAV) is characterised by excessive neutrophil lung infiltration as well as concomitant induction of oxidative stress and tissue damage. Neutrophils are major producers of Reactive Oxygen Species (ROS) via the enzyme NADPH oxidase 2 (Nox2) which contributes to oxidative stress levels during pulmonary infection. However, the physiological role of neutrophils in respiratory virus infection remains controversial as neutrophils also confer protection by controlling virus dissemination and secondary bacterial co-infection. Using a novel neutrophil-specific Nox2 knockout mouse model, we show that the majority of superoxide production in response to IAV infection is triggered by neutrophil Nox2. Moreover, absence of neutrophil Nox2 activation significantly improved IAV clearance 3 days post infection. Elevated amounts of pro-inflammatory cytokines IL-1beta, IL-17 as well as gamma delta T (gdT) cell counts in the lung assessed by ELISA and flow cytometry on bronchoalveolar lavage and lung tissue at 3 days post infection, were indispensable for improved virus clearance in neutrophil-specific Nox2 knockout mice demonstrated through in vivo blocking experiments. In ex vivo neutrophil gd T cell co-cultures neutrophil Nox2-mediated ROS production induced oxidative stress specifically in adjacent gd T cells via direct cell contact thereby skewing gd T cell cytokine production towards IL-17 signalling. In turn, increased gd T cell derived IL-17 production conferred antiviral protection early during IAV infection. Our results illustrate how neutrophil Nox2-mediated ROS production controls the anti-viral immune response via redox-signalling.

P115 HERMANSKY-PUDLAK SYNDROME TYPE 2 NEUTROPHILS CULTURED FROM PATIENT-DERIVED INDUCED PLURIPOTENT STEM CELLS REVEAL A PHENOTYPE OF HEMOPHAGOCYTOSIS

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Hermansky-Pudlak Syndrome Type 2 (HPS2) is an extremely rare autosomal recessive disorder caused by mutations in the AP3B1 gene, showing partial albinism combined with severe neutropenia. Studies on the consequence of a defective cytoplasmic adapter-protein complex 3 due to absence of the B3A subunit in these patients are lacking. Patients and animal models differ considerably, complicating the interpretation of neutrophil development in HPS2. In order to have a model closer to patients, we cultured neutrophils from an HPS2 patient-derived induced pluripotent stem cell (iPSC) line. We investigated the cultured neutrophils by FACS-based analysis of surface markers, morphology, and assays for ROS production, degranulation, protease release, and live-cell imaging. Compared to WT iPSC neutrophils and PMNs, ROS production was comparable forHPS2 iPSC neutrophils. In contrast, HPS2 iPSC neutrophils showed increased expression of azurophil granule-derived CD63 compared to WT iPSC neutrophils, and reduced degranulation, mimicking the phenotype of HPS2 patient neutrophils. Moreover, cytospins showed "bloated" macrophage-like cells containing cellular and nuclear remnants indicating a hemophagocytic phenotype. Live-cell imaging of iPSC neutrophils with healthy donor M-CSF macrophages showed that while WT iPSC-derived neutrophils were not taken up, whereas HPS2 iPSC neutrophils were strongly interacting with and taken up by the healthy donor macrophages. In short, our work suggests that the neutropenia in HPS2-patients might be related to an underlying hemophagocytic phenotype, as well as the potential of iPSC-derived neutrophils as a powerful tool to study rare diseases of neutropenia.

P116 TSP-1 SUPPORTS NEUTROPHIL TRAFFICKING TO INFLAMED TISSUE BY PROMOTING INTERACTIONS WITH PLATELETS AND ENDOTHELIAL CELLS

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Thrombospondins (TSPs) represent a family of five matricellular proteins critically involved in a variety of biological processes. The role of TSPs for neutrophil trafficking to inflamed tissue remains largely unclear. Here, we demonstrate that among these proteins predominantly TSP-1 is present in inflamed tissues, as evidenced by immunostaining and confocal microscopy of different organs of septic mice. Intraperitoneal application of recombinant TSP-1 induced a significant increase in numbers of neutrophils recruited to the peritoneal cavity of mice. Multi-channel in vivo microscopy on the mouse cremaster muscle further revealed that TSP-1 particularly promotes interactions of neutrophils, platelets, and endothelial cells in venular microvessels. In this context, flow cytometry documented that TSP-1 does not directly change the activation status of neutrophils or microvascular endothelial cells, but potently activates platelets. Hence, our experimental data indicate that TSP-1 promotes neutrophil trafficking to inflamed tissue by supporting a multicellular interplay with platelets and endothelial cells in the microvasculature. This study is supported by the Collaborative Research Center 914 of Deutsche Forschungsgemeinschaft (DFG).

