INTERNATIONAL SYMPOSIUM ON INNATE IMMUNITY AND INFLAMMATION
in conjunction with
THE 5th NATIONAL CONFERENCE OF INFECTIOUS DISEASES
"INFECTION NOWADAYS. THERAPY, WHERE TO?"

5th – 8th September 2018

Cluj-Napoca

Coordinating Editor:
Mihaela Lupse, MD, PhD

The authors have the responsability for the content of these paper abstracts

"Clujul Medical" Editorial Office
Str. Moţilor, nr. 33
RO-400609 Cluj-Napoca,
România
Tel/fax: +40-264-596086

E-mail
clujulmedical@umfcluj.ro

Site
www.clujulmedical.umfcluj.ro

Indexed
PubMed
PubMed Central
SCOPUS
CAB Abstracts
EBSCO
Index Copernicus
getCited
JournalSeek
Open Access Directory
InfoBase
SCIPIO

Publisher
Iuliu Haţieganu Medical University Publishing House

- Clujul Medical - Journal of Medicine and Pharmacy

Supplement No. 5, Vol. 91, 2018
e-ISSN 2066-8872

CONTENTS

- Clujul Medical - Journal of Medicine and Pharmacy

Supplement No.5 , Vol. 91, 2018
e-ISSN 2066-8872

AN INTEGRATIVE ANALYSIS OF TRANSCRIPTOMICS AND LIPIDOMICS DATA FROM HUMAN CD14$^+$ MONOCYTES

IOANNA GEMÜND[1], MARIE OESTREICH[1], LEA SEEP[1], ANNA-LENA HARDT[1], THOMAS ULAS[1], CHRISTOPH THIELE[2,*], JOACHIM L. SCHULTZE[1,3,*], ANNA C. ASCHENBRENNER[1,4,*]

[1]Genomics and Immunoregulation, Life & Medical Sciences (LIMES) Institute, University of Bonn, 53115 Bonn, Germany
[2]Biochemistry and Cell Biology of Lipids, Life & Medical Sciences (LIMES) Institute, University of Bonn, 53115 Bonn, Germany
[3]Platform for Single Cell Genomics and Epigenomics at the German Center for Neurodegenerative Diseases and the University of Bonn, 53127 Bonn, Germany
[4]Department of Internal Medicine and Radboud Center for Infectious Diseases (RCI), Radboud University Medical Center, Nijmegen 6525, The Netherlands
***These authors contributed equally to this work.**
*Corresponding author: *e-mail: a.aschenbrenner@uni-bonn.de*

The use of transcriptomics to support biological analyses has increased tremendously in the last years. In immunology, it has been of great help to identify, distinguish and functionally describe cell populations and subsets as well as to determine differences in various disease contexts. Lipidomics, however, has not been used to similar extent for these questions – albeit the fact that in recent years, elevated levels of nutrients (such as cholesterol, triglycerides and free fatty acids), that are often contained in disproportionate amounts in contemporary diet, have been shown to represent anthropogenic stimuli that have been implicated to cause low-level inflammation, termed 'metaflammation', and cue metabolic disorders. A crucial constituent in the development and progression of this pathology are cells of the innate immune system, particularly the myeloid compartment consisting of monocytes, tissue macrophages, and dendritic cells.

Previous analyses by our lab had provided initial insights into changes in the transcriptional signatures of myeloid cells following exposure to free fatty acids. Depending on the nature of the fatty acid, the cells were specifically reprogrammed exemplifying their ability to react to such anthropogenic stimuli.

As lipids can act as important signaling mediators aside from their structural function in membranes or metabolic role in energy storage, we now used advanced mass spectrometry-based assessment of lipid species to integrate lipidomics into our analyses by analyzing transcriptomes and lipidomes of human CD14+ monocytes after exposure to saturated or unsaturated fatty acids. Transcriptional signatures were screened for differences in metabolic pathways and overlaid with the observed changes in various lipid species. Culturing of monocytes in the presence of external free fatty acids increased transcripts favoring degradation of excess fatty acids via mitochondrial β-oxidation, di- and tri-acylglyceride (DAG and TAG, respectively) synthesis, and PPARγ signaling target genes. Lipidomics data was analyzed by k-means clustering of group fold change patterns for similarly regulated lipid species and screened for specific modules for the different experimental conditions. Transcriptional changes of key lipid metabolism components were reflected in the respective lipidomes as DAG and TAG levels were specifically increased lipid species in the FFA-treated conditions. More specifically, monocytes exposed to palmitate showed specific hallmarks described for pro-inflammatory/TLR4 polarized cells: isocitrate dehydrogenase (IDH1) of the citric acid cycle was downregulated, pro-inflammatory cytokines TNF and IL1B as well as several ceramide species were induced.

Integrating the assessment of transcriptomic and lipidomic profiles provides a powerful workflow for generating a more complete understanding of the general biological and metabolic processes in immune cells in various contexts. In the future, this approach will help study pathophysiological conditions and thus gain insight into molecular mechanisms of disease.

Keywords: transcriptomics, lipidomics, myeloid cells

CRELD1 REGULATES IMMUNE CELL FATE AND FUNCTION BY CONTROLLING THEIR ACTIVATION THRESHOLD AND SURVIVAL

LORENZO BONAGURO[1], MAREN KOEHNE[2], LISA SCHMIDLEITHNER[2], JONAS SCHULTE-SCHREPPING[3], ELVIRA MASS[4], MARC BEYER[2], YANG LI[5], ROB TER HORST[6], CHRISTINE FALK[7], MIHAI G. NETEA[6,8], MICHAEL HOCH[1], JOACHIM L. SCHULTZE[3,9], ANNA C. ASCHENBRENNER[3,6]

[1]Developmental Genetics & Molecular Physiology, Life & Medical Sciences (LIMES) Institute, University of Bonn, 53115 Bonn, Germany
[2]Molecular Immunology in Neurodegeneration, German Center for Neurodegenerative Diseases, 53127 Bonn, Germany
[3]Genomics and Immunoregulation, Life & Medical Sciences (LIMES) Institute, University of Bonn, 53115 Bonn, Germany
[4]Developmental Biology of the Innate Immune System, Life & Medical Sciences (LIMES) Institute, University of Bonn, 53115 Bonn, Germany
[5]Department of Genetics, University Medical Center Groningen, University of Groningen, 9700 RB Groningen, The Netherlands
[6]Department of Internal Medicine and Radboud Center for Infectious Diseases (RCI), Radboud University Medical Center, Nijmegen 6525, The Netherlands
[7]Institute of Transplant Immunology, Integrated Research and Treatment Center Transplantation, Hannover Medical School, 30625 Hannover, Germany
[8]Immunology & Metabolism, Life and Medical Sciences Institute (LIMES), University of Bonn, Bonn 53115, Germany
[9]Platform for Single Cell Genomics and Epigenomics at the German Center for Neurodegenerative Diseases and the University of Bonn, 53127 Bonn, Germany
*Corresponding author: *e-mail: a.aschenbrenner@uni-bonn.de*

Life or death of a cell is literally the most critical state a cell must control. It needs to maintain its activation state within a physiological window providing at least a low, but sufficient level of activation to prevent destruction, yet safeguarding itself from overactivation to provide adequate subsequent functions. This is particularly important for cells of both, the innate and adaptive immune system. As a first attempt, we have chosen T cells as the experimental model, since the immune system needs to meticulously control the balance of launching an effective adaptive immune response against pathogens while ensuring protection from self-reactive T cells, curtailing immune responses after clearance, as well as maintaining a diverse naïve and/or memory T cell pool. The molecular control of this balance is still a matter of active research.

We here identify Cysteine-rich with EGF-like domains 1 (Creld1) as a previously unrecognized surface molecule that functions as an essential regulator of proliferation and survival of naïve CD4+ T cells, as well as a critical influencer of CD4+ T cell activation and thus T helper cell differentiation.

On the molecular level, overexpression of Creld1 induces translocation of NFATc1 into the nucleus in different cell culture lines. This effect is dependent on Calcineurin as it can be prevented by inhibition of this phosphatase with Cyclosporine A (Mass and Wachten, et al., 2014). Fluorescence protease protection assays revealed an extracellular N- and cytoplasmic C-terminus for Creld1. By cloning several deletion constructs, we demonstrate that this effect on NFATc1 is mediated by the Creld1 C-tail and that the protein needs to be localized at the plasma membrane to exert its impact on nuclear NFAT translocation. As NFAT transcription factors are critically involved in proliferation, we monitored cell division by CFSE labeling and found that overexpression of full length Creld1 or a mutant containing only the transmembrane region + C-tail accelerated proliferation compared to the mutant lacking the C-terminus. We also observed the effect of Creld1 on cell proliferation and survival in CD4+ T cell-specific *Creld1* knockout mice. Proliferation after T cell receptor (TCR) stimulation *ex vivo* as well as CD4+ T cell numbers in aged mice were profoundly reduced. *In vitro* studies further revealed increased cell death under standard culture conditions as well as early hyper-responsive behavior seen in intracellular calcium levels, glycolytic activity, NFAT activity assays, and early activation markers on the cell surface after TCR stimulation. Similarly, using data from the Human Functional Genomics Project, we identified several human SNPs as eQTLs for CRELD1 expression influencing IFNγ secretion from LPS-stimulated PBMCs. Transcriptomic analyses of murine control and Creld1 knockout CD4+ T cells revealed minor differences in naïve T cells while the results after 8h of *ex vivo* culture with TCR stimulation recapitulated the hyperactivation of Creld1-deficient T cells. The greatest difference was seen in the unstimulated 8h control cells where loss of Creld1 decreased many signaling pathways necessary for basic cellular maintenance, thus substantiating the importance of Creld1 as a regulator of activation threshold and cell survival. It will be of utmost importance to assess whether the effect seen for T cells and fibroblast cell culture lines is similarly apparent in innate immune cells and what the signaling pathways in these cells are that might be linked to CRELD1 regulation.

Keywords: activation, proliferation, signaling, cell maintenance, cell survival

ROLE OF HEPATIC MACROPHAGES IN LIVER METABOLISM

NÓRA RÉKA BALZER, AMIR HOSSEIN KAYVANJOO, CORNELIA CYGON, ELVIRA MASS

Life and Medical Sciences Institute (LIMES), Department of Developmental Biology of the Innate Immune System, Bonn, Germany

The recent discovery that most tissue-resident macrophages have a fetal origin and are self-maintained in postnatal tissues, independently of definitive hematopoietic stem cells, places these cells in a unique position. In addition of being sensors of chronic sterile inflammation in the mother, e.g. caused by high calorie intake, they may experience the maternal inflammatory state in the fetus. The global epidemic of obesity has led to an increasing number of obese women of childbearing age. While it is now understood that maternal obesity may have harmful effects on fetal and offspring metabolic programming, the underlying mechanisms remain elusive. During embryogenesis, the fetal liver is the major metabolic organ. Yet, whether hepatic macrophages play a role in fetal liver metabolism remains to be investigated. Moreover, whether macrophage development and function changes upon maternal obesity result in persistent molecular changes in the offspring, is absolutely unclear.

To test if fetal liver metabolism depends at all on hepatic resident macrophages we used a loss-of-function mouse model that is deficient for resident macrophages. Our results suggest that macrophages are indeed important for the glucose metabolism before birth, as livers lacking macrophages accumulate glycogen. As a gain-of-function model, we use a mouse model for maternal obesity and examine the diet-related changes of liver macrophages. Further, we use cross-fostering experiments to address whether the observed changes are reversible. Our preliminary results indicate that maternal nutrition has indeed an impact on the immune cell composition and lipid metabolism of the developing liver. Taken together, fetal hepatic macrophages are a good candidate to serve as gatekeeper for transgenerational metabolic alterations caused by maternal diet.

MAPPING THE HUMAN IMMUNE LANDSCAPE IN THE ALVEOLAR SPACE OF HEALTHY DONORS AND COPD PATIENTS

KEVIN BASSLER[1], WATARU FUJII[1], THEODOROS KAPELLOS[1], KRISTIAN HÄNDLER[2], SIMONE PICELLI[2], COLLINS OSEI-SARPONG[2], MARC BEYER[2], PAWEL BIERNAT[1], KATHRIN KLEE[1], MATTHIAS BECKER[1], CARMEN PIZARRO[3], DIRK SKOWASCH[3], JOACHIM L. SCHULTZE[1,2]

[1]Genomics and Immunoregulation, Life & Medical Sciences (LIMES) Institute, University of Bonn, Bonn
[2]Platform for Single Cell Genomics and Epigenomics at the German Center for Neurodegenerative Diseases and the University of Bonn, Bonn
[3]Medical Clinics and Policlinics, University Hospital Bonn, Section of Pneumology

Chronic obstructive pulmonary disease (COPD) is ranked the fourth leading cause of death worldwide and hence constitutes a significant burden, both medically and financially. The disease is characterized by a poorly reversible airway obstruction which is mainly caused by chronic inflammation of the lung followed by destruction of the parenchyma. However, the cellular mechanism underlying COPD is poorly understood and thus no effective therapy is currently available.

To broaden the current knowledge of the immune landscape in the lung, we performed single cell mRNA-Seq of CD45-enriched bronchoalveolar lavage cells obtained from five COPD patients and five healthy controls. In all donors, we found that alveolar macrophages (AMs) constitutes the major immune cell population and together with mast cells, B cells, T cells and dendritic cells, they form the basic immune compartment in the alveolar space. Interestingly, we detected an enrichment of neutrophils and eosinophils in COPD patients. This finding is in agreement with data we obtained from a large-scale multi-color flow cytometry study, which comprised 26 COPD patients and 20 healthy controls. Further analysis revealed sub-populations within the AM compartment in both healthy and COPD. To our knowledge, these sub-populations have not been described before in the alveolar space and we classified them into proliferating BIRC5+ AMs, immature AMs, mature AMs and monocyte-derived cells. Using diffusion maps, we were able to model a developmental trajectory, which indicates that AM are replenished in at least two different ways, namely via proliferating $BIRC5^{+}$ AMs (self-replenishment) and differentiating monocytes.

Collectively, single cell mRNA-Seq data allowed new insight into the human immune landscape of the alveolar space. Based on our initial findings we postulate that sinlge cell mRNA-seq of BAL and lung tissue will lead to a better understanding of the pathophysiology of chronic lung diseases such as COPD and might even guide subclassification and staging of these diseases.

FACING THE GROWING COMPUTATIONAL NEEDS FOR SYSTEMS IMMUNOLOGY

MATTHIAS BECKER[1], STEFANIE HERRESTHAL[1], KATHRIN KLEE[1], THOMAS ULAS[1], JONAS SCHULTE-SCHREPPING[1], PAWEL BIERNAT[1], PATRICK GÜNTHER[1], KEVIN BASSLER[1], MILIND CHABBI[3], ROCKY CRAIG[3], HARTMUT SCHULTZE[4], SHARAD SINGHAL[3], JOACHIM L. SCHULTZE[1,2]

[1]Genomics and Immunoregulaton, Life & Medical Sciences (LIMES) Insttute, University of Bonn, Bonn, Germany
[2]Platform for Single Cell Genomics and Epigenomics at the German Center for Neurodegeneratve Diseases and the University of Bonn, Bonn, Germany
[3]Hewlet Packard Labs, Palo Alto, USA
[4]Hewlet Packard Enterprise, Ratngen, Germany & Portland, USA

Systems immunology approaches combining experimental and computational approaches are targeted at understanding the innate immune system as a whole. In studies such as the Human Functional Genome Project (HFGP), or even larger studies such as the German Rhineland study (30.000 participants) or the UK biobank (500.000 participants), large datasets including clinical, genomic, functional and molecular data are collected and can be utilized for systems immunology approaches to better understand innate immune cells in health and disease. However, the avalanche of data generated in these large population studies are computationally challenging and it can already be foreseen that our current hardware and software infrastructures are insufficient to handle such large, multi-dimensional and heterogenous data. To address this foreseeable bottle neck in systems immunology, we teamed up with computational scientists developing a radically new compute infrastructure that should allow to accelerate large data analysis significantly thereby allowing to computationally address scientific questions in systems immunology so far unreachable.

This new infrastructure – so-called memory-driven computing (MDC) - is memory-centric, highly flexible in directly connecting different types of processors to the central memory and it is extremely scalable. To exemplify the power of MDC we used an algorithm recently introduced as near-optimal computing for transcriptome alignment, a pre-processing step of genomics data often used in systems immunology approaches. Here we demonstrate that MDC increases the speed of even the most highly optimized algorithm by more than 100-fold. For example, a large transcriptome derived from any type of immune cell was aligned in about 10 seconds using MDC, a process that took more than 16 minutes on a baseline single-core computer. Such significant reduction in data processing time will radically change the way we will analyze large data in the future. Moreover, it will become more feasible to align genome and transcriptome data several times with different data models, even in large datasets as they are presented in HFGP, the Rhineland study or UK biobank. Another area in systems immunology where we foresee significant improvements of our analyses by accelerating processing time and allowing for higher data scaling is single cell RNA-sequencing, a technology that will revolutionize our understanding of the innate immune system. Clearly, these improvements in data analysis are not restricted to the analysis of the immune system and we predict these improvements to impact on all of the biomedical sciences.

PERSISTENT MONOCYTE ACTIVATION IN PATIENTS WITH ELEVATED LDL CHOLESTEROL LEVELS DURING STATIN TREATMENT

SIROON BEKKERING[1], SOPHIE BERNELOT MOENS[2], SIMONE L VERWEIJ[2], BORIS NOVAKOVIC[3], LOTTE CA STIEKEMA[2], JEANINE ROETERS VAN LENNEP[4], LEO AB JOOSTEN[1], HENK STUNNENBERG[3], MIHAI G NETEA[1], ERIK SG STROES[2], NIELS P RIKSEN[1]

[1]Department of Internal Medicine, Radboud University Medical Center, Nijmegen, The Netherlands
[2]Department of Vascular Medicine, Academic Medical Centre, Amsterdam, The Netherlands
[3]Department of Molecular Biology, Radboud University Medical Center, Nijmegen, The Netherlands
[4]Department of Vascular Medicine, Erasmus Medical Center, Rotterdam, The Netherlands

Aim. Monocytes can adopt a longterm proinflammatory phenotype after brief exposure to atherogenic stimuli, such as oxidized LDL. This is mediated via epigenetic reprogramming and metabolic rewiring, and is called trained innate immunity. Statins can prevent trained immunity in vitro. Here we studied the inflammatory profile of monocytes from patients with elevated LDL cholesterol levels, before and after statin treatment and compared this to matched controls with normal LDL cholesterol levels.

Methods. Monocytes were isolated from 20 patients with LDL-c>4.9 mmol/l before and after 3 months of statin treatment as well as from matched controls. We measured systemic inflammation, monocyte subsets and expression of activition markers by FACS, and cytokine production capacity after 24 hours of ex vivo stimulation. Monocytes from five patients before/after treatment and matched controls were subjected to RNA sequencing.

Results. Monocytes from patients (LDL-c 6.7±1.7 mmol/l) showed increased expression of activation markers measured by FACS (CCR2, CD11b, CD11c and CD29) compared to controls subjects (LDL-c 2.8±0.8 mmol/l) but monocyte subsets were similar between groups. Monocytes from patients showed an increased production of pro-inflammatory cytokines TNFα, Interleukin (IL)-1β and IL-6 after stimulation with different TLR ligands. Furthermore, anti-inflammatory cytokines IL-10 and IL-1Ra were significantly increased in patients compared to control subjects. Statin treatment significantly lowered LDL-c to 4.0±1.8 mmol/l, reversed levels of some activation markers (CCR2 and CD29), and the production of anti-inflammatory cytokines. The production of pro-inflammatory cytokines remained unaffected. RNAsequencing showed broad inflammatory activation of monocytes of patients vs. controls and no change upon statin treatment.

Conclusion. A hyperlipidaemic environment induces long-term changes in the inflammatory activity of monocytes, which persisted even 3 months after lipid lowering. Therefore, additional studies on the mechanism of this trained immunity phenotype are needed to design novel additional pharmacological strategies.

ASSOCIATIONS BETWEEN SKIN REACTION UPON BCG VACCINATION AND INFANT *IN VITRO* CYTOKINE RESPONSES

M.L.T. BERENDSEN[1,2,3], K.J. JENSEN[1,2], F. SCHALZ-BUCHHOLZER[1,2], S. BIERING-SØRENSEN[1,2], I. MONTEIRO[2], P. AABY[2], C.S. BENN[1,2,4]

[1]Research Center for Vitamins and Vaccines (CVIVA), Bandim Health Project, Statens Serum Institut, Copenhagen, Denmark
[2]Projecto de Saúde Bandim, Indepth Network, codex 1004, Bissau, Guinea-Bissau
[3]Department of Internal Medicine, Radboud Center for Infectious Diseases, Radboud University Medical Center, Nijmegen, The Netherlands
[4]OPEN, University of Southern Denmark/Odense University Hospital, Denmark

Introduction. Bacillus Calmette–Guérin (BCG) vaccination has been shown to have non-specific beneficial effects on neonatal mortality, possibly via a process called 'trained immunity'. The majority of vaccinees develop a local reaction (papule, pustule or ulcer) following vaccination, which in most cases results in the formation of a scar. Beneficial effects of BCG are most profound in children that develop such a scar. In the present explorative cross-sectional study, we explored associations between type and size of the local reaction and *in vitro* cytokine responses at age 4 weeks.

Methods. Within a randomized trial comparing early BCG vs postponed BCG in low-birth-weight (LBW) infants in Guinea-Bissau, 4 weeks after randomization, a subgroup had their local BCG reactions assessed and was bled. Levels of innate and adaptive cytokine responses to purified protein derivative (PPD), PMA/ionomycin and Toll-like receptor (TLR)-2,-4 and -7/8 agonists were measured in whole-blood assays. Values out of range were imputed via Tobit-regression based multiple imputation. Linear regression models of log-transformed cytokine concentrations were fitted to generate geometric mean ratios (GMR).

Results. Among 196 infants randomized to early BCG, 19 had no local reaction, 148 a papule, 29 a pustule/ulcer. None had yet developed a scar. For stimulation with PPD, both papules and pustules/ulcers were associated with higher cytokine responses over no reaction, except for Interleukin (IL)-1β. Similar associations were found with local reaction size. For the TLR agonists, associations with a common upward trend were found for interferon (IFN)-γ and IL-5 (papules and pustules/ulcers) and IL-17 (only papules). Upward trends for pustules/ulcers were also seen for IL-1β, IL-6 and tumor necrosis factor (TNF)-α after CL075 stimulation. Associations with local reaction size were found for IL-1β, IL-6, TNF-α and IFN-γ only after CL075 stimulation. Girls tended to have stronger associations for both type and size of local reaction.

Conclusion. This explorative study suggests positive associations for both type and size of the local reaction after BCG vaccination with *in vitro* cytokine responses after PPD stimulation. For TLR agonists, a common upward trend was suggested for T-cell derived cytokines, while a trend for monocyte derived cytokines was only found after CL075 stimulation. These trends should be examined in higher-powered studies.

APPLYING TOPIC MODELS TO COMPARE IMMUNE CELL POPULATIONS ACROSS MULTIPLE BATCHES OF SINGLE-CELL RNA-SEQ EXPERIMENTS

PAWEŁ BIERNAT[1], KEVIN BASSLER[1], PATRICK GÜNTHER[1], JONAS SCHULTE-SCHREPPING[1], STEFANIE HERRESTHAL[1], KATHRIN KLEE[1], THOMAS ULAS[1], MATTHIAS BECKER[1], JOACHIM L. SCHULTZE[1,2]

[1]Genomics and Immunoregulation, Life & Medical Sciences (LIMES) Institute, University of Bonn, Bonn, Germany
[2]Platform for Single Cell Genomics and Epigenomics at the German Center for Neurodegenerative Diseases and the University of Bonn, Bonn, Germany

Recent outburst of single-cell techniques has enabled much more fine-grained view into the immune cells heterogeneity. The increasing popularity of single-cell techniques, with single-cell RNA-Seq (scRNA-Seq) being the most widely used one, calls for easy to use analysis methods. However, the typical scRNA-Seq analysis is a combination of several independent steps: filtering, dimensionality reduction, clustering, batch effect removal and others. At each of these steps human intervention is necessary to pick the right parameters and algorithm suitable for the task and the data. Although eventually such workflow yields desired information, it is also prone to unintended bias and requires constant attention and scrutiny of an experienced bioinformatician. We propose an alternative approach: we develop a topic model encompassing several aspects of scRNA-Seq, that can be used in place of the whole scRNA-Seq analysis pipeline.

Topic models are a class of Bayesian models used for extracting human-interpretable topics from documents. Before applying the model the documents are first summarized by their word counts. The word counts in documents bear a striking resemblance to the gene counts in cells, the starting point of the scRNA-Seq analysis. Despite the similarities, there are, however some issues specific to scRNA-Seq data that are not addressed in the existing topic models. For example, standard topic models do not take into account batch effects. The housekeeping genes, present in all cells, also need to be accounted for as they can overshadow the relevant cell-specific genes. Lastly, the topics are not automatically labeled so the results still need expert intervention.

Our model, based on a hierarchical Latent Dirichlet Allocation, addresses all of the above points and can be used to perform analysis on data with batch effects. One of the hallmarks of our model is the use of known cell signatures for topic labeling. As a case study we present the analysis of PBMCs obtained from four donors and show how the model can remove the batch effects, extract specific cell populations and find relevant genes differentially expressed between donors. We are convinced that the application of advances in computational sciences to questions in systems immunology will greatly accelerate our understanding of the immune system as a whole.

BORRELIA-INDUCED INHIBITION OF ANTIGEN PRESENTATION: A NOVEL ESCAPE MECHANISM FROM THE HOST DEFENSE SYSTEM

M. A.E. BROUWER, L.A.B. JOOSTEN

Department of Experimental Internal Medicine, Radboudumc, Nijmegen, The Netherlands

Lyme disease is the most common human vector borne disease caused by spirochetes of the *Borrelia* genus in Western Europe and the United States, and is transferred by tick. Lyme disease starts in the skin, where the causal bacteria *Borrelia sensu lato* first encounters host immune cells at the localization of the tick bite. After escaping these immune cells, the pathogen can disseminate throughout the human body, using blood and lymphatic systems, causing further complications for the host. *Borrelia* strongly downregulates genes and proteins involved in antigen presentation. Inhibition of antigen presentation interferes with the crucial steps necessary for optimal T-cell and B-cell responses towards *Borrelia*. Possibly explaining the lack of a proper immune response in Lyme disease patients against *Borrelia* spirochetes.

CANDIDA AURIS: UNDERSTANDING THE MECHANISMS OF HOST IMMUNE RESPONSE

M. BRUNO[1], M. JAEGER[1], M.G. KRUPPA[2], M. ZUCHAO[3], Y. NING JIAO[2], D.W. LOWMAN[2], E.J. GIAMARELLOS-BOURBOULIS[4], D.L. WILLIAMS[2], A. CHOWDHARY[5], J.F. MEIS[6], M.G. NETEA[1]

[1]Radboud University Medical Center, Nijmegen, Netherlands (Presenting author)
[2]East Tennessee State University, Johnson City, USA
[3]Center of Excellence in Inflammation, Infectious Disease, and Immunity, Johnson City, USA
[4]National and Kapodistrian University of Athens Medical School, Athens, Greece
[5]University of Delhi, Delhi, India
[6]Canisius Wilhelmina Hospital (CWZ), Nijmegen, Netherlands

Objectives. *Candida auris* is an emerging fungal pathogen that presents a serious global health threat. Its intrinsic resistance to common antifungal drugs and the rising incidence of outbreaks in healthcare settings leads to the need for urgent investigation of potential novel treatment options. Practically nothing is known about the mechanisms of innate host defense against *C. auris*. The aim of our research was to identify the mechanisms through which cells of the innate immune system recognize C. auris, initiate the innate antifungal immune response, and protect the host against *C. auris* infection.

Methods. In vitro stimulation assays with both live, as well as heat-killed *C. auris* compared to *C. albicans* were used to evaluate differences in the innate host response between both fungi. Specific blockage and inhibition of known Candida pattern recognition receptors was applied to investigate the potential of both strains to activate the specific pathways. Finally, we evaluated the differences of virulence and pathogenesis of both strains *in vivo*. Immunocompetent mice were intravenously infected and survival rate and fungal burdens in different organs was determined.

Results. Levels of TNFα, IL-6 and IL-1β were significantly increased in PBMCs stimulated with live *C. auris* compared to *C. albicans* strains. Conversely, there were no significant differences in cytokine production in PBMCs stimulated with heat-killed *C. albicans* and *C. auris*, as well as no differences in ROS induction and lactate production, even after blocking the principal PRRs or the major intracellular signaling pathways known to be crucial for fungal recognition and signaling. Mice infected with *C. auris* had an increased survival compared to *C. albicans* infected mice (median survival *C. auris* 13.5 days, *C. albicans* 9.5 days; $p < 0.001$), and showed less fungal burden in both liver and in kidneys ($p < 0.05$).

Conclusion. By comparing the host immune response against both *C. albicans* and *C. auris* we found that live *C. auris* is capable in activating a more powerful pro-inflammatory cytokine response probably leading to an increased fungal killing, which is most likely also the reason why in a systemic *C. auris* infection model, animal infected with this fungus showed an increase survival and a decrease in fungal burden compared to *C. albicans*. The differences in immune cell stimulation may be due either to differences in the structure of mannans of *C. auris* which are known to be exposed on the surface of the cell wall, or differences in which these mannans shield the interaction of beta-glucans with the pattern recognition receptors on the surface of immune cells. Further experiments using extracted cell wall components as well as investigation of the exact cell wall architecture are warranted in order to elucidate these findings.

THE LONG PENTRAXIN-3 RESTRAINS INFLAMMATION IN SARCOIDOSIS BY REGULATING LEUKOCYTE RECRUITMENT AND GRANULOMA FORMATION

CLÁUDIA F. CAMPOS[1,2], HELDER N. BASTOS[1,2,3], OKSANA SOKHATSKA[4], JOANA GAIFEM[1,2], CLÁUDIO DUARTE-OLIVEIRA[1,2], CLÁUDIA S. RODRIGUES[1,2], SAMUEL M. GONÇALVES[1,2], CECILIA GARLANDA[5], LUIS DELGADO[4], ANTÓNIO MORAIS[3], SANDRA COSTA[1,2], RICARDO SILVESTRE[1,2], EGÍDIO TORRADO[1,2], ALBERTO MANTOVANI[5,6], CRISTINA CUNHA[1,2], AGOSTINHO CARVALHO[1,2]

[1]Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Campus Gualtar, 4710-057 Braga, Portugal
[2]ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal
[3]Serviço de Pneumologia, Centro Hospitalar São João, Faculdade de Medicina, Universidade do Porto, Porto, Portugal
[4]Serviço de Imunologia, Faculdade de Medicina, Universidade do Porto, Porto, Portugal
[5]Humanitas Clinical and Research Center, Rozzano (Milan), Italy
[6]Humanitas University, Rozzano (Milan), Italy

Sarcoidosis is a systemic inflammatory disease of unknow etiology characterized by the presence of non-caseating granulomas, with lung involvement in almost all cases. Although the histological landscape of human sarcoid granulomas has been extensively studied, the genetic, molecular and inflammatory signatures underlying macrophage transformation into epithelioid cells that aggregate, initiate and sustain granulomatous inflammation remain elusive. Consequently, available treatment options are scarce and currently, there are no therapeutic approaches targeting sarcoidosis pathogenic mechanisms. Among the fluid-phase molecules with immunoregulatory properties, the long pentraxin-3 (PTX3) has been shown to play a pivotal role at the crossroads of innate immunity and inflammation, with a central role in the pathogenesis of several lung diseases. Herein, we developed an integrative translational approach to elucidate the PTX3-mediated mechanisms that control leukocyte recruitment and inflammation in sarcoidosis. By resorting to a murine model of granulomatous inflammation, we identified PTX3 as an integral component of sarcoid granulomas that is required to control leukocyte recruitment. Macrophages were identified as the predominant PTX3-expressing immune cell in the granuloma, playing a central role in the regulation of the inflammatory process. More importantly, individuals carrying loss-of-function genetic variants in human PTX3 were found to have an increased risk of developing sarcoidosis. Accordingly, the genetic deficiency of PTX3 in these patients was characterized by a markedly increased leukocyte recruitment to the pulmonary microenvironment. These results reveal a previously unanticipated key role of PTX3 during granulomatous inflammation in sarcoidosis, pinpointing this protein as a promising immunotherapeutic target for effective medical interventions in sarcoidosis patients.