P117 VITRONECTIN PROMOTES IMMUNOTHROMBOTIC DYSREGULATION

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Microvascular immunothrombotic dysregulation is a key event in the pathogenesis of severe systemic inflammatory disease. Besides platelets and plasmatic coagulation, neutrophils contribute to microvascular clot formation. The molecular mechanisms controlling immunothrombosis in inflamed microvessels remain largely unclear. Here, we report that particularly the matricellular protein vitronectin (VN) is enriched in the pulmonary microvasculature of patients with severe non-infectious (pancreatitis-associated) or infectious (coronavirus disease 2019-associated) systemic pathologies, as evidenced by (immuno)histochemistry of tissue samples. In vivo microscopy on the mouse cremaster muscle documented severely compromised interactions of neutrophils, platelets, and endothelial cells during photochemical injury-elicited microvascular thrombus formation in VN-deficient animals. Results of in vitro adhesion, spreading, and rotational thrombelastometry assays further suggest that VN establishes an intravascular scaffold supporting interactions of aggregating platelets with neutrophils and endothelial cells. Blockade of the VN receptor glycoprotein (GP)IIb/IIIa interfered with this multicellular interplay and effectively prevented microvascular clot formation. Targeting the VN-GPIIb/IIIa axis hence appears as a promising strategy to counteract microvascular immunothrombotic dysregulation in systemic inflammatory disease. This study is supported by Collaborative Research Center 914 of Deutsche Forschungsgemeinschaft (DFG).

P118 GSDMD- AND PAD4-INDEPENDENT NET FORMATION MEDIATES VASCULAR OCCLUSIONS IN SEPTICEMIA

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Background: The generation of Neutrophil Extracellular Traps (NETs), also termed NETosis, contributes to host defense; however, excessive NETosis leads to hyperinflammatory, autoimmune and thrombotic disease states. Histone citrullination by peptidyl arginine deiminase 4 (PAD4), and pore formation in the plasma membrane by gasdermin D (GSDMD) are believed to be involved in the formation and release of NETs. Aim: In this study, we aimed to investigate the role of GSDMD and PAD4 in NET formation in experimental models of septicemia and sterile neutrophilia. Methods: We generated Gsdmd-/- and Pad4-/- gene deficient mice on a Dnase1/Dnase1l3-/- deficient background to amplify NET occlusions. Mice were challenged in two models of NET formation, sterile neutrophilia, and septicemia, triggered by transgene expression of granulocyte colony stimulating factor (G-CSF) and injection of LPS and heat-killed E. coli, respectively. Results: Gsdmd/Dnase1/Dnase1l3-/- and Pad4/Dnase1/Dnase1l3-/- mice were not protected from death in sterile neutrophilia, and septicemia. Histological analysis of lung sections showed that NET-derived clots occluded blood vessels in both mouse strains. Conclusion: Taken together, we challenge the current role of GSDMD by newly reporting that intravascular NETosis is independent of GSDMD in experimental models of sterile neutrophilia and septicemia.

P119 EVALUATION OF THE PHAGOCYTE ACTIVITY OF PERIPHERAL PHAGOCYTES OF PATIENTS WITH COMMON VARIABLE IMMUNODEFICIENCY

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OBJECTIVE: To evaluate the phagocytic activity of peripheral phagocytes of patients with common variable immunodeficiency (CVID) RESULTS: Using flow cytometry a sample of a patients with suspected CVID will be taken to carry out tests with marked bacteria with pH Rhodo green™ to evaluate phagocytosis capacity. 23 samples were analyzed, 11 patients with CVID, 5 of these patients were under gamma globulin replacement therapy, and 12 healthy subjects. No changes were observed in the distribution of circulating leukocytes or phagocytes compare to healthy subjects. Although phagocytic capacity is decreased in some patients with CVID, nevertheless as a group it is similar to the group of healthy subjects, so are the frequencies of monocyte subsets.