IDENTIFICATION OF RARE CODING VARIANTS IN IL-1-RELATED PATHWAYS IN PATIENTS WITH ADULT-ONSET STILL'S DISEASE

G. CAVALLI*[1,2], R. VAN DEUREN[2], P. ARTS[2], M. STEEHOUWER[2], C. GILISSEN[2], P. SFRISO[3], S. COLAFRANCESCO[4], R. PRIORI[4], L. CANTARINI[5], O. M. LUCHERINI[5], E. BALDISSERA[6], F. VAN DE VEERDONK[2], L. DAGNA[1], A. HOISCHEN[2], C. A. DINARELLO[2,7]

[1]Vita-Salute San Raffaele University, Milan, Italy
[2]Radboud University Medical Center, Nijmegen , Netherlands
[3]University of Padua, Padua
[4]Sapienza University of Rome, Rome
[5]University of Siena, Siena
[6]Unit of Immunology, Rheumatology, Allergy and Rare Diseases (UniRAR), Vita-Salute San Raffaele University, Milan, Italy
[7]University of Colorado Denver, Aurora, CO, United States

Background. Adult-onset Still's disease (AOSD) is a rare autoinflammatory disease characterized by fever, arthritis, and multi-organ involvement. Inflammation in AOSD is mediated by interleukin (IL)-1β, as confirmed by the dramatic clinical efficacy of selective blockers of this cytokine. The genetic predisposition to this rampant IL-1-driven inflammation remains nevertheless elusive. Previous studies failed to identify associations between polymorphisms in the genes encoding IL-1 and AOSD, thus pointing at more complex genetic mechanisms. This 'missing heritability' cannot be adequately investigated with traditional techniques for genetic partitioning, such as GWAS, which only assess common variants and polymorphisms. Studies focusing on highly penetrant rare variants or different types of mutations (i.e. small copy-number variations; insertions/deletions) are warranted.

Objectives. We hypothesized that genetically determined changes in IL-1-related pathways resulting in excessive IL-1β activity lead to the development of autoinflammation in AOSD. Scope of this study was to unravel the combined mutational variation of a network of IL-1-related receptors, pathways, counter-regulators, and cellular processes possibly involved in the pathogenesis of AOSD and IL-1-mediated inflammation in general.

Methods. We collected clinical, demographic, and genetic data from a large cohort of 76 AOSD patients and developed an innovative platform based on molecular inversion probes (MIP) technology for performing highly multiplexed targeted-resequencing. This allows efficient sequencing of the coding sequence of 48 genes related to the IL-1-pathway, and allows studying rare and common variants in one assay. We have also screened 500 healthy controls, and 1000s of samples with other disorders using the same assay.

Results. We identified rare and unique (i.e. private variants) in the IL1 pathway in several individuals with AOSD. Whether any these are involved in a strong predisposition to AOSD is currently followed-up. Rare genetic variants have been identified in six IL-1-pathway 'clusters':

1. Deregulated activation of the inflammasome and release of IL 1β and IL-18.
2. IL-1 family receptors and intracellular signaling mediators.
3. Other pro-inflammatory cytokines and receptors.
4. Regulatory molecules, including IL-1Ra or IL-37.
5. Cellular processes regulating production of IL-1 and IL-18 (i.e. autophagy).
6. Production of ROS, which function as markers of cellular damage and trigger inflammation.

Conclusions. Unraveling the genetic bases of inflammation in AOSD deepens our understanding of the human innate immunome. Of note, this study platform may serve for the genetic analysis of other IL-1-mediated conditions, including gout and other autoinflammatory diseases, whose genetic predisposition remains elusive. Equally important, the identification of pathways amenable to targeting with small molecules or biologics may translate into remarkable clinical implications.

STAT6-MEDIATED DIRECT REPRESSION OF INFLAMMATORY ENHANCERS LIMITS INFLAMMASOME ACTIVATION IN ALTERNATIVELY POLARIZED MACROPHAGES

ZSOLT CZIMMERER[1,*], BENCE DANIEL[2,*], ATTILA HORVATH[1,*], DOMINIK RÜCKERL[3,*], GERGELY NAGY[1], MATE KISS[1], MARIETTA M. BUDAI[4], LASZLO STEINER[5], BELA NAGY JR.[6], SZILARD POLISKA[7], CSABA BANKO[8], ZSOLT BACSO[8], JUDITH E. ALLEN[3], SZILVIA BENKO[4], LASZLO NAGY[1,2,9]

[1]Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary
[2]Sanford-Burnham-Prebys Medical Discovery Institute, 6400 Sanger Road, Orlando, FL 32827 USA
[3]Faculty of Biology, Medicine and Health, School of Biological Sciences, University of Manchester, Manchester, United Kingdom
[4]Department of Physiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary
[5]UD-Genomed Medical Genomic Technologies Ltd., Debrecen, Hungary
[6]Department of Laboratory Medicine, Faculty of Medicine, University of Debrecen, Debrecen, Hungary
[7]Genomic Medicine and Bioinformatic Core Facility, Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary
[8]Department of Biophysics and Cell Biology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary
[9]MTA-DE "Lendület" Immunogenomics Research Group, University of Debrecen, Debrecen, Hungary
***These authors contributed equally to this work.**

Cellular response to environmental cues requires reorganization of the transcriptional program by activating novel pathways and turning off unneeded ones. The molecular basis of signal-dependent transcriptional activation has been extensively studied in macrophage polarization, however our understanding remains quite limited regarding the relevance and molecular determinants of repression.

Here we show that IL-4-activated STAT6 is required for the direct transcriptional repression of a large number of genes during *in vitro* and *in vivo* alternative macrophage polarization. At the molecular level, repression results in decreased lineage-determining transcription factor, p300 and RNA polymerase II binding followed by reduced enhancer RNA expression, H3K27 acetylation and chromatin accessibility. In addition, STAT6-repressed enhancers show extensive overlap with the NF-κB/p65 cistrome and exhibit decreased responsiveness to LPS following IL-4 stimulus on a subset of genes. As a consequence, macrophages exhibit dampened inflammatory response, including diminished inflammasome activation, decreased IL-1β production and pyroptosis.

Our findings reveal a novel molecular circuit and biological activity of IL-4/STAT6 signaling in establishing and maintaining the alternative polarization-specific epigenetic signature and rendering macrophages less responsive to inflammatory stimuli.

Keywords: Il-4, STAT6, transcriptional repression, macrophage, enhancer, inflammation

COMMON- AND RARE GENETIC VARIATION IN THE INTERLEUKIN-1 PATHWAY

ROSANNE C. VAN DEUREN[1], PEER ARTS[1], LEO JOOSTEN[1], ROMANA NETEA[1], THEO PLANTINGA[1], CHARLES A. DINARELLO[1,2], MIHAI NETEA[1], FRANK L. VAN DE VEERDONK[1], ALEXANDER HOISCHEN[1]

[1]Department of Internal Medicine and Department of Human Genetics, Radboud Institute for Molecular Life Sciences,
Radboud University Medical Center, 6525 HP Nijmegen, the Netherlands
[2]Department of Medicine, University of Colorado, Aurora, Colorado, USA

The interleukin-1 (IL-1) pathway has a fundamental role in infection and inflammation. The IL-1 family members comprise both agonists and antagonists that together are responsible for maintaining a balance in immunological defense. Common Single Nucleotide Polymorphism (SNP) variants in the underlying genes have previously been associated with infection, inflammation, cardiovascular disease and cancer [1]. The effect of rare genetic variants, however, remains to be determined. This study was designed to provide elucidation on the role of both common- and rare genetic variation in the IL-1 pathway in immune responses in health and disease.

A highly-multiplexed targeted re-sequencing technology termed Molecular Inversion Probes (MIPs) [2] enabled a genetic screen for the complete coding sequence of the IL-1 pathway (ligands, receptors, signaling molecules, and IL-1 regulating genes), consisting of 48 genes that can be further subdivided into sub-pathways for analysis. This assay is effective in the identification of rare genetic variation and therefore provides an ultra-high sensitivity in the identification of coding genetic variation [3].

Here we present our data on large cohorts (N>1000) of patients with Gout and Thyroid Cancer, diseases that have been previously linked to IL-1 defects, and in 500 healthy individuals for which extensive immunophenotyping assays have been performed [4,5,6].

The first technical results of the MIP-assay on 48 IL-1 pathway genes in 472 individuals show, after an appropriate quality filtering, a uniform coverage with high sensitivity in detecting both common- and rare genetic variation for almost all IL-1 pathway genes. We have designed a statistical framework to calculate the burden of these common- and rare variants together on the immune response as measured by cytokine measurements in specific populations of both stimulated and unstimulated immune cells.

The approach of this association will be twofold: 1) Genotype-first - linking individuals with rare, private variants with protein damaging effects to their immunophenotypic signature; 2) Phenotype-first - burden testing for common- and rare variants combined by looking at individuals with extreme immunophenotypic signatures.

This study shows that the MIP-assay of all 48 genes of the IL-1 pathway is effective in providing a genetic screen for the complete coding sequence. Furthermore, we provide a framework that will aid in the elucidation of the role of both common- and rare genetic variation in the IL-1 pathway and subsequently on immune responses in health and disease.

β-GLUCAN REVERTS THE ITACONATE-INDUCED BLOCKADE OF TRAINED IMMUNITY BY RESTORING SDH EXPRESSION

JORGE DOMÍNGUEZ-ANDRÉS[1], BORIS NOVAKOVIC[2], YANG LI[3], BRENDON P. SCICLUNA[4], MARK S. GRESNIGT[1], ROB TER HORST[1], LEO A.B. JOOSTEN[1], VINOD KUMAR[3], TOM VAN DER POLL[4], HENK STUNNENBERG[2], MIHAI G. NETEA[1,5]

[1]Department of Internal Medicine and Radboud Center for Infectious diseases (RCI), Radboud University Nijmegen Medical Centre, Geert Grooteplein 8, 6500HB Nijmegen, the Netherlands
[2]Department of Molecular Biology, Faculty of Science, Radboud University, 6525 GA Nijmegen, the Netherlands
[3]Department of Genetics, University Medical Center Groningen, Groningen, The Netherlands
[4]Center of Experimental & Molecular Medicine, Division of Infectious Diseases, Amsterdam Medical Center, University of Amsterdam, Amsterdam, the Netherlands
[5]Human Genomics Laboratory, Craiova University of Medicine and Pharmacy, Craiova, Romania

Sepsis frequently leads to hyperactivation of the immune system and organ dysfunction within the first hours. However, some patients evolve to an immunoparalysis state in which the immune system is unable to respond to the infection. In the last years, itaconate, one of the most highly induced metabolites after LPS stimulation, has been witnessed as a key molecule contributing to the development of immune tolerance in sepsis.

In this work we describe how itaconate contributes to the induction of tolerance in human monocytes and how β-glucan counteracts this effect by blocking the expression of IRG1, the enzyme that drives itaconate synthesis. Furthermore, treatment with β-glucan improves the expression of the different isoforms of succinate dehydrogenase contributing to keep the integrity of TCA cycle and leading to an enhanced cytokine response after secondary stimulation.

β-GLUCAN INDUCED TRAINING IMMUNITY RESULTING IN PROTECTION AGAINST *LEISHMANIA* INFECTION: A CRUCIAL ROLE FOR IL-32

JÉSSICA C. DOS SANTOS[1,2,*], ANA MARINA BARROSO[2], MURIEL V. TEODORO[2], MICHELLE S. M. A. DAMEN[1], RODRIGO S. GOMES[2], SAMUEL T. KEATING[1], MIHAI G. NETEA[1], FÁTIMA RIBEIRO-DIAS[2], LEO A. B. JOOSTEN[1,2]

[1]Department of Internal Medicine and Radboud Center of Infectious Diseases (RCI), Radboud University Medical Center, Nijmegen, The Netherlands
[2]Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Goiânia, Goiás, Brazil
*Corresponding Author: *e-mail: jessica.dossantos@radboudumc.nl*

American Tegumentary Leishmaniasis (ATL) is a vector-borne parasitic disease caused by *Leishmania* protozoan. Recent studies demonstrate that innate immune cells undergo long-term functional reprogramming in response to infection or vaccination via a process called *trained immunity*, which is dependent on metabolic and epigenetic reprogramming. This adaptive agency is encapsulated by the observation that stimulation of innate immune cells with a pathogen augments the subsequent immune response to similar or unrelated immunological stimuli, thereby conferring non-specific protection from secondary infections. Recent reports describe clinical improvement in the treatment of ATL with Bacillus Calmette-Guérin by processes related with an increase of NK cells and proinflammatory monocytes. Based on this, we hypothesized that activation of innate cells might play a role in controlling infections caused by *Leishmania*. Here we demonstrate for the first time that monocytes trained with β-glucan (a component of the *Candida albicans* cell wall) confers protection against infections caused by *L. braziliensis*. Primary human monocytes were trained with β-glucan, thereafter these cells were infected with promastigotes forms of *L. braziliensis*. During early stages of the infection, trained monocytes increased phagocytosis capacity compared to controls. However, 4 hours and 24 hours following infection, the infection index was lower in trained monocytes compared to the RPMI control. Accordingly, when β-glucan-mediated trained immunity was blocked by rhIL-1Ra, an increase in the infection index was observed after 24 h of infection with *L. braziliensis*, paralleling a decrease in IL-32γ expression. In this same context, up-regulation of IL-32γmRNA was observed in cells trained with IL-1β, which reflects the induction of IL-32 dependent on the IL-1β activation in trained monocytes. Further biochemical investigation using molecular approaches and transgenic animal models highlight the potential clinical implications of these findings *in vivo*. Paralleling the in vitro findings, β-glucan-trained IL-32γTG mice showed a significant increase in lesion size after 3 weeks of infection compared to controls, but from the 5th week of infection a significant decrease in lesion size was observed in these mice trained, with consequent decrease in parasite load, increased production of IL-32 and TNFα. In addition, IL-32γTG mice treated with anakinra (IL-1 receptor antagonist) prior to β-glucan training, which was found to abrogate the effects of β-glucan by increasing parasite load. This study represents the first definitive characterization of the role of IL-32γ in the trained phenotype induced by β-glucan, the results of which improve our understanding of molecular mechanisms governing trained immunity and *Leishmania* infection control.

DIFFERENTIAL CYTOKINE RESPONSES IN MONONUCLEAR CELLS OF GOUT PATIENTS BASED ON THERAPY AND DISEASE STATUS

DRAGOȘ MĂRGINEAN, TANIA O. CRIȘAN, MAARTJE C.P. CLEOPHAS, VIOLA KLUCK, RADU A. POPP, TIM L. JANSEN, MIHAI G. NETEA, LEO A. B. JOOSTEN AND HINT CONSORTIUM

Background. Gout is an inflammatory disease whose biological cause is represented by monosodium urate (MSU) crystals being formed in patients with hyperuricemia and triggering inflammatory events. Recent studies concluded that hyperuricemia is associated with a pro-inflammatory status by directly affecting the peripheral blood mononuclear cells. Our objective was to ascertain whether the gout medications used in common medical practice influence *ex vivo* cytokine production.

Methods. We evaluated whether freshly isolated mononuclear cells of patients treated with urate-lowering agents or with gout anti-inflammatory medication exhibit a differential ex-vivo IL1β and IL6 response to several inflammatory stimuli (Pam3Cys, C16 and C16+C4), in the presence or absence of MSU crystals. The cytokine levels were assessed using specific sandwich ELISA kits for IL-1β and IL-6. The patients used in the study were part of the 250Gout cohort from the Human Functional Genomics Project Nijmegen, The Netherlands. The time that passed after a patient's acute gout attack was recorded and patients were re-evaluated after certain time intervals. The data was analyzed using general linear models.

Results. Our findings suggest that for patients treated with allopurinol there might be a stronger cytokine response, which is also exacerbated by the addition of MSU crystals. There is a statistically significant higher difference in mean cytokine levels between cells from allopurinol-treated patients and those without, as assessed by ANOVA.

Conclusion. Differential responses due to certain urate-lowering drugs have been observed. This could be a direct effect of therapy or be in line with the higher severity of the disease in treated patients. Further studies would be required to clarify the clinical reproducibility and significance of these findings.

Keywords: gout, cytokine response, therapy

CD36 REPRESSES CD14 LICENSED INTERLEUKIN-1B RELEASE AND INFLAMMATION BY DRIVING EICOSANOID METABOLISM TOWARDS LTB4 PRODUCTION

KARINA F. ZOCCAL[1], LUIZ G. GARDINASSI[1], CARLOS A. SORGI[1], ALYNE F. G. MEIRELLES[1], KARLA C. F. BORDON[2], ISAIAS GLEZER[3], PALMIRA CUPO[4], ALESSANDRA K. MATSUNO[4], VALDES R. BOLLELA[5], ELIANE C. ARANTES[2], FRANCISCO S. GUIMARÃES[6], LÚCIA H. FACCIOLI[1]

[1]Departamento de Análises Clínicas, Toxicológicas e Bromatológicas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto - SP, Brasil
[2]Departamento de Física e Química, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto - SP, Brasil
[3]Departamento de Bioquímica, Escola Paulista de Medicina, Universidade Federal de São Paulo, São Paulo - SP, Brasil
[4]Departamento de Puericultura e Pediatria, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto - SP, Brasil
[5]Departamento de Clínica Médica, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto - SP, Brasil
[6]Departamento de Farmacologia, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto - SP, Brasil

Envenomation by the scorpion *Tityus serrulatus* induces interleukin IL-1β production, which causes inflammation, lung edema and death. Bioactive lipids such as prostaglandin (PG)E_2 and leukotriene (LT)B_4 modulate the production of IL-1β by innate immune cells. However, pattern recognition receptors that perceive *T. serrulatus* venom (TsV) and inform cells to produce LTB4 or PGE2 to regulate IL-1β release remain poorly studied. Furthermore, whether molecular mechanisms observed in experimental models also translate in human cell responses to TsV remain underexplored.

Objective. We sought to investigate the roles of CD36 and CD14 on the recognition of TsV and whether these receptors control the production of eicosanoids during scorpion envenomation in mice and by human cells *in vitro*.

Results. We identified that CD14 induces PGE_2/cAMP/IL-1β release and inflammation. CD14-deficient mice produced high amount of LTB4 and are extremely resistant to envenomation, whereas treatment with LTB4 receptor antagonist reverses the susceptibility to TsV. Interestingly, CD36 drives the metabolism of eicosanoids for a distinct pathway that culminates in LTB_4 production, repressing PGE_2/cAMP/IL-1β axis and mortality. Indeed, CD36-deficient mice produce less amount of LTB_4 and are highly susceptible to TsV envenomation, and treatment with LTB4 rescues fatal outcomes. Of importance, we demonstrate that the molecular mechanisms governing resistance and susceptibility to scorpion envenomation in mice strongly translate into human cell responses to TsV.

Conclusion. This study provides major insights into molecular mechanisms controlled by the interplay between CD14 and CD36, differential eicosanoid metabolism and regulation of IL-1β release.

Funding. This work was supported by grants from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP; grant #2014/07125-6 and EMU #2015/00658-1 to LF, #2014/03332-7 to KFZ) and from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

INFLAMMATORY RESPONSE microRNAs EXPRESSION IS MODIFIED EXTRA-VESICULARLY AND IN MICROVESICLES FROM PLASMA OF FRUCTOSE AND CAFETERIA MODELS

FAUSTO SÁNCHEZ-MUÑOZ, ADRIAN HERNANDEZ, LUIS VALDERABANO-FAJARDO, JULIO ALMANZA-PEREZ, GUILLERMO CARDOSO-SALDAÑA, RICARDO MARQUEZ-VELASCO, MIGUEL CRUZ, JAIME H GÓMEZ-ZAMUDIO, MARTHA LÁZARO-SUÁREZ

Western diets and processed food have been associated with the increase of obesity and its complications. In this scenario, low grade inflammation has been implicated in the development of insulin resistance, endothelial dysfunction, hypertension, and type 2 diabetes. Chronic ingestion of high fructose as well as Cafeteria promoted diets have been useful models in this scenario. Little is known about abundance of plasma miRNAs free and in microvesicles in obesogenic diets. Therefore, we explored the circulating levels of miR-16, miR-21, miR-146a, miR-155 and miR-223 in rats consuming cafeteria diet and fructose in drinking water. In plasma, Leptin, TNF-α, IL-6 and IL-1β was also measure. Sprague Dawley rats were divided into three groups. The control group consumed standard diet, the cafeteria group consumed cafeteria diet for 15 weeks and the fructose group consumed 10% fructose in the drinking water for 15 weeks and miRNAs expression was determined in total plasma and in microvesicles, and in the extravesicular fraction by RT-qPCR with TaqMan probe based assays for miR-16, miR-21, miR-146a, miR-155, and miR-223, using cel-miR-39 (as spike in control and reference). Fructose-fed animals showed lower cholesterol total and HDL serum levels than controls ($P<0.05$). In plasma, TNF-α levels showed increased ($P<0.05$) in both fructose and cafeteria diet groups, while IL-1β levels decreased ($P<0.05$) in cafeteria diet group. In EVs miR-21 and miR-223 expression was upregulated ($P<0.05$). The extravesicular fraction showed a decrease of miR-155 expression ($P<0.05$). In conclusion, our results showed that cafeteria diet and fructose in drinking water modulated miR-21, miR-223 and miR-155 expression.

LOSS OF MOTILITY AND PHAGOCYTOSIS OF ALVEOLAR MACROPHAGES IN CHRONIC OBSTRUCTIVE RESPIRATORY DISEASE

WATARU FUJII[1], KEVIN BASSLER[1], THEODOROS KAPELLOS[1], ANNA C.ASCHENBRENNER[1,2], KRISTIAN HÄNDLER[3], JOACHIM L. SCHULTZE[1,3]

[1]Genomics and Immunoregulation, Life & Medical Sciences (LIMES) Institute, University of Bonn, Bonn, Germany
[2]Department of Internal Medicine and Radboud Center for Infectious Diseases (RCI), Radboud University Medical Center, Nijmegen, The Netherlands
[3]Platform for Single Cell Genomics and Epigenomics, German Center for Neurodegenerative Diseases and the University of Bonn, Bonn, Germany

Background. Cells of the innate immune system, e.g. alveolar macrophages (AMs) have been implicated to play a major role in the pathogenesis of chronic obstructive pulmonary disease (COPD) and asthma. Although changes in distribution and cellular functions of immune cells in these diseases have been reported, the cellular components and potential dysfunctions of the innate immune system are still not fully understood in human chronic respiratory diseases.

Method. We performed multi-color flow cytometry (MCFC), single-cell RNA sequencing (scRNA-seq) and functional assays to establish a detailed characterization of innate immune cells from the bronchoalveolar lavage (BAL) of 55 individuals (26 controls, 20 COPD patients (pts), 9 asthma pts).

Result. Based on MCFC, there does not seem to be an absolute increase of AMs in COPD and asthma pts, while the number of neutrophils significantly increased in BAL of COPD (2.4-fold) and asthma pts (3.8-fold) as compared to controls. Furthermore, the number of group 1 innate lymphoid cells (ILC1s) was increased in BAL of COPD pts (6.3-fold). In addition, scRNA-seq revealed unexpected mast cell population in BAL of control and COPD pts. Although the number of AMs was not altered, AMs from COPD patients showed less phagocytosis of E. coli as well as migration towards CCL3, a chemokine known to attract AMs. Gene expression analysis revealed that the expression of CCR5, a receptor for CCL3, was significantly decreased in AMs of COPD pts. Moreover, expression of transcription factor PPARγ, an upstream candidate for CCR5 was also reduced in AMs of COPD pts.

Conclusion. This study reveals elevated levels of neutrophils and ILC1s in BAL of COPD pts, while absolute numbers of AMs were unchanged but functionally showed less phagocytosis of E. coli, reduced motility towards CCL3, as well as less CCR5 and PPARγ expression. These observed dysfunctions of innate immunity could be a consequence or a pre-requisite for COPD, and also be linked to the susceptibility of the pts to recurrent infections.

MOLECULAR AND FUNCTIONAL MEMORY IN DECIDUAL NK CELLS OF PAROUS WOMEN

MORIYA GAMLIEL[1], DEBRA GOLDMAN-WOHL[2], OFER MANDELBOIM[1], SIMCHA YAGEL[2]

[1]The Lautenberg Center for General and Tumor Immunology, IMRIC, Hebrew University Faculty of Medicine. Jerusalem, Israel
[2]The Magda and Richard Hoffman Center for Human Placenta Research, Obstetrics and Gynecology, Hadassah Hebrew University Medical Center, Jerusalem, Israel

Introduction. Natural killer (NK) cells are abundant in the human decidua. These decidual NKs (dNK) produce cytokines, growth and angiogenic factors beneficial for the development of the placental bed. Preeclampsia and several of the "Great Obstetrical Syndromes" with a basis of poor placental development, are associated with first pregnancies. Thus, we investigated differences in dNKs in pregnancies of primigravid vs parous women.

Hypothesis. Human dNK cells remember pregnancy, thus better supporting subsequent pregnancies.

Methods. dNKs isolated from elective pregnancy terminations of primigravid and parous women (450 samples) were characterized by assays including FACs, RNA-seq and epigenetic analysis (ATAC-seq, CHIP-seq) followed by angiogenic and growth factor functional experiments. Endometrial NK cells from menstrual blood were also tested before and following pregnancies.

Results. We discovered a dNK population unique to pregnancies of parous women, possessing a novel transcriptome and epigenetic signature, characterized with high expression levels of the receptors NKG2C and LILRB1. Activation of these receptors leads to increased production per cell and secretion of IFNγ and VEGFa, the latter found to support vascular sprouting and trophoblast-tumor growth. Higher expression of these receptors was found on endometrial NK cells following first pregnancies. Epigenetic characterization of the endometrial NK cells strengthens the hypothesis that the precursors of these 'memory' cells are found in the uterus between pregnancies.

Discussion. We propose that this population termed as Pregnancy Trained decidual NK cells (PTdNKs) represents NK “memory” of pregnancy. We further suggest that the precursors of PTdNKs are found in the endometrium. These findings lend molecular and functional support for the observation that pregnancy is more robust in parous women. Our study offers an explanation of first pregnancy as a risk factor for development of preeclampsia. PTdNKs may prove useful in understanding and treating disorders of poor placentation.

HIGH-DIMENSIONAL PROFILING OF EARLY IMMUNE EVENTS FOLLOWING ACELLULAR PERTUSSIS BOOSTER VACCINATION

GILLARD J[1,2], BRAZDA P[3], ATLASY N[3], SUFFIOTTI M[4], DE JONGE M.I.[1,2], SIMONETTI E[1,2], JANSSEN-MEGENS EM[3], TEODOSIO C[5], DE GROOT R[1,2], PANTALEO G[4], BERBERS G[6], FENWICK C[4], STUNNENBERG H[3], DIAVATOPOULOS DA[1,2]

[1]Section Pediatric Infectious Diseases, Laboratory of Medical Immunology, Radboud Institute for Molecular Life sciences, Nijmegen, Netherlands
[2]Radboud Center for Infectious Diseases, Radboudumc, Nijmegen, Netherlands
[3]Department of Molecular Biology, Radboud University, Faculty of Science, Nijmegen, Netherlands
4Swiss Vaccine Institute, Lausanne, Switzerland
[5]Leiden University Medical Center, Department of Immunohematology and Blood Transfusion, Leiden, Netherlands
[6]National Institute for Public Health and the Environment (RIVM), Centre for Infectious Disease Control (Cib), Biltoven, Netherlands

Many countries continue to experience pertussis epidemics in spite of widespread vaccination.Moreover, increasing disease incidence has been observed in completely vaccinated children, adolescents andadults. It is thought that the first vaccine dose given during infancy programs long-term immunity to pertussis,with acellular (aP) and whole cell pertussis (wP) vaccines inducing distinct immune profiles. Thus the objectiveof this study is to apply systems vaccinology to study the early innate immune response to aP boostervaccination in young adolescents primed with either aP or wP vaccines during infancy. We characterized earlyimmune events before, and 24 hours after booster vaccination using complementary tools. Deep phenotypingof circulating immune cells was performed with a specialized mass cytometry (CyTOF) panel for innateresponses. In parallel, flow cytometry was used to further characterize the immune response and to obtainsingle innate immune cells by index sorting, thereby bridging our cytometry dataset and downstream geneexpression analysis through single-cell RNA sequencing (scRNAseq). We found that both cytometry datasetsdisplay high concordance, including shifts in granulocyte and myeloid populations post-vaccination. scRNAseqand correlation analysis of early innate immunity with long-term pertussis-specific immunity is ongoing. Thisstudy provides novel insights into the molecular mechanisms underlying the immune response to aP boostervaccination and provides an important framework for the development of new pertussis booster vaccines.

IL-6 AND TNFα CONCENTRATIONS ARE HIGHER IN FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY THAN IN HEALTHY CONTROLS

ANNA GRECO[1,2], KARLIEN MUL[1], BAZIEL VAN ENGELEN[1], LEO JOOSTEN[2]

[1]Department of Neurology, Radboud University Medical Center and [2]Department of Experimental Internal Medicine, Radboud University Medical Center, Nijmegen, The Netherlands

Background. Facioscapulohumeral dystrophy (FSHD) is one of the most prevalent inherited myopathies and, although the role of inflammation in its pathogenesis is suggested, evidence in literature remains equivocal. A further understanding of the inflammatory mechanism involved is crucial, especially as it may open up new possibilities for pharmaceutical interventions, anti-inflammatory drugs being available. The aim of this study was to analyze and compare concentration levels of inflammatory cytokines detected in serum samples of FSHD patients and healthy controls.

Methods. We measured IL-6, TNFα, IL-1β and IL-1α in serum samples of 150 genetically confirmed FSHD patients (73 male, 77 female; mean age 48 ± 15). IL-1β and IL-6 were measured in serum samples of 480 healthy controls (202 male, 278 female; mean age 27 ± 12). IL-1α and TNFα were measured in serum samples of 10 healthy controls (9 male, 1 female; mean age 22 ± 3). Experiments are currently on progress on the healthy controls cohort. Concentration levels of cytokines were detected using the multi-analyte Simple Plex Cartridge Kit for IL-1α, IL-1β, IL-6, TNF-α (ProteinSimple, San Jose, CA, USA) following manufacturer's instructions.

Results. Results are expressed as mean ± standard deviation. IL-6 concentration was higher in the patient group than in the controls group (3.29 pg/ml ± 2.61 pg/ml vs 1.29 ± 1.35 pg/ml, $p < 0.0001$). TNFα concentration was also significantly higher in the patient group compared to the healthy controls group (6.87 ± 1.82 pg/ml vs 5.10 ± 1.46 pg/ml, $p = 0.0022$). IL-1β concentration was not significantly different between the two groups (0.18 ± 0.12 pg/ml vs 0.34 ± 0.84 pg/ml, $p = 0.8773$). Finally, IL-1α average concentration was significantly higher in the controls group than in the patients group, based on partial data from 10 healthy controls (0.33 ± 0.40 pg/ml vs 0.12 ± 0.70 pg/ml, $p = 0.0002$).

Discussion. An inflammatory response is present in FSHD patients and IL-6 and TNFα concentrations were higher than in the controls group. Muscle is indeed a well known target of IL-6 whose signaling has been associated with stimulation of myogenesis through regulation of the proliferative capacity of muscle stem cells. It is also known that IL-6 and TNFα can also have negative consequences, such as promotion of atrophy and muscle waste. We propose that IL-6 and TNFα pathways may play a role in progression of FSHD by promoting an active phase of muscle inflammation. The significantly lower concentration of IL-1α in FSHD patients could be explained by the presence of genetic variants of IL-1α. A molecular inversion probe assay for the IL-1 superfamily members is currently being performed to test this hypothesis.

Keywords: cytokines, facioscapulohumeral muscular dystrophy, inflammation

EXPERIMENTALLY-INDUCED IMMUNOTOLERANCE COINCIDES WITH LOSS OF MONOCYTE METABOLIC PLASTICITY AND REDUCED ANTIFUNGAL IMMUNITY

INGE GRONDMAN[1], ROB J.W. ARTS[1], REBECCA M.KOCH[2], ROSALIE KEMPKES[1], ROB TER HORST[1], MATTHIJS KOX[2], PETER PICKKERS[2], MIHAI G. NETEA[1], MARK S. GRESNIGT[1,3]

[1]Laboratory of Experimental Internal Medicine, Department of Internal Medicine and Radboud Center for Infectious Diseases (RCI), Radboud University Medical Center, Nijmegen, the Netherlands
[2]Intensive Care Medicine, Radboud University Medical Center, Nijmegen, the Netherlands
[3]Current affiliation: Department of Microbial Pathogenicity Mechanisms, Leibniz Institute for Natural Product Research and Infection Biology, Hans Knöll Institute, Beutenbergstraße 11a, 07745, Jena, Germany

During severe sepsis circulating immune cells can exhibit a hypo-inflammatory state, also called immunotolerance, characterized by reduced cytokine responses and antigen presentation. In this state patients are highly vulnerable to secondary infections, including *Candida-infections*. Candidemia is a growing problem seen in the ICU, especially in immunocompromised patients and those treated with broad spectrum antibiotics. The exact molecular mechanisms leading to immunotolerance are unknown, but it has become apparent that cellular metabolism exerts a great influence on immune cell function. Therefore we investigated, the metabolic changes in circulating immune cells during experimentally induced immunotolerance. Additionally, we validated the influence of cellular metabolism on essential immune cell functions in host defense against *C. albicans*.