P120 NEUTROPHIL REVERSE MIGRATION FROM LIVER FUELS NEUTROPHILIC INFLAMMATION TO TISSUE INJURY IN NONALCOHOLIC STEATOHEPATITIS

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Patients with NAFLD are characterized by a chronic low-grade systemic metabolic inflammation and exacerbated, dysfunctional innate immune response. Here, we investigated how neutrophil response to tissue injury is altered by the presence of NASH. We found that our diet-induced NASH zebrafish model exhibits metainflammation characterized by upregulation of inflammatory and metabolic genes and infiltration of neutrophils into tissues and organs (e.g., liver, intestine, skin, brain). Moreover, neutrophils from NASH larvae show hyperactive and aberrant neutrophil inflammatory response to injury with increased neutrophil recruitment, migratory speed, ROS production, NETosis, altered swarming behavior and delayed resolution of inflammation. Interestingly, we showed that neutrophils undergo reverse migration from the NASH liver to the wounded area. Finally, treatment of NASH with Pentoxifylline and Metformin significantly reduced systemic chronic inflammation and the exacerbated recruitment of neutrophils. Together, our findings suggest that NASH exacerbates neutrophilic inflammation via neutrophil priming at the liver, which can further undergo reverse migration and respond to secondary inflammatory triggers such as tissue injury. Reverse migration of primed neutrophils from the liver might be an important mechanism that fuels the exacerbated neutrophil response observed in NASH conditions and associated metainflammation contributing to poor prognosis and increasing death in high-risk groups.

P121 ATYPICAL ANTI-NEUTROPHIL CYTOPLASMATIC ANTIBODIES ARE ANTIBODIES AGAINST NEUTROPHIL EXTRACELLULAR TRAPS: A NOVEL PATHOPHYSIOLOGICAL MECHANISM IN ULCERATIVE COLITIS

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Introduction: The number of mucosal neutrophils, as well as the presence of neutrophil extracellular traps (NETs), correlate with disease activity in Ulcerative colitis (UC), an inflammatory bowel disease. Most (~80%) UC patients present atypical Anti-Neutrophil Cytoplasmatic Antibodies (a-ANCAs) to a yet unknown antigen, which shows a different immunofluorescence staining pattern when compared to cytoplasmatic (c-ANCA) and perinuclear (p-ANCA) that target proteinase (PR3) and myeloperoxidase (MPO), respectively. Here, we analyzed whether a-ANCAs specifically bind to NETs. Methods: Blood neutrophils were treated to form NETs using LPS-treated platelets (non-lytic NETs) or PMA (lytic NETs). Patient biopsies and in vitro-formed NETs were incubated with patient and control serum and processed for immunofluorescence microscopy. Macrophage-mediated clearance of serum-pretreated NETs was analyzed in real-time using Incucyte microscopy. Results: Sera containing c-ANCA, p-ANCA or a-ANCA showed the expected IF staining pattern on ethanol-fixed neutrophils, while the a-ANCA pattern was lost in paraformaldehyde(PFA)-fixed neutrophils. In contrast, all ANCA-types reacted with NETs. Either DNAse or trypsin pre-treatment of NETs abolished a-ANCA staining of NETs, while this was observed for c-ANCA and p-ANCA only with trypsin. In inflamed colonic tissue of UC patients, a-ANCA co-stained with extracellular DNA and neutrophil elastase covering the intestinal epithelium. In vitro, NETs were efficiently opsonized by macrophages, which was strongly inhibited after pre-decorating NETs with a-ANCAs. Macrophages exposed to a-ANCA-decorated NETs expressed higher CXCL-8 and IFN-B levels than the NETs exposed to control serum (both $p<0.05$). Conclusion: a-ANCAs specifically bind to de novo DNA-protein antigens in NETs, which prevents efficient macrophage-mediated clearance and induces a pro-inflammatory (M1) phenotype. This inflammatory milieu may contribute to the pathophysiology of UC.