During immunotolerance, monocytes lost their capacity to mount cytokine responses to *C. albicans*, which was associated with a reduced capacity to alter cellular metabolism. In particular, pathways such as glycolysis, TCA cycle, pentose phosphate pathway, and glutaminolysis were affected. These pathways were validated by in vitro modulation of these metabolic pathways in naïve cells, and their modulation resulted in significantly altered cytokine responses to *C. albicans*. Furthermore, these pathways affected oxidative burst, and in particular modulation of glutamine metabolism influenced *Candida*-killing.

Collectively, our data show that change of cellular metabolism in immunotolerance may play a crucial role in the susceptibility to secondary *Candia*-infections by modulating key antifungal immune pathways.

PROSPECTIVE MODULATION OF THE GUT MICROBIOME BOOSTS ROTAVIRUS VACCINE IMMUNOGENICITY IN A RANDOMIZED CONTROLLED TRIAL

VANESSA C HARRIS[1], BASTIAAN W HAAK[1], SCOTT A HANDLEY, BAOMING JIANG, DANIEL E VELASQUEZ, GUY AM BERBERS, ELLES M KEMPER, ESTER MM VAN LEEUWEN, MICHAEL BOELE VAN HENSBROEK. JOOST WIERSINGA

Department of Medicine, Division of Infectious Diseases and Center for Experimental and Molecular Medicine (CEMM), Academic Medical Center, Amsterdam, The Netherlands. (VC Harris MD, BW Haak MD, Prof WJ Wiersinga MD)
Amsterdam Institute for Global Health and Development (AIGHD), Amsterdam, the Netherlands. (VC Harris MD, Prof M Boele v Hensbroek MD)
Department of Pathology and Immunology, Washington University School of Medicine, St Louis, Missouri, USA (SA Handley PhD)
Department of Experimental Immunology, Academic Medical Center, Amsterdam, The Netherlands. (EMM van Leeuwen PhD)
Department of Pharmacology, Academic Medical Center, Amsterdam, The Netherlands. (EM Kemper, PhD)
Emma Children's Hospital, Academic Medical Center, Meibergdreef 9, 1105, Amsterdam, The Netherlands. (Prof M Boele v Hensbroek MD)
Laboratory for Infectious Diseases and Perinatal Screening, Netherlands National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands (G Berbers PhD)
Division for Viral Diseases, Center for Disease Control and Prevention (CDC), Atlanta, Georgia, USA (B Jiang PhD, DE Velaszquez)
[1]Shared first authorship
*Corresponding author: *e-mail: v.c.harris@amc.nl. 2*

Background. Rotavirus (RV) is a leading cause of severe childhood gastroenteritis and diarrheal deaths. Rotavirus vaccines (RVV) have lower effectiveness in developing countries where most rotavirus-related deaths occur. The intestinal microbiome correlates with RVV immunogenicity in developing-country settings. This proof-of-concept study evaluates if prospective modulation of the intestinal microbiome can improve RVV immunogenicity.

Methods. This open-label randomized control trial enrolled healthy men aged 18-35 in the Netherlands. Subjects were randomized to 7-days broad-spectrum (oral vancomycin, ciprofloxacin, and metronidazole), narrow-spectrum (oral vancomycin), or no antibiotics and then vaccinated with RVV (RotarixTM), polysaccharide-pneumococcal (Pneumo 23), and tetanus-toxoid vaccine. The primary study endpoint was difference in 28 days-post-vaccination anti-RV IgA levels. Secondary endpoints were proportion of volunteers with day 7 anti-RV IgA boosting (>2-fold increase), absolute and proportion of RV-antigen shedding, anti-RV, pneumococcal, and anti-tetanus IgG and correlations between microbiome and outcomes. Clinicaltrials.gov, NCT02538211.

Findings. Between 2015-2017, 66 healthy volunteers were randomly assigned: 23 broad-spectrum, 21 narrow-spectrum, and 22 no antibiotics. 21/23 (91%) broad-spectrum, 21/21 (100%) narrow-spectrum, and 21/22 (95%) control group volunteers completed the study per-protocol. Baseline anti-RV IgA was high in all groups. There were no 28-day anti-RV IgA differences but anti-RV IgA boosting was significantly higher in the narrow-spectrum group (8/21 vs. 1/21 each, RR=0.125, 95% CI 0.02-0.67, p=0.021). Higher proportions of volunteers shed RV in the narrow and broad-spectrum groups than control (8/21 each vs. 1/21, RR 8, 95% CI 1.5-47.4, p=0.02) and narrow-spectrum antibiotics had significantly higher OD-shedding overall (p= 0.0027). There were no differences in anti-RV, tetanus or pneumococcal total IgG. Both antibiotic treatments decreased Bacteroidetes; only vancomycin reduced Firmicutes and expanded Proteobacteria abundance.

Interpretation. Despite the negative primary endpoint, this study clearly demonstrates that targeted alteration of the intestinal microbiota boosts rotavirus vaccine response in healthy sero-positive adults. These findings support a role for microbiome manipulation in improving rotavirus vaccine immunogenicity in developing countries.

Funding. Emma Children's Hospital Foundation (Stichting Steun Emma)

TRANSCRIPTOMIC CHARACTERIZATION OF PBMCS IN PATIENTS WITH STAT1 GAIN-OF-FUNCTION MUTATION AT SINGLE-CELL RESOLUTION

HÄNDLER K.[1,2], BASSLER, K.S.[1], BIERNAT P.[1], OSEI-SARPONG C.[3], FUJI W.[1], CIROVIC B.[1], VAN DE VEERDONK F.[4], PICELLI S.[2], ULAS T.[1], BEYER, M.D.[1], JOOSTEN L.[4], NETEA M.[4,5], SCHULTZE J.L.[1,2]

[1]Genomics and Immunoregulation, Life and Medical Sciences Institute, University of Bonn, Germany
[2]PRECISE Platform for Single Cell Genomics and Epigenomics, German Center of Neurodegenerative Disease, Bonn, Germany
[3]Molecular Immunology & Neurodegeneration, German Center of Neurodegenerative Disease, Bonn, Germany
[4]Radboudumc, Radboud University Nijmegen, The Netherlands
[5]Immunology and Metabolism, Life and Medical Sciences Institute, University of Bonn, Germany

Background. Signal transducer and activator of transcription 1 (STAT1), is an important immune regulator. STAT1 can be phosphorylated by multiple receptor associated kinases in response to various ligands including type I interferons IFN-α and IFN-β and type II IFN-γ. STAT1 phosphorylation causes homo- or heterodimerization and translocation to the nucleus where STAT1 homodimers formed upon IFN-γ stimulation are upregulating gene expression by binding to the Interferon-Gamma-Activated Sequence (GAS) promoter element. In response to either IFN-α or IFN-β stimulation, STAT1 forms a heterodimer with STAT2 that can bind the Interferon-Stimulated Response Element (ISRE) promoter element. Gain-of-function (GOF) mutations of STAT1 were first discovered in patients with chronic mucocutaneous candidiasis (CMC). It was shown that a heterozygous mutation of STAT1 that impairs dephosphorylation in the nucleus and therefore permanently activates STAT1 dimers is the cause of more than half of CMC cases. Patients with a STAT1 GOF mutation and CMC also commonly show viral and bacterial infections, and develop autoimmunities or even carcinomas. So far, the exact mechanism of the disease is poorly understood, low levels of IL-17A producing T-cells in CMC patients have been reported but STAT1 is a ubiquitously expressed transcription factor in immune cells and a transcriptional impact of the mutation is expected in various immune cell types.

Methods. We analyzed transcriptomic data of 12.000 single PBMCs from two patients and two healthy individuals generated by the Seqwell approach and performed a targeted RNA-Seq immune panel analysis in 15.000 single PBMCs from the same cohort using a BD Rhapsody. Cellular heterogeneity was analyzed using computational approaches including correlation matrixes and t-distributed stochastic neighbor embedding (tSNE). A hierarchical latent dirichlet allocation topic model (hLDA) was employed to merge multiple patient data. Clusters of single cells with expression profiles corresponding to PBMC cell types were identified. Differentially expressed (DE) genes were determined between STAT1 GOF mutation patients and healthy controls in all clusters.

Results. Under unstimulated conditions used in this study, expression differences between cells with STAT1 GOF mutation and non-mutated cells were generally subtile, allowing for clustering according to cell type rather than mutation. Still, genes with GAS and IRES regulatory sequences were on average higher expressed in STAT1 GOF mutation patients as compared to healthy control individuals in all PBMC cell types. Most notably STAT1 itself was upregulated in most clusters in GOF mutation individuals. DE genes significantly differentiating STAT1 GOF mutated from unmutated cells could be identified for B cells, NK cells and CD8+ T cells where T cell receptor and proinflammatory IL32 expression were increased and for monocytes where cytokine/interferon response genes were upregulated. Collectively, single cell RNA-sequencing allowed for the identification of mutation-mediated functional changes in all immune cell types simultaneously in a single experiment, clearly demonstrating the power of single cell analysis for clinically applied systems immunology.

HUMAN CYTOKINE RESPONSES DURING CHRONIC STABLE HIV

WOUTER A. VAN DER HEIJDEN, LISA VAN DE WIJER, ROB TER HORST, MARTIN JAEGER, MIHAI G. NETEA, QUIRIJN DE MAST, ANDRÉ J.A.M. VAN DER VEN

Department of Internal Medicine and, Radboud University Medical Center, Nijmegen, The Netherlands

Introduction. Chronic inflammation and immune dysfunction play a key role in the development of non-AIDS related comorbidities. Underlying mechanisms by which a HIV infection perturbs the immune system during successful HIV treatment are complex and still need to be elucidated. The Human functional genomics project (www.humanfunctionalgenomicsproject.com) provides a proven platform and a well-characterized healthy cohort to explore possible new pathways responsible for the persistent inflammatory state during a chronic HIV infection in both innate and adaptive compartment.

Methods. In this prospective cohort study, we included virologically suppressed HIV-infected individuals on stable antiretroviral therapy (cART > 6 months). The controls (50FG cohort) were simultaneously sampled every three months during the inclusion period of the HIV-cohort. In this study we studied the association between circulating mediators, immunophenotyping and the ex vivo cytokine production after stimulation with bacterial, fungal and viral stimuli. We used a linear regression model with age, gender, BMI and seasonality as covariates to compare both cohorts and correct for possible confounders.

Results. Between December 2015 and March 2017, a total of 211 HIV-infected individuals and 56 healthy controls were included. HIV-infected individuals were older than controls, with median (IQR) age of 52.5 (13.2) years vs. 30.0 (27.1) years ($p<0.001$), and more often male (91.0% vs 60.7%, $p<0.001$). The median (IQR) duration of known HIV-infection and cART were 8.1 (9.3) and 6.5 (7.9) years. Levels of circulating pro-inflammatory markers were increased in HIV-infected individuals compared to controls (alfa-1-antitrypsin, IL-18, CRP, I-FABP, sCD14 and βTG, FDR-corrected p-values <0.05). Upon stimulation with various pathogens, HIV-infected individuals showed a predominantly pro-inflammatory profile in monocyte-derived cytokines. This increase in cytokine production was most pronounced for IL-1B. We did not observe differences in T-cell-derived cytokines between groups. To further explore these findings immunophenotyping, using flowcytometry, will be incorporated.

Discussion/conclusion. In this study we comprehensively assessed inflammation and immunity in long-term treated virologically suppressed HIV-infected individuals and healthy controls and found that HIV was associated with a more pro-inflammatory profile and upregulation of monocyte-derived cytokines. Interestingly, this increased ex vivo cytokine production capacity in HIV could in part be due to intrinsic monocyte reprogramming. Our findings highlight the relevance for innate immune system as a potential therapeutic target for inflammation-related comorbidities in chronic HIV.

BCG INDUCED TRAINED IMMUNITY FOR PREVENTION OF POSTOPERATIVE INFECTIONS IN PATIENTS UNDERGOING ELECTIVE LAPAROTOMIC SURGERY

HELDER LS[1], KOCH RM[3], JOOSTEN LAB[2], NETEA MG[2], SCHEFFER, GJ[1]

[1]Department of Anesthesiology, Radboudumc, Nijmegen, the Netherlands
[2]Department of Internal Medicine, Radboudumc, Nijmegen, the Netherlands
[3]Department of Intensive Care Medicine, Radboudumc, Nijmegen, the Netherlands

Introduction. Despite strictly implemented perioperative infection-preventive strategies, postoperative infection rates are still high (up to 20%). Surgery-induced immunosuppression, indicated by altered immune cell functionality and cytokine release, contributes to such infections, which are associated with high mortality rates. This study aims to improve perioperative host-defence mechanisms by induction of trained immunity due to Bacillus Calmette-Guérin (BCG) vaccination 2 weeks prior to surgery.

Objective. Trained immunity, or innate immune memory, is based on initial exposure of monocytes to microbial ligands (β-glucan, BCG) which increases their responsiveness to secondary infections through epigenetic modifications. BCG-induced trained immunity is effective in-vivo, but the concept has never been applied in the surgical context, and its effect on SSI rate has never been investigated. The inflammatory response after elective laparotomic surgery in BCG-vaccinated patients will be compared with non–vaccinated patients in a double-blind randomized placebo-controlled trial. We expect that BCG vaccination will result in epigenetic reprogramming of circulating immune cells. This will be accompanied by a stronger innate response to pathogens, leading to a lower incidence of surgical site infections, pneumonia and sepsis.

Methods. The study will include 104 patients (≥ 18 years), scheduled for elective laparotomic procedures at the Radboudumc. Blood will be drawn during the perioperative period (before vaccination, before and after surgery, 30 post-operatively and 90 days post-operatively). Ex vivo cytokine production by immune cells before and after surgery upon stimulation with different pathogens will be measured by ELISA, immune cell subpopulations will be assessed by FACS, and epigenetic and transcriptomic profiles before and after surgery will be compared between intervention groups.

Outcomes. The main study endpoint is the inflammatory cytokine response to laparotomic procedures after BCG vaccination, compared to the control group. Epigenetic and metabolic reprogramming of innate immune cells after BCG vaccination will lead to the acquisition of a trained phenotype, facilitating a more efficient elimination of infectious pathogens during the perioperative period. Secondary endpoints are the occurrence of surgical site infection (superficial/ deep incisional and deep organ space infections) and the incidence of other postoperative complications (pneumonia, sepsis).

USING SYSTEMS BIOLOGY TO STUDY THE DIAGNOSTIC VALUE OF BLOOD GENE EXPRESSION-BASED CLASSIFIERS AS EXEMPLIFIED FOR ACUTE MYELOID LEUKEMIA

STEFANIE HERRESTHAL[1], KONSTANTINOS PERRAKIS[2], BERND TASCHLER[2], MATTHIAS BECKER[1], LEA SEEP[1], KEVIN BASSLER[1], PATRICK GÜNTHER[1], KATHRIN KLEE[1], THOMAS ULAS[1], TORSTEN HAFERLACH[3], SACH MUKHERJEE[2], JOACHIM L. SCHULTZE[1,4]

[1]LIMES-Institute, Department for Genomics and Immunoregulation, University of Bonn, Carl-Troll-Str. 31, 53115 Bonn, Germany
[2]Statistics and Machine Learning, German Center for Neurodegenerative Diseases, Sigmund-Freud-Str. 27, 53127 Bonn, Germany
[3]MLL, Münchner Leukämielabor GmbH, Max-Lebsche-Platz 31, 81377 München
[4]PRECISE Platform for Single Cell Genomics and Epigenomics at the German Center for Neurodegenerative Diseases and the University of Bonn, Sigmund-Freud-Str. 27, 53127 Bonn, Germany

Research on innate immunity in health and disease has been revolutionized by the advances of systems biology approaches, which try to understand biological processes in a global and integrative manner. One disease which has been heavily studied particularly on the transcriptomic level is Acute Myeloid Leukemia (AML), a severe, mostly fatal malignancy of innate immune cells. Early promising results introduced in 1999 by Golub and colleagues already suggested that strategies of global gene expression profiling could not only be used for disease subclassification, but also for disease prediction.

However, recent international recommendations for diagnosis and differential diagnosis of AML are still solely based on classical approaches including assessment of morphology, immunophenotyping, cytochemistry, and cytogenetics.

In contrast, the exciting new developments in the computational sciences and an ever-increasing pool of publicly available gene expression data demand the evaluation of this long-sought question whether gene expression profiling could become part of the integrated genomic approach for diagnosis, differential diagnosis, subclassification, outcome prediction, and monitoring disease activity in AML.

Utilizing 12,029 samples from 105 individual studies and applying several machine learning approaches we unequivocally demonstrate in a global, systems biology approach that AML can be robustly classified with high accuracy, specificity and sensitivity. Robustness was demonstrated across different study settings, technology platforms (microarray, RNA-seq), and computational approaches. Furthermore, the large data size also allowed us to demonstrate enrichment of genes associated with AML biology within class predictors.

Collectively, our study forms a strong foundation for finally considering transcriptome assessment as part of an integrated genomic approach in cancer diagnosis and treatment to be implemented early on for diagnosis and differential diagnosis of AML, and most likely also other leukemias and possibly other diseases of the innate immune system.

COPY NUMBER VARIATION OF THE CCL3L1 GENE ENCODING MIP1-ALPHA ISOFORM LD78BETA AND VIRAL PATHOGEN DIVERSITY

ADEOLU ADEWOYE, MANUELA SIRONI, EDWARD HOLLOX

The chemokine MIP1-alpha is encoded by two genes in humans, each generating a distinct isoform. The LD78alpha isoform of MIP1-alpha is encoded by the *CCL3* gene and the LD78beta isoform is encoded by *CCL3L1*. The beta isoform differs by three amino acids and has six-fold higher affinity to the *CCR5* receptor. Knockout mouse studies have shown that MIP1-alpha is an important mediator of virus-induced inflammation.

The *CCL3L1* gene (and the *CCL4L1* gene encoding MIP1beta) are on a copy number variable region of the genome that varies between 0 and 14 copies per diploid genome. There is a gene dosage effect, such that increasing copies increases expression of the gene.

Here, we determine *CCL3L1* copy number in three-generation pedigrees to estimate the mutation rate of the locus at about 0.5% per meiosis per generation, consistent with the diversity in copy number we observe in Europeans. We determine the variation of *CCL3L1* copy number across the globe using 1279 individuals from 13 populations from the 1000 Genomes project and combining it with 961 individuals from a further 52 populations. By using information from the GIDEON database of global infectious diseases, we show evidence of a positive correlation between *CCL3L1* copy number and population virus diversity, but not with diversity of other pathogens. Taken together this suggests that high *CCL3L1* copy number and/or high *CCL4L1* copy number may have been selected by past viral infections in human prehistory.

DECIPHERING MYELOID CELL ACTIVATION BY ARTIFICIAL TRANSCRIPTION FACTORS

ARIK HORNE[1], KEVIN BASSLER[1], THEODOROS KAPELLOS[1], ANNA ASCHENBRENNER[1], WATARU FUJII[1], THOMAS ULAS[1], MARC BEYER[2], JOACHIM L. SCHULTZE[1,3]

[1]Genomics and Immunoregulation, Life and Medical Sciences Institute, University of Bonn, Germany
[2]Molecular Immunology & Neurodegeneration, German Center of Neurodegenerative Disease, Bonn, Germany
[3]PRECISE Platform for Single Cell Genomics and Epigenomics at the German Center for Neurodegenerative Diseases and the University of Bonn, Bonn, Germany

Innate immune cells, including myeloid cells such as dendritic cells, monocytes or macrophages are guided in their differentiation and function via precise transcriptional programs. Some key transcription factors (TFs) have already been associated to these programs, for example C/EBPα and PU.1 for myeloid cell fate determination or STAT1 for INFγ-mediated activation of these cells. But it is also known that transcription is guided by networks of transcription regulators and not a single regulator is responsible for a complex cell response.

Can we define systems that allow us to study individual transcriptional regulators in isolation? We postulated that it will be essential to dissect such networks by studying single transcription regulators in isolation and combine them later again to induce complex cell responses. To answer this question, we used reductionist approach in well-defined cellular systems such as HEK or BLaER1 cells and combined these with CRISPR/Cas mediated epigenetic engineering, including the generation of artificial TFs. We focused first on two transcription factors PPARγ and IRF-1, since they are well known to shape the transcriptome of the myeloid cell compartment. We showed that the selected genes could be regulated by CRISPR/Cas based artificial TFs in these reductionist systems. Furthermore, we identified specific promotor regions for each gene, which elucidated the highest level of activation after CRISPR/Cas mediated gene activation.

Collectively, we provide a powerful epigenetic perturbation system that allows us to decipher complex transcriptional regulator networks in innate immune cells.

INTER-INDIVIDUAL AND INTRA-INDIVIDUAL VARIATION OF THE IMMUNE SYSTEM

ROB TER HORST

Background. We all encounter countless pathogens every day. Luckily, our immune system protects us from these invaders, and keeps us healthy. However, not all immune systems are created equal, not even within a "healthy" population. Some of use might be slightly more likely to suffer from certain infections because of diminished immune responses, whereas others might suffer from allergies because of an overactive immune system. In some people these variations are more extreme and can become pathogenic, with the patients either being immunocompromised or suffering from severe auto-immune diseases. To improve treatments, we need to better understand what the factors are that influence the immune system.

In a recent set of papers in which we analyzed data for ~500 healthy individuals we shed some light on possible causes for this variation between individuals (inter-individual variation). For circulating cytokines, cytokine production capacity and immuno-phenotypes the most important influencing factors included genetics, age and gender. Importantly, we also observed that specific immune responses were diminished responses in either winter or summer.

However, because we only measured one timepoint per individual our power for picking up seasonal changes was suboptimal. Additionally, we could not estimate what part of the unexplained variation in immune responses was caused by non-seasonal changes within individuals from day-to-day (intra-individual variation).

Experimental setup. To overcome this problem 50 of the original 500 healthy volunteers were recruited to be followed up 4 more times about three months apart. This allowed us to evaluate what immunological characteristics are stable within an individual over time, and which are strongly influenced by the environment.

Results. We observe that specific pathogens (e.g. Rhizopus oryzae) always induce similar responses in the same individual, whereas responses to other pathogens (e.g. Influenza) vary greatly. These marked differences in longitudinal stability also translate to immuno-phenotypes, with for instance specific T-cell counts showing great stability, whereas B-cell numbers vary strongly.

These findings are important for several reasons. For one, they should help researchers select pathogens for some of their experiments, e.g. if they want to minimize the effect of environmental factors. But more importantly, our results could have clinical implications. For instance, seasonal variation could direct prevention/treatment plans for certain conditions.

MEAN PLATELET VOLUME: IS IT A FEASIBLE INFLAMMATORY BIOMARKER IN GOUT?

IOANA HOTEA[1,2], CRISTINA PAMFIL[1,2], TANIA CRISAN[2], DRAGOS MARGINEAN[2], RADU POPP[2], SIMONA REDNIC[1,2], LEO AB JOOSTEN[2,3]

[1]Rheumatology Department of Emergency County Hospital Cluj-Napoca, Romania
[2]Iuliu Hatieganu University of Medicine and Pharmacy Cluj-Napoca, Romania
[3]Department of Internal Medicine, Radboud University Medical Centre, Nijmegen, The Netherlands

Background. Gout is the most common form of inflammatory arthritis that is usually presented with recurrent acute attacks in between intercritical periods [1]. Recent studies focused on the potential role of the mean platelet volume (MPV) as a possible biomarker of inflammation in various autoimmune diseases [2,3].

Objectives. To determine whether MPV is a feasible measure of inflammation and disease activity in gout.

Methods. We reviewed the hospital records of 139 patients with gout, evaluated between September 2016 and January 2018. All patients met a minimum of 8 points of the ACR/EULAR 2015 classification criteria and/or had a documented presence of uric acid crystals in the synovial fluid.

We correlated the MPV with inflammatory markers, respectively ESR and CRP, and also with the level of uric acid and clinical parameters (acute attack, intercritical gout, severity of attack, frequency of gouty attacks). Statistical analysis was performed using IBM SPSS.

Results. The mean (standard deviation, SD+) age of patients was 62 ±12 years, the majority of them were male (n=107 ,79%). The mean (SD+) level of serum uric acid was 7.78 ±1.2 mg/dl. The majority of patients were assessed during the intercritical period (85/139, 61.15%); the rest were evaluated during a flare (54/139, 38.8%). There was a statistically significant correlation between MPV and CRP (r=-0.23, p=0.04). No correlations were noted between MPV and ESR or the level of uric acid (r=-0.188, p=0.07 and respectively, r=0159, p=0.12). Moreover, MPV did not correlate with clinical parameters of disease activity, such as the clinical form of gout, or the severity of gouty attacks (p=0.68). Similarly, MPV was not associated with a higher number of flares per year (more than 7: p=0.699 eta-sq=0.029).

Conclusion. MPV was inversely correlated with the CRP, however MPV did not prove to reflect the disease activity or severity in patients with gout. More studies are needed to determine the role of MPV as a biomarker of inflammation in gouty arthritis.

THE ROLE OF GUT MICROBIOTA AND SHORT-CHAIN FATTY ACIDS IN LUNG IMMUNITY

Max C. Jacobs, W. Joost Wiersinga's group

The gut microbiota refers to the trillions of bacteria residing in animal and human intestines that provide numerous essential function in their host including metabolism, protection against enteric pathogens and development of immunity. By making use of both preclinical and clinical models, our group has recently provided evidence for an important role for the gut microbiota in defence against bacterial pneumoniae. In humans, bacterial pneumoniae is the prime cause of sepsis. No cure currently exists for sepsis, which explains this condition's high mortality-rate of approximately 25% persisting even in high-income countries. However, the mechanisms underlying the interaction between the gut microbiota and the lungs, popularly coined as the gut – lung axis, has remained largely unknown. Our current work sheds light on how gut microbiota or gut-derived metabolites such as short-chain fatty acids (SCFAs) influences systemic as well as lung immunity. We show that antibiotic induced gut microbiota depletion negatively affects relative presence of alveolar macrophages in bronchoalveolar lavage fluid (BALF) and levels of the proinflammatory cytokine interleukin (IL)-6. Besides, we provide evidence that SCFAs modulate both activity of alveolar macrophages and neutrophils by regulating expression of a range of plasma membrane markers as well as levels of tumor necrosis factor alpha (TNFα).

THE ROLE OF IL-32 IN THE INNATE HOST RESPONSE AGAINST *CANDIDA ALBICANS*

MARTIN JAEGER[1*], JESSICA C. DOS SANTOS[1,2*], BAS HEINHUIS[1], RODRIGO SAAR-GOMES[2], MARK GRESNIGT[1], MICHELLE DAMEN[1], FATIMA RIBEIRO-DIAS[2], MELISSA JOHNSON[3], YANG LI[4], VINOD KUMAR[4], CHARLES A. DINARELLO[1,5], MIHAI G. NETEA[1], LEO A.B JOOSTEN[1,2]

[1]Department of Internal Medicine and Radboudumc Center for Infectious Diseases (RCI), Radboud University Medical Center, Nijmegen, The Netherlands
[2]Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Goiânia, Goiás, Brazil
[3]Division of Infectious Diseases, Duke University School of Medicine, Durham, North Carolina, USA
[4]University of Groningen, University Medical Center Groningen, Department of Genetics, Groningen, The Netherlands
[5]Department of Infectious Diseases, University of Colorado, Denver, Denver, Colorado, USA

Infections with *Candida albicans* are still a major problem in immunocompromised and hospitalized patients leading to a large number of death and costs for health care systems worldwide. Extensive genetic and population wide studies have identified important components in *Candida* host response pathways that are dysregulated that are responsible for disease susceptibility. The cytokine/transcription factor IL-32 has recently been described to be important in several bacterial infections but nothing is known about its role in fungal infections.

Methods and Results. By performing experiments with primary human cells *in vitro* as well as murine *in vivo* experiments, we here for the first time, identified interleukin (IL)-32 as a novel mediator in the host response against *C. albicans*. This is most likely not through direct antifungal effect of IL-32 on *Candida* itself but rather an immunomodulatory effect on cells of the innate immune system. A intronic polymorphism in the *IL-32* gene strongly influences *C. albicans* induced IFNγ production in human PBMCs *in vitro* and addition of recombinant human IL-32γ led to increased concentrations of this cytokine as well as an increase in *C. albicans* phagocytosis whereas other IL-32 isoforms did not. In a large cohort of patients suffering from Candidemia, the TT genotype of the described genetic variant led to strongly diminished serum levels of IFNγ whereas other cytokines remained unaffected. In the following we show that *C. albicans* infected human IL-32γ transgenic mice show an improved 14-day survival compared to wild type mice.

Conclusion. In summery these results identify IL32 as a novel component in the immune response against *C. albicans.* These insights may help to improve existing therapies and will eventually lead to benefits for (immunocompromised) patients suffering from *Candida* infections.

UNDERSTANDING IMMUNOLOGICAL RESPONSES TO FUNGAL PATHOGENS IN TYPE 1 DIABETES

A.W.M. JANSSEN[1], R. STIENSTRA[1,2], M.G. NETEA[1,3], N.P. RIKSEN[1], C.J.J. TACK[1]

[1]Radboud University Medical Centre, Department of Internal Medicine (463), PO Box 9101, 6500 HB Nijmegen, The Netherlands
[2]Division of Human Nutrition and Health, Wageningen University, Wageningen, The Netherlands
[3]Radboud Center for Infectious Diseases, Radboud University Medical Center, Nijmegen, The Netherlands
*Corresponding Author: *e-mail: Anouk.Janssen@radboudumc.nl*

Background/Rationale: Type 1 diabetes is an auto-immune disease, which renders the affected individual at increased risk of developing complications. A common denominator of many diabetes-associated complications are dysregulated innate immune responses. For example, it has been well documented that the presence of diabetes leads to an increased risk for fungal infections with more complications compared to non-diabetic individuals. Recent epidemiological studies revealed that among individuals diagnosed with type 1 diabetes those at highest risk for infection events and worse outcomes are older, obese and have a longer duration of diabetes. Besides that, patients with type 1 diabetes are at approximately double the risk of patients with type 2 diabetes for infection-related death. However, the precise factors leading to perturbed innate immune function in patients with diabetes are poorly understood.

Methods: During a single outpatient clinic visit we collected blood samples for immunological phenotyping from n=244 patients with type 1 diabetes. PBMCs were isolated and exposed to fungal pathogens. In addition to analyzing specific immune responses using cytokine/chemokine release measurements (IL-1β, IL-6, TNFα, IL-8, IL-10, IL-1RA and MCP-1), extensive clinical data from these patients was collected (including age, gender, BMI, duration of diabetes, HbA1c, glycemic burden , number and type of diabetic complications, co morbidities and medication use).

Preliminary results: Between February 2016 and June 2017, 244 type 1 diabetes patients were recruited in the Radboudumc Nijmegen. 54.5% of the patients were male, the mean age was 51.6 years (min 19.7, max 84.4, SD 16.3). The mean BMI was 25.8 kg/m2 (min 16.7, max 41.8, SD 4,3). The average duration of diabetes was 28.4 years (min 1, max 71, SD 15.7). The average HbA1c was 64 mmol/mol (range 34-136).The various cytokines released by the immune cells ex vivo after fungal stimulations have been measured.

We are currently studying the association between various factors (including glycemic burden, presence of diabetic complications, the duration of diabetes) and the immunologic responses to fungal pathogens.

HEME INDUCES TRAINED IMMUNITY

E. JENTHO[1,2], B. NOVAKOVIC[3], H.G. STUNNENBERG[3], F. RÖSTEL[1,2], J. GUERRA[1,2], LAB. JOOSTEN[3], M.G. NETEA[3,4], M. BAUER[1,2], S. WEIS[1,2]

[1]Universitätsklinikum, Jena, Germany
[2]Center for Sepsis Control and Care, Jena, Germany
[3]Medical Centre and Radboud University, Nijmegen, Netherlands
[4]Life&Medical Science Institute (LIMES), University of Bonn, Germany

Background. Trained immunity (TRIM) refers to a recently discovered adaptive response of cellular components of the innate immune system to subsequent inflammatory stimuli (Netea et al., 2016). It is based on long lasting epigenetic and transcriptional changes, induced by microbial or endogenous molecules (Saeed et al., 2014). Yet, in the perspective of Matzingers danger hypothesis, cells of the innate immune system do not exclusively differentiate between foreign and self but also sense host derived molecules released during infection associated damage (Matzinger, 1994). Heme, an iron containing tetrapyrol, acts as a potent damage associated molecular pattern that can modify macrophage function and fate (Soares and Bozza, 2015; Soares and Weiss, 2015) and disrupts parenchymal homeostasis in severe bacterial infections (Larsen et al., 2010; Weis et al., 2017). During infections, heme is released e.g. by red blood cells in the case of malaria or by damaged tissue, e.g. in the case of bacterial sepsis.