P122 MESALAZINE INHIBITS BOTH LYTIC AND NON-LYTIC NETS; RELEVANCE FOR ITS THERAPEUTIC ACTION IN ULCERATIVE COLITIS

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Introduction: Neutrophil extracellular traps (NETs) have been found in the inflamed mucosa of ulcerative colitis (UC), an inflammatory bowel disease, and its components are amplified in inflamed tissue. Increased neutrophil counts are connected with disease activity in UC. Mesalazine (5-asa) is one of the first drugs used and the first line for sustaining remission. There are several suggested modes of action, but the specific mechanism remains unknown. Methods: Blood was drawn with EDTA/citrate tubes. Neutrophils were pretreated for 30 min with 5-asa (1, 2.5 and 5 mM). Washed and activated with platelets with LPS for 45 min (non-lytic) or PMA for 3 hours (lytic). The IncuCyte NETosis assay, which records 18 images per well every 2 minutes for three hours and analyzes them in their own system to produce a total green object integrated intensity (RCU X um/image), and Indirect Immunofluorescence (IIF) with probes against neutrophil elastase (NE) and DAPI (4′,6-diamidino-2-phenylindole) for DNA were used to measure NET formation. Results: Mesalazine pre-treatment inhibited NET formation in neutrophils treated with PMA and activated platelets treated with LPS in a dose-dependent manner. In both real-time and IIF tests. With statistical significance when was compared to the lytic formation with 5-asa (1, 2.5 and 5 mM) (22,659 vs 9,350 vs 7,817 vs 5,095 RCU X um/image; $p<0.05$) and non-lytic formation (18,482 vs 12,534 vs 9,405 vs 8,346 RCU X um/image; $p<0.05$). Conclusion: The strong NET inhibitory property of the medication may be its route of action. If the prevention of NET growth leads to remission, other therapies that assist remove NETs, such DNAse, may be beneficial to UC patients.

P123 IMAGE ANALYSIS AND CELLULAR MORPHOMETRY OF BLOOD SMEAR NEUTROPHILS: COMPUTATIONAL AUTOMATIZATION

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Currently, the morphological classification of cells, as well as their morphometry, depend on human expertise; however, this implies a slowdown in the process and is prone to subjectivity depending on several factors, including human error. Changes in the cellular structure, such as shape, size, area, volume, chromatin texture, and nuclear segmentation, can help us detect alterations at a molecular level depending on the pathology or process we are facing. The identification of such changes could be automatized and significantly reduce both the cell measurement error rate and the analysis time. We aim to do this by implementing algorithms and creating a computational program with digital image processing techniques that automatically calculate most of the measurable cell changes. For this, we prepare polymorphonuclear-rich smears of healthy subjects and stain them with Wright´s solution for image acquisition at 100x with a digital camera attached to a Zeiss microscope. We are developing a program able to identify cell and nuclear perimeters, which then can identify cell and nuclear patterns, measure diameters, axis ratios, and stain density to characterize leukocytes, especially neutrophils, in Python. Finally, with machine learning models, we aim to train the system so that neutrophil identification and measurement can be done automatically when any blood smear is presented. This software could also be potentially used in nucleomics, topographic hematology, and chronobiology