We hypothesised that heme, besides its established acute effects on myeloid cells, induces innate immune memory.

Findings/Novelty. In this study we used human primary monocytes and mouse models in combination with functional immunology, metabolomic, epigenomic and transcriptomic techniques, to study heme-induced TRIM. Our findings include:

1.We provide experimental evidence derived from primary murine and human myeloid cells that heme is a potent inducer of trained immunity.

Trained immunity induction was specific to heme, with other damage-associated molecular patterns (DAMPs), such as ATP or the Toll-like receptor 4-ligand Lipopolysaccharid, not leading to trained macrophages. In addition, while many biological functions of heme are associated to the redox-active central iron atom (Larsen et al., 2012), this does not apply to heme-mediated innate immune training.

2.Using time-course RNAseq and ChipSeq analysis in human primary monocytes we show that heme and β-Glucan (a strong trainer) induced gene expression and epigenetic profiles i.e. H27K4ac, can be grouped in ‘common’, ‘heme’ and ‘β-Glucan’ specific clusters. Indicating a potential ‘common set of transcriptional events’ that are necessary for TRIM in human monocytes, which involves genes involved in metabolism/lysosome/phagocytosis.

3.Heme also up-regulated the expression of several hundred genes, which can be separated into two groups – pro-inflammatory cytokines and chemokines and phagocytosis/proteasome.

4.Despite similarities between heme and β-glucan in the response after the first stimulus, heme induced trained immunity does not rely on the activity of mechanistic Target of Rapamycin (mTOR). Instead, heme training strictly relies on the activation of the spleen tyrosine kinase (Syk)/c-Jun-N-terminal kinase (JNK) mediated pathway and ultimately leads to increased release of inflammatory cytokines after second hit.

In summary, we provide evidence that heme induces trained immunity via distinct pathways and reveal the underlying molecular mechanisms.

NEUTROPHILS WITH AN EXTRAVASATION PHENOTYPE ARE DETECTABLE IN THE PERIPHERAL BLOOD OF PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

THEODORE S. KAPELLOS[1], WATARU FUJII[1], KEVIN BASSLER[1], KRISTIAN HÄNDLER[1,2], CHRISTINE FALK[3], DIRK SKOWASCH[4], JOACHIM L. SCHULTZE[1,2]

[1]Genomics and Immunoregulation, Life & Medical Sciences (LIMES) Institute, University of Bonn, Bonn
[2]Platform for Single Cell Genomics and Epigenomics at the German Center for Neurodegenerative Diseases and the University of Bonn, Bonn
[3]Institute for Transplantation Immunology, Medical School Hannover, Hannover
4Medical Clinics and Policlinics, Section of Pneumology, University Hospital Bonn, Bonn

Chronic obstructive pulmonary disease (COPD) is a chronic respiratory disease projected to surpass prevalent diseases in Western countries, such as lung cancer and neurodegeneration by 2020. It is characterized by excessive inflammation and destruction of the alveolar tissue and irreversible small airway obstruction which leads to dyspnoea and chest tightness. Due to the continuous deterioration of patients' quality of life and the enormous hospitalization costs, it is urgent that we identify reliable and informative biomarkers for diagnosis and outcome prediction.

To address these questions, we characterized the myeloid (neutrophils, CD14+ monocytes, NK cells) and lymphoid (T, B, NK cells) immune populations in the blood of control patients and patients with COPD by multi-colour flow cytometry (MCFC) including 11 markers and performed whole transcriptome analysis of sorted cells. We did not find significant differences in the numbers of blood immune cells between control and COPD subjects. Furthermore, studying the immune cell types that are within the peripheral blood mononuclear cell fraction (PBMC) by bulk RNA-sequencing (RNA-seq) also did not reveal major differences. However, when analyzing neutrophils by RNA-seq, changes in gene expression were revealed which pointed towards an increased capacity of extravasation into tissues in patients with COPD.

To functionally test whether the alterations in blood-derived neutrophils translate into changes within the lung, we assessed the recruitment of neutrophils to the lung and detected a significant increase in their numbers in the bronchoalveolar lavage (BAL) fluid of patients with COPD. These findings are also in accordance with elevated levels of the plasma levels of the chemokines CCL11 and the main neutrophil-recruiting chemokine CXCL8. Collectively, we demonstrate that changes in neutrophils are already apparent in peripheral blood of COPD patients, which might open new avenues towards blood-based disease-associated biomarkers. Furthermore, widely-used approaches such as the application of MCFC or the focus on PBMC might have missed important biology of this detrimental disease in the past. We postulate that the application of unbiased higher resolution methodologies, such as scRNA-seq of whole blood samples will be necessary to better understand the disease and to develop better biomarkers.

TARGETING SEPSIS-INDUCED ACUTE LUNG INFLAMMATION BY MODULATING THE CALCINEURIN/NFATC3 PATHWAY IN MACROPHAGES

KARPURAPU MANJULA*, QIAN ZIQING, NIRUJOGI TEJA SRINIVAS, PEI DEHUA, CHRISTMAN JOHN

The Ohio State University, USA

Our previous studies indicate that the transcription factor NFATc3 mediates sepsis-induced ALI pathogenesis and can be blocked with a specific calcineurin inhibitory peptide 11R-VIVIT. Here, we report that mouse pulmonary microvascular endothelial cell monolayer maintained a tighter barrier function when co-cultured with NFATc3 deficient but not wild type macrophages. NFATc3 deficient mice subjected to the cecal ligation and puncture (CLP) model of polymicrobial sepsis showed decreased neutrophilic influx, improved alveolar capillary barrier function, pulmonary arterial oxygen saturation and survival benefit. Furthermore, CP9-ZIZIT, a highly potent, cell-permeable peptide inhibitor of calcineurin inhibited NFATc3 activation at 50 fold lower concentration compared to 11R-VIVIT. CP9-ZIZIT effectively reduced sepsis induced inflammatory cytokines and pulmonary edema in mice. Studies are underway to delineate the role of NFATc3 in pulmonary alveolar fluid clearance. Thus, our data demonstrates that genetic ablation or pharmacologic inhibition of NFATc3 activation in pulmonary macrophages protects the lung from inflammation and injury and improves survival in the mice subjected to CLP polymicrobial sepsis. These data provide preclinical support for blocking NFATc3 as a therapeutic option for improving the outcome of patients with severe sepsis and at high risk for developing ARDS.

CONTRIBUTION OF RESIDENT MACROPHAGES TO THE HSC NICHE IN THE FETAL LIVER

AMIR HOSSEIN KAYVANJOO, HARUTAI NIRAMIS, NÓRA RÉKA BALZER, CORNELIA CYGON, ELVIRA MASS

Department of Developmental Biology of the Innate Immune System, Life and Medical Sciences Institute (LIMES), University of Bonn, Germany

Tissue-resident macrophages are a heterogeneous population of phagocytes found in almost all tissues of the body. Recent studies indicate that most resident macrophages originate from yolk sac progenitors and are maintained by local proliferation independent from hematopoietic stem cells (HSC). Their heterogeneity across different organs is important for tissue-specific niches and cellular interaction during tissue development as well as tissue homeostasis. Yet, their heterogeneity within one organ, especially during development, has not been addressed in detail. In the fetal liver, hepatic macrophages are known to play an important role for the maturation of erythroblasts. However, whether resident macrophages can also contribute to the fetal liver HSC niche remains enigmatic.

Using multicolor flow cytometry, we show that the hepatic macrophage population is indeed heterogeneous. Further, we observe that macrophages serving as a platform for erythroblast maturation in the fetal liver also interact with HSC using 3D reconstructions from immunofluorescent-labeled sections. To test the functionality of hepatic macrophages in HSC development and maintenance, we made use of in vitro and in vivo models where macrophages are lacking. Re-aggregation cell cultures containing all hepatic cells but macrophages as well as a mouse model lacking macrophages indicate that hepatic macrophages are important players in the HSC niche. Interestingly, we observe not only a decrease of HSC in fetal livers that are deficient for resident macrophages, but also a differentiation bias of HSC towards the myeloid lineage. Our findings support the hypothesis that fetal hepatic macrophages play a crucial role for liver function as a hematopoietic organ.

TIME COURSE TRANSCRIPTOMIC AND FUNCTIONAL ASSESSMENT OF INNATE IMMUNE SYSTEM RESPONSE IN *IN VIVO* MODELS OF ENDOTOXEMIA

FARID KERAMATI[1], BORIS NOVAKOVIC[1], GUUS LEIJTE[2], PETER PICKKERS[2], MATTHIJS KOX[2], HENK STUNNENBERG[1]

[1]Department of Molecular Biology, Faculty of Science, Radboud University, Nijmegen, Netherlands
[2]Department of Intensive Care Medicine, Radboud University Medical Center, Nijmegen, Netherlands

As frontiers of the immune response against foreign contaminants, innate immune cells identify and react to bacterial endotoxin, especially LPS, via their surface receptors. Conventionally, monocytes are isolated from peripheral blood and studied *in vitro*. However, our results from the comparison of *in vitro* versus *in vivo* response of monocytes to a LPS challenge showed a significant difference, which emphasizes the necessity to assess the response of monocytes *in vivo*. To investigate the monocyte response *in vivo*, we intravenously administered LPS (2 ng/kg) to elicit a systemic inflammatory response in healthy volunteers. Interestingly, the LPS challenge caused a rapid disappearance of monocytes from the blood stream, and a strong increase in the number of low-density granulocytes. Further time course assessment of the blood cells, at 4, 8, and 24 hours after LPS administration, showed reappearance of monocytes around 8 hours post injection mainly as classical monocytes (CD14+ CD16-). Strikingly, a second LPS infusion, one week after the first challenge, did not have a substantial effect on monocyte counts, suggesting a tolerized, unresponsive state of these cells. Monocyte RNA expression profiles of the samples from RNA-seq data also showed significantly different and unique cell profiles at each time point, demonstrating the memory effect of LPS challenge on monocytes at later time points. Furthermore, at each time point monocytes were isolated from peripheral blood and exposed to LPS in an ex vivo manner. RNA-seq data of these re-exposed monocytes at each time point unravels their functional potential. We will study the (epi)genomic and transcriptomic basis of the innate immune memory, especially for monocytes, and discuss pertaining post *in vivo* LPS challenge effects from a transcriptomics point of view.

CANONICAL AND NON-CANONICAL RNA SPLICING IN SPECIFIC PERIPHERAL BLOOD MONONUCLEAR CELLS OF CRITICALLY ILL PATIENTS WITH SEPSIS

HINA N. KHAN[1,2], XANTHE BRANDS[1], SIMONA AUFIERO[2], TJITSKE S.R. VAN ENGELEN[1], BAS HAAK[1], LONNEKE A. VAN VUGHT[1], AEILKO H. ZWINDERMAN[2], JANNEKE HORN[3], MARCUS J. SCHULTZ[3,4], ARIE J. HOOGENDIJK[1], TOM VAN DER POLL[1,5], BRENDON P. SCICLUNA[1,2]

[1]Center for Experimental Molecular Medicine, Academic Medical Center, University of Amsterdam, the Netherlands
[2]Department of Clinical Epidemiology, Biostatistics and Bioinformatics, Academic Medical Center, Amsterdam, the Netherlands
[3]Department of Intensive Care Medicine, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands
[4]Mahidol Oxford Tropical Medicine Research Unit, Bangkok, Thailand
[5]Division of Infectious Diseases, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

Introduction. Dysregulation of the host immune response is a pathognomonic feature of sepsis. Canonical (linear) RNA splicing is an important characteristic of protein-coding RNA regulation, which ultimately provides functional diversity to proteins. Abnormal physiological conditions are understood to shift efficient linear splicing of protein-coding RNA towards inefficient non-canonical splicing, characterized by the accumulation of non-coding circularized (circ)RNA. An evaluation of RNA splicing dynamics in specific immune cells of sepsis patients and healthy subjects is lacking.

Objective. We here sought to firstly map the canonical and non-canonical RNA splicing patterns in specific immune cell-types of sepsis patients relative to healthy subjects. Secondly, evaluate the accumulation of circRNA by *in vitro* stimulation of monocytes.

Methods. The study comprised a discovery cohort of six critically ill patients diagnosed with sepsis due to community-acquired pneumonia and four age, gender matched healthy subjects. Peripheral blood mononuclear cells were isolated and fluorescence activated cell sorting was used to purify CD14+ monocytes, CD4+, CD8+ T cells and CD19+ B cells for RNA sequencing. CD14+ monocytes from independent six healthy volunteers (functional validation cohort) were purified and stimulated in vitro for 2- and 24-hours with lipopolysaccharide (LPS), *Streptococcus (S.) pneumoniae* and *Klebsiella (K.) pneumoniae.*

Results. Analysis of canonical RNA splicing revealed predominantly unique alternative splicing events per cell-type. Alternative splicing was prevalent in monocytes of sepsis patients relative to health, with 151 alternative splicing events detected. *SRGN, RNASE2* and S100A12 transcripts were highly altered protein-coding genes. We subsequently turned our attention to non-canonical RNA splicing analysis. Overall, 735, 753, 636 and 430 putative back-splice events producing circRNAs were predicted in monocytes, CD4+, CD8+ T cells and B cells, respectively. Our analysis also showed that proposed features of RNA circularization, that is, interspersed *Arthrobacter luteus* restriction endonuclease repeats, were significantly enriched (>20%) in the flanking introns of predicted circRNA. Principal component analysis of circRNA expression revealed clearly distinct monocyte, T cell and B cell clusters. CircRNA accumulation in monocytes, CD4+, CD8+ T cells and B cells of sepsis patients was increased by 1.7-, 2.2-, 1.5- and 1.4-fold relative to health. *VCAN* (chr5: 83519349-83522309) and *CHD2* (chr15: 93000512-93014909) circRNA were highly abundant in monocytes of sepsis patients, which were partially induced by in vitro stimulation of healthy monocytes with LPS, *S. pneumoniae* and *K. pneumoniae*.

Conclusion. Monocytes, CD4+ and CD8+ T cells and B cells of sepsis patients engage in predominantly distinct canonical and non-canonical RNA splicing events relative to health. Immune cell subsets of sepsis patients were predicted to accumulate circRNA, which suggests part of the protein-coding gene output may be inefficient for protein synthesis. CircRNA production in monocytes may be induced by clinically relevant causal pathogens and endotoxin.

GENETIC VARIANTS IDENTIFY IL-37 AS AN IMPORTANT ANTI-INFLAMMATORY CYTOKINE IN GOUT IN HUMANS

VIOLA KLÜCK, ROSANNE C. VAN DEUREN, AMARA SHAUKAT, MAARTJE CLEOPHAS, TANIA CRISAN, NICOLA DALBETH, LISA STAMP, ANNA-KATHRIN TAUSCHE, TIMOTHY RADSTAKE, TIM JANSEN, MATTHIJS JANSSEN, PHILLIP RICHES, JENNIE HARRÉ HINDMARSH, ALEX HOISCHEN, FRANK VAN DE VEERDONK, MIHAI NETEA, CHARLES DINARELLO, TONY MERRIMAN, LEO JOOSTEN

Background. During a gout flare, MSU crystals induce, in presence of a secondary stimulus, acute joint inflammation characterized by the recruitment of predominantly neutrophils. These neutrophils account for a release of more chemokines and inflammatory mediators further amplifying the inflammatory reaction of gout. IL-37 emerges as a fundamental inhibitor of the innate immunity and was shown to inhibit MSU-crystal induced inflammatory responses in cell lines and murine models of gout [1,2]. To further elucidate the role of *IL-37* in the pathogenesis of gout, we examined DNA of gout patients for genetic variations in *IL-37* and assessed the functional consequences of these mutations.

Methods. Exons of the *IL-37* gene were sequenced using Molecular Inversion Probes (MIPs) [3] in 698 patients with crystal-proven gout. The burden of rare genetic variants detected in IL37 was compared to a cohort of 469 healthy controls (also sequenced with MIPs) and the Exome Aggregation Consortium database (ExAC) [4].