P124 EVALUATION OF CYTOKINES, ALPHA SYNUCLEIN AND ION CHANNELS AS POTENTIAL BLOOD MARKERS FOR PARKINSON'S DISEASE

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Parkinson´s Disease is the second most common neurodegenerative disease and is cause of an increase of medical attendance in individuals over 60 years old. Diagnosis of the disease is usually during an advanced stage and treatments are non-curative and aim only to control disease development. We propose to study leukocyte surface protein expression patterns during different stages of the disease and associate them with the concentration of inflammatory cytokines. For this we will measure the inflammatory status by quantifying the pro-inflammatory cytokines IL-1β, IL-6 and TNF-α, as well as the quantification of α-synuclein and the change in the expression of ion channels KV1.3, KCa3.1, TRPC6 and TRPM2, in neutrophils from samples from patients with Parkinson's disease and samples from healthy subjects. To achieve the primary objective, the concentration of alpha synuclein, IL-1 beta, IL-6 and TNF- α in plasma from samples from PD patients and controls will be quantified by ELISA; RT-qPCR will analyze the gene expression of channels KV1.3, KCa3.1, TRPC6 and TRPM2 in neutrophils samples of patients with PD and controls, using the Patch Clamp technique electrophysiological studies will be conducted on the effects of the inflammatory response and alpha synuclein on the conductivity of ion channels in heterologous and native systems. After the biochemical identification of the main ion channels of neutrophils affected by PD we will perform electrophysiological studies to evaluate the function of ion channels in the presence of cytokines and alpha synuclein.

P125 CYTOSOLIC PCNA INTERACTS WITH S100A8 AND CONTROLS AN INFLAMMATORY SUBSET OF NEUTROPHILS IN COVID-19

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Neutrophils are key players in the hyperinflammatory response upon SARS-CoV-2 infection. This is associated with the aberrant neutrophil subpopulations and increased circulating levels of neutrophil-derived calprotectin (S100A8/A9). We have previously described that cytosolic proliferating cell nuclear antigen (PCNA) controls neutrophil survival and NADPH oxidase-dependent reactive oxygen species (ROS) production. The aim of the present study was to examine whether the cytosolic proteins PCNA and S100A8/A9 could be involved in the dysregulated function of neutrophils from COVID-19 patients. We show that both PCNA and S100A8 expression and interaction were elevated in neutrophils from patients with COVID-19 compared to healthy donors (HD) and this was correlated with the disease severity. Increased PCNA expression was accompanied by decreased apoptosis and increased NADPH-oxidase activity in neutrophils from COVID-19 patients compared to HD. Remarkably, these effects, as well as the interaction between PCNA and S100A8, were potently counteracted by T2 amino alcohol (T2AA), a PCNA inhibitor, thus demonstrating that the PCNA scaffold orchestrated neutrophil activation. Notably, the interaction between PCNA-S100A8 was more intense in an activated neutrophil subset defined by a CD16high-CD62Llow phenotype. PCNA scaffold is a decisive component of neutrophil activation and heterogeneity in COVID-19. Our findings uncover PCNA-S100A8 complex as potential driver for neutrophil dysregulation in COVID-19 thereby highlighting a novel host target for dampening the deleterious systemic inflammation associated with SARS-COV2 infection.

P126 IMPACT OF DPP1 INHIBITION ON NEUTROPHIL SERINE PROTEASES IN NAÏVE RODENT MODELS

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Neutrophil serine proteases (NSPs) are associated with pathogen destruction and inflammatory mediation; their dysregulated activation can result in excess secretion of active NSPs causing damaging inflammation, and contributing to neutrophil-mediated inflammatory diseases. Brensocatib is a novel, oral, selective, reversible inhibitor of dipeptidyl peptidase 1 (DPP1), which activates several NSPs including neutrophil elastase (NE), proteinase 3 (PR3), and cathepsin G (CatG). Thus, administration of brensocatib may attenuate the damaging effects of chronic inflammatory diseases by inhibiting the downstream activation of NSPs; a completed Phase 2 trial in non-cystic fibrosis bronchiectasis supports this thesis. To support a range of preclinical programs, an extensive naïve dosing study was undertaken in a variety of rodent species and strains to define brensocatib's pharmacodynamic effect on NE, PR3, and CatG when administered orally at different dosing levels, frequencies, and durations. Overall, mice showed greater reduction in NSPs compared to rats. Both mice and rats dosed once daily (QD) had equivalent NSP reduction compared to BID (twice a day) dosing when the QD dose was 1.5-times the BID daily dose. NE, PR3, and CatG were reduced approximately 77%, 71%, and 90% in C57BL/6 mice, 62%, 61%, and 88% in BALB/c mice, 37%, 70%, 40% in SD rats, and 19%, 53%, and 37% in Wistar rats, respectively, at 20/30 (BID/QD) mg/kg/day brensocatib. Maximum reduction in NSPs was observed after ~7 days and recoveries were nearly symmetrical. These results may facilitate future in vivo brensocatib study dosing considerations, such as timing of prophylactic or therapeutic administration, choice of species, dosage and dosing frequency.