Neutrophils were isolated from healthy Dutch donors, pretreated with recombinant IL-37, a genetic variant of IL-37 or IL-37Fc for 1h and stimulated with opsonized MSU crystals. ROS were quantified by adding luminol and measuring the intensity of the chemiluminescence at ~425nm. IL-8 was assessed in supernatant after 18h using a specific sandwich ELISA kit.

For the validation of genetic variants, a total of clinically-ascertained 2202 gout cases and 2295 controls (further stratified into 424 hyperuricemic (≥0.41mmol/L) controls) drawn from various populations of European and NZ Polynesian ancestry were utilized. Taqman® genotyping was carried out, followed by the multivariate-adjusted (age, sex and GPancestry in Polynesian) association analysis in R 3.2.2 with gout as the outcome.

Results. MIP-sequencing identified four non-synonymous, rare variants in exon 5 of IL-37 in 6 gout patients (p.A144P; p.H172HX; p.N182S; p.C181*), whereas none were detected in our cohort of healthy controls. Two of these variants were also not observed in ExAC. To elucidate the effect of these mutations in *IL-37*, we produced a recombinant protein based on one of the found mutations (p.C181*). This mutant was studied in an *in vitro* model in which full length IL-37 (46-218) significantly decreased ROS and IL-8 production. The mutant of IL-37 demonstrated diminished anti-inflammatory effects compared to the full length protein.

In our validation cohort the rs*752113534* variant (p.N182S) was monomorphic in the European sample set but the minor G-allele exhibited a frequency of 0.05 in the NZ Eastern and Western Polynesian sample sets. A meta-analysis of Eastern and Western Polynesian showed a significant association of the G-allele with gout risk using hyperuricemic controls (OR= 1.81, P_{OR}=0.03).

As a potential therapeutic IL-37Fc was tested in our in vitro model of gout and strongly inhibited ROS and IL-8 production.

Conclusion. Rare genetic variants in *IL-37* were found in patients with gout. One of these *IL-37* mutants demonstrated a loss of the anti-inflammatory function *in vitro*. Moreover, the association of IL-37 rare variant *rs752113534* in developing gout in NZ Polynesian supports a role for IL-37 in an inflammatory pathway leading to gout in the presence of hyperuricemia. Futhermore, IL-37Fc was identified as a potential new therapeutic in the treatment of gout.

SURVIVAL OF TUBERCULOUS MENINGITIS IS LINKED TO CEREBROSPINAL FLUID VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF); A SYSTEMS APPROACH

VALERIE A. C. M. KOEKEN[1], ARJAN VAN LAARHOVEN[1], RAÚL AGUIRRE-GAMBOA[2], SOFIATI DIAN[1,3], MIHAI G. NETEA[1], BACHTI ALISJAHBANA[3], AHMED R. GANIEM[3], VINOD KUMAR[2], REINOUT VAN CREVEL[1]

[1]Radboud university medical center, Department of Internal Medicine, Nijmegen, the Netherlands
[2]University Medical Centre Groningen, Department of Genetics, Groningen, the Netherlands
[3]Universitas Padjadjaran, TB-HIV Research Center, Bandung, Indonesia

Introduction. Tuberculous meningitis (TBM) is the most severe form of tuberculosis (TB), resulting in death or neurological disability in >30% of adult patients. Damaging or ineffective host immune responses contribute to the high mortality of TBM, but the underlying mechanisms remain largely unknown. Using unbiased cerebrospinal fluid (CSF) metabolomics we have recently shown that tryptophan metabolism contributes to poor outcome of TBM (*van Laarhoven et al., Lancet Inf Dis 2017 in press*). We now extend this approach to CSF proteomic measurement.

Methods. Using a multiplex immunoassay, a panel of 92 inflammation-related proteins were measured in CSF samples from 131 TBM patients and 45 controls from Bandung, Indonesia. Next, genome-wide SNP typing was used to identify quantitative trait loci (QTLs) to link protein levels to genetic polymorphisms. Finally, QTLs were linked to patient survival in 377 TBM patients.

Results. Overall, 68 of the 74 markers included in the analyses (92 %) were significantly different between the controls and the TBM group, and the vast majority of the proteins was elevated in the patient group. Five proteins significantly predicted survival in our group, including vascular endothelial growth factor (VEGF) (Fig. 1). Furthermore, QTLs for VEGF were determined to identify a genetic foundation for the VEGF levels in the CSF of these patients. One SNP was genome-wide significantly ($p < 10_{-8}$) associated with survival (Fig. 2), which also predicted survival in TBM patients in an independent patient group.

Conclusions. Based on our systems approach, VEGF likely contributes to poor outcome of TBM, possibly because of its effects on vascular permeability, dysfunction of tight junction proteins, and brain edema. Future trial should determine if anti-VEGF (bevacizumab) as host-directed therapy can improve outcome of TBM patients.

COLD EXPOSURE AND MONOCROTALINE INDUCE THE REPRESSION OF MIR-146A-5P IN THE HEART RIGHT VENTRICLE OF RATS

JOSE L SANCHEZ-GLORIA, FAUSTO SÁNCHEZ-MUÑOZ, ROXANA CARBÓ, ADRIÁN HERNÁNDEZ, JULIO SANDOVAL, RUBIO I.

Cold exposure increases the severity of many cardiovascular and respiratory conditions. The effects of cold stress on the molecular physiopathology involved in Pulmonary hypertension has not been assessed. Thus we aimed to characterize the general effects of cold exposure and the involvement of miR-21, miR-146a and miR-155 regulation of gene expression. Four experimental groups were conducted, 24 Male Sprague Dawley weight 250 g were divided in control, Monocrotaline (60 mg/kg four weeks and Monocrotaline plus cold stress for 10 days (MCT+Cold) groups. Weigh of the heart and heart right ventricle, Fulton index RV/(LV+S) was assed and microRNA expression of mature, miR-146a, miR-21 and miR-155 was measured by RT-qPCR. Results: Heart weight normalized against total body weight showed an increase in the heart weight in the experimental groups: Control 0.0032±0.00117, MCT 0.0041± 0.00031 and MCT+Cold 0.005±0.00064 ($p<0.05$ vs control). Right ventricle weight normalized against total body weight Control 0.00075±0.000064, MCT 0.0041± 0.00031 and MCT+Cold 0.005±0.00064 ($p<0.05$ vs control) Fulton index was increased in the MCT treated groups: Control 0.35±0.017, MCT 0.62± 0.08 and MCT+Cold 0.75±0.13 ($p<0.05$ vs control). Whereas, lower expression of miR-146a-5p was found in the MCT treated groups and MCT+F: Control 1±0.08, MCT 0.33±0.05 and MCT+Cold 0.46±0.09 ($p<0.05$ vs control). Also, the MCT group showed lower levels of both miR-21 and miR-155 than the other groups Control 1±0.13, 0.99±0.15; MCT 0.48±0.04, 0.57±0.08; and MCT+Cold 1.04±0.31 and 0.92±0.24 folds respectively ($p<0.05$ vs control). In conclusión, rats exposed to 10 days of cold and with pulmonary artery hypertension induced by monocrotaline had more right ventricle weight and heart weight with lowered expression of miR-146a-5p.

A SCREENING APPROACH TO IDENTIFY EPIGENETIC COMPOUNDS THAT MODULATE MACROPHAGE ACTIVATION

ROSARIO LUQUE-MARTIN[1], J VAN DEN BOSSCHE, AE NEELE, S. VAN DER VELDEN, PALWINDER MANDER[2], MENNO P.J DE WINTHER[1]

[1]Academic Medical Centre, Amsterdam, The Netherlands
[2]GlaxoSmithKline, Stevenage, UK

Over the years it has been shown that macrophages are important immune regulators in a wide variety of diseases. In disease conditions the capacity of macrophages to develop either to a more anti-inflammatory or pro-inflammatory phenotype can improve or worsen the outcome of disease. This capacity to change phenotype is closely linked to the epigenetic landscape of macrophages. Epigenetics changes are carried out by proteins commonly divided according to their function in 3 subtypes: readers, writers or erasers. Writers and erasers are the enzymes responsible for either adding or removing the different chemical groups to histones or to the DNA producing a change in the expression of genes. The readers recognize epigenetics marks and will recruit epigenetics writers or erasers to these marks in order to add or remove them. Knowing the importance of epigenetics in macrophages under disease conditions, we screened an "epigenetic tool-box" generated by GSK that contains 16 inhibitors targeting the 3 subtypes. This screen was performed with the aim of assessing the effect of inhibitors on macrophage polarisation. To screen the effects of these compounds, monocytes isolated from peripheral blood from 4 different donors were differentiated into MCSF macrophages and then treated with the different compounds at 3 concentrations (10 μM, 1 μM, 100 nM) in combination with pro-inflammatory stimuli (LPS or LPS+IFN-γ). After 24h cytokine production (IL1β, IL-6, IL-12, TNFα and IL-10) was analysed. Five compounds consistently increased or decreased cytokine production (e.g. ESM-iHDAC showed a reduction in the response). For these selected compounds the effect on cell surface markers was investigated in response to LPS, LPS+IFN-γ, IL-4 or IL-10. The markers used for the anti-inflammatory response were CD206, CD200R and CD163 and for the pro-inflammatory CD86, CCR7, CD64 and HLA-DR, whilst CD14 and CD16 were used as macrophage markers for both. On-going studies are currently assessing the consequences of inhibiting selected epigenetic targets for macrophage activation and function in disease.

Funding. This work was funded by the Marie Sklodowska-Curie EpiMac project (Grant No. SEP-210163258)

DISTINCT LIPID MEDIATOR INFLAMMATORY PROFILES IN HIV-INFECTED AND UNINFECTED SUBJECTS

MAGDALENA SIPS[1], LAURA DE CLERCQ[1], ROMAIN A. COLAS[2], JESMOND DALLI[2], LINOS VANDEKERCKHOVE[1]

[1]Ghent University, Department of internal medicine, Ghent, Belgium
[2]Lipid Mediator Unit, William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, UK

Up to date, with no prophylactic HIV vaccine available, HIV incidence rates remain undefeated. Due to new infections and longer life expectancy of HIV-infected individuals, a number of people aging with HIV constantly increase. Despite full virological suppression, HIV-positive individuals exhibit higher rate of cardiovascular disorders and cancers, among other HIV-associated non-AIDS conditions (HANA). The occurrence of HANA is thought to result from the residual levels of immune activation and inflammation ultimately leading to immune dysfunction.

We have recently established Virological and Immunological Monitoring (VIM) platform with an aim to characterize markers of inflammation and immune activation, and assess the immune responsiveness (IR) of anti-viral effector cells with a subsequent aim to identify immunomodulators that can effectively restore dysfunctional immunity in HIV+ patients.

50 VIM samples were extensively phenotyped and plasma was used to quantify pro-inflammatory eicosanoids and the specialized pro-resolving mediators, hypothesizing defects in immune resolution pathways in HIV-infected patients. Activated NK cells and neutrophils were detected in immunological non-responders while normal immune-phenotype was observed in immunological-responders at the level of neutrophils, NK cells and cytotoxic T cells. Interestingly, we found distinctive lipid mediator profiles between HIV+ and HIV- subjects, indeed suggesting distortions in pro-inflammatory/ pro-resolution processes.

Overall, this data suggest that while multiple cellular markers of immune activation are normalized in treated HIV+ subjects, plasma concentrations of specialized pro-resolving mediators are altered, demonstrating defects in inflammation resolution pathways that may contribute to the immune dysfunction observed in HIV+ individuals.

NLRP3 INFLAMMASOME INHIBITION LIMITS MELANOMA PROGRESSION AND MYELOID-DERIVED SUPPRESSOR CELL EXPANSION

ISAK W. TENGESDAL[1], PRESTON NEFF[1], TANIA AZAM[1], MAYUMI FUJITA[2], CHARLES A DINARELLO[1,3], CARLO MARCHETTI[1]

[1]Department of Medicine, University of Colorado Denver, Aurora, CO
[2]Department of Dermatology, University of Colorado Denver, Aurora, CO
[3]Department of Medicine, Radboud University Medical Center, Nijmegen, The Netherlands

Does the NLRP3 inflammasome-derived inflammation facilitate tumor progression? We hypothesized that IL 1β and NLRP3 inflammasome activation contribute to the tumor progression by the expansion of myeloid-derived suppressor cells (MDSCs), which contribute to tumor-induced immune suppression. *In vitro*, the NLRP3 inflammasome inhibitor OLT1177 reduced IL 1β release in the human metastatic melanoma cell line 1205Lu (IC50 at 1μM), without suppressing NLRP3 or the IL 1β precursor protein level. To observe the role of OLT1177 in vivo, mice were fed with standard chow or OLT1177-enriched diet for 14 days and challenged with the mouse B16F10 melanoma cell line. Then both groups received B16F10 cells mixed in a matrigel matrix injected subcutaneously and sacrificed after 14 days. Treatment with OLT1177 reduced plasma IL 6 (-60%) and primary tumor size ($p<0.05$; Figure A) compared to the mice fed a standard diet. Analysis of bone marrow PMN-MDSCs revealed that tumor bearing mice treated with OLT1177 exhibited similar PMN-MDSCs populations to that of mice not bearing tumors ($p<0.001$; Figure B). In the spleen, the level of PMN-MDSCs in chow fed tumor-bearing mice was increased compared to mice not bearing tumors ($p<0.05$; Figure C). However, in tumor-bearing mice fed OLT1177 a normalization in the population of PMN-MDSC was observed comparable with mice not bearing tumors (Figure B-C; $p<0.05$).

These data suggest that inhibition of the NLRP3 inflammasome with OLT1177 prevents the tumor-induced expansion of MDSCs, thereby limiting immunosuppression, and restores immune mediated control of tumor growth.

GENETIC TARGETING OF HEMATOPOIETIC PROGENITORS ALLOWS TRACING OF MONOCYTE DYNAMICS AND FUNCTION IN STROKE

YVES WERNER[1], ELVIRA MASS[2,3], DAGMAR SCHÜTZ[1], KATHRIN KLEE[4], CHRISTIAN HÄNDLER[4], JOACHIM SCHULTZE[4], FREDERIC GEISSMANN[2], RALF STUMM[1]

[1]Institute of Pharmacology and Toxicology, Jena University Hospital, Jena, Germany
[2]Sloan Kettering Institute, Memorial Sloan Kettering Cancer Center, NY, USA
[3]Developmental Biology of the Innate Immune System, Life & Medical Sciences (LIMES) Institute, University of Bonn, Bonn, Germany
[4]Genomics and Immunoregulation, Life & Medical Sciences (LIMES) Institute, University of Bonn, Bonn, Germany

Monocyte-derived and tissue-resident macrophages belong to independent lineages with distinct functions. However, phenotype and morphological changes during inflammatory conditions make it difficult to assess their respective roles in pathology. In the brain, genetic fate-mapping and bone-marrow chimeras have been used to study the functions of microglia and monocytes, respectively. However, tools to permanently target bone-marrow derived monocytes independent of promoter-driven reporters and irradiation are lacking. Here, we report that inducible expression of *Cxcr4* allows specific labelling of hematopoietic stem cell-derived monocytes during inflammation. In two stroke models we found that monocytes infiltrate intact brain tissue surrounding the infarct but do not proliferate and engraft in this region. After completed repair, monocytes were enclosed in scar tissue by glial cells. Transcriptional signatures of brain monocytes and microglia after stroke reflect major differences in signaling pathways, paracrine mediators, and proliferative behavior. We further show that initial monocyte infiltration and subsequent exclusion of monocytes from the glial scar and the infarct surrounding require Cxcr4. In summary, we provide a genetic fate-mapping model to unequivocally label monocytes throughout disease progression in the brain and show that Cxcr4 signaling regulates a monocyte-depended contribution to injury and repair in stroke.

INVOLVEMENT OF *SOCS3* REGULATION IN URATE INDUCED PROINFLAMMATORY EFFECTS – ASSESSMENT OF DNA METHYLATION AT GENE BODY OF *SOCS3*

MEDEEA BADII[1], ORSOLYA GAAL[1], LOREDANA PECA[1], CRISTIAN G. ANDRA[1], RADU POPP[1], TANIA OCTAVIA CRIȘAN[1], VIOLA KLUCK[2], LEO A. B. JOOSTEN[1,2] AND HINT CONSORTIUM

[1]Department of Medical Genetics, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania
[2]Department of Internal Medicine and Radboud Instituten