P127 PNEUMOLYSIN-INDUCED PMN TRANSMIGRATION AND DISRUPTION OF AIRWAY EPITHELIUM ADHERENS JUNCTIONS

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Streptococcus pneumoniae is a major cause of pneumonia, where infection of respiratory mucosa results in a robust influx of polymorphonuclear cells (PMNs) and extensive tissue damage. Dissemination of *S. pneumoniae* across the lung epithelium into the bloodstream can lead to lethal septicemia and meningitis, however the mechanisms underlying this process have not been fully elucidated. Here we found that apical *S. pneumoniae* infection of polarized respiratory epithelial monolayers disrupted the organized peripheral localization of the adherens junction (AJ) protein E-cadherin, likely compromising airway epithelial integrity. We created an open source, automated Python computer script called "Intercellular Junction Organization Quantification" (IJOQ) to analyze E-cadherin disruption. IJOQ can handle a high degree of sample-sample staining variability and robustly measure intercellular junction integrity. Analysis of a *S. pneumoniae* mutant deficient in the pore-forming toxin pneumolysin (PLY) revealed that AJ disruption and neutrophil recruitment were PLY-dependent. *S. pneumoniae* dissemination across polarized respiratory epithelial monolayers was promoted by PLYs and this bacterial transit was dramatically enhanced by neutrophil transepithelial migration. Infection of monolayers with mixtures of wild type and PLY-deficient *S. pneumoniae* demonstrated that the pro-dissemination activities of PLY functioned in trans. These data indicate that PLY is an important factor that promotes neutrophil recruitment and disruption of pulmonary epithelium integrity, linked events that are crucial in promoting bacterial dissemination and disease progression during *S. pneumoniae* infection.

P128 STAT1 DEFICIENCY INDUCE SUPPRESSION OF C8+ LYMPHOCYTES IN COLITIS ASSOCIATED COLON CANCER DEVELOPMENT

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Signal Transducer and Activator of Transcription 1 (STAT1), mediates intracellular control of a variety of cellular processes, including several antitumor mechanisms such as apoptosis induction, cell cycle arrest, and the regulation of antitumor immunity. However, the activity of this protein remains controversial in different cancer types including Colorectal cancer. In some reports of colitis-associated colon cancer (CAC), the absence of STAT1 has been reported to accelerate tumor formation, increasing the presence of neutrophils in the blood, and recruitment to the spleen. Tumor-associated neutrophils are a heterogeneous cell population that promotes or inhibits tumor growth depending on their activation. Here, we evaluated the participation of neutrophils in AOM/DSS model in STAT1-deficient mice. We showed that STAT1 expression was not relevant in the tumors of numbers between mice with STAT1 WT expression and deficiency mice group. However, the STAT1 lack of expression favored the levels of markers related to invasiveness and inflammation, β-catenin, NF-κB, and STAT3, and increase the recruitment of neutrophils and CD8+lymphocytes into the tumors at day 48. These neutrophils showed ROS and NETs downregulation. Finally, we showed that STAT1 deficiency suppressed CD8+ lymphocyte proliferation, suggesting that tumor CAC development in STAT1 deficiency mice was supported by neutrophil-mediated CD8+ suppression.

P129 SIMULTANEOUS RECRUITMENT OF TALIN-1 AND KINDLIN-3 TO PLASMA MEMBRANE DURING NEUTROPHIL ARREST

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Neutrophils arrive at sites of inflammation from the bloodstream by first rolling and then arresting. Arrest is triggered by chemokines that induce the conformation of high-affinity beta2 integrins. Two FERM domain proteins, kindlin-3 and talin-1, are required for neutrophil arrest. It is well known that talin-1, an adaptor protein that binds the actin cytoskeleton, activates integrins by binding to membrane lipids, the small GTPase Rap1 and the beta2 cytoplasmic domain, which alters the topology of the beta transmembrane domain and the conformation of the extracellular domains. However, the mechanism underlying neutrophil arrest and the role of kindlin-3 in this process are poorly understood. Moreover, how kindlin-3 cooperates with talin-1 to activate integrins remains unknown. We recently reported that kindlin-3 is recruited to the plasma membrane of neutrophil-like HL-60 cells through its pleckstrin homology (PH) domain prior to arrest. To confirm this in primary neutrophils, we crossed kindlin-3+/- mice with our recently generated human beta2 integrin (hITGB2) knockin activation reporter mice. From the offspring, we harvested kindlin-3 KO embryos at E17.5 and retrovirally transduced fetal liver cells with EGFP-kindlin-3. The fetal liver cells were used to reconstitute lethally irradiated recipient mice. As expected, kindlin-3 deficiency abolished high affinity integrin activation and arrest in primary mouse neutrophils. Reconstitution with wildtype, but not deltaPH kindlin-3 rescued the phenotype. To address the temporal relationship between kindlin-3 and talin-1 recruitment to the plasma membrane, we generated EGFP-talin-1 knockin HL-60 cells expressing TagRFP-kindlin-3. Flow chamber experiments revealed that talin-1 and kindlin-3 are simultaneously recruited to the plasma membrane of HL-60 cells from rolling to arrest. These data suggest kindlin-3 and talin-1 cooperate in inducing high affinity integrin activation.

P130 NEUTROPHILS ARE NEEDED FOR RESOLUTION OF ORAL MUCOSITIS

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Oral mucositis (OM), characterized by a massive inflammation and ulceration of oral cavity, is a common complication of anti-cancer treatments. OM severely decreases quality of life, and can become life-threatening, if it prevents oral hydration, or if it serves as an entry for systemic spread of oral bacteria. The OM is initiated by chemotherapy-induced death of oral mucosal cells, but the exact role of the neutrophils that subsequently infiltrate OM lesions remains unclear. On the one hand, infiltrating neutrophils could worsen the excessive local inflammation. On the other, they could aid in preventing bacterial entry into tissues and stimulate wound healing. The objective of this study was to determine the role of neutrophils in OM induction and resolution by using anti-Ly6G mediated neutrophil depletion in a mouse model of OM. In the absence of neutrophils, the healing of oral ulcers was delayed, the inflammation increased, and bacteria invaded the surrounding deeper tissues. Surprisingly, the beneficial effect of neutrophils on ulcer healing was not directly linked to their anti-bacterial activity, as prevention of bacterial entry into tissues with antibiotics did not compensate for neutrophil depletion. In conclusion, we demonstrated that neutrophils play in fact a beneficial role in resolution of OM, probably linked to their wound-healing abilities.

P131 COMPARISON OF THE EFFECTS OF G-CSF TREATMENT ON NEUTROPHILS FROM PATIENTS WITH CONGENITAL NEUTROPENIA AND THE GLYCOGEN STORAGE DISEASE

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The glycogen storage disease type Ib (GSD-1b) is a rare inborn dysfunction of carbohydrate metabolism. This disorder causes neutropenia with functionally deficient neutrophils and exposes patients to recurrent infections. Like severe congenital neutropenia (SCN) patients, GSD-1b patients are treated with the granulocyte colony-stimulating factor (G-CSF) but its capacity to fully restore neutrophil immune functions has been poorly investigated. Therefore, the goal of our study was to compare the effects of G-CSF administration on the neutrophil population and functionality in SCN and GSD-1b. In SCN patients the increase of neutrophil counts positively correlated with plasma concentration of G-CSF. Similarly, neutrophil's viability, production of NETs, the chemotaxis response, and intracellular levels of alarmin and defensin were also correlated with [G-CSF]. Conversely, the phagocytic activity and the levels of intracellular myeloperoxidase and lactotransferrin were not affected by G-CSF. In stark contrast to SCN, in GSD-1b patients, no clear correlation was found between [G-CSF], neutrophil count, viability, NETs production, phagocytosis, and chemotaxis response. Except for defensins, the level of other antimicrobial compounds also was not affected by the treatment. Altogether, our results indicate that G-CSF is mostly ineffective to restore a functional neutrophil population in GSD-1b patients contrary to its positive effects in SCN.

Supported by the Foundation for Polish Science (FIXNET:POIR.04.04.00-00-1603/18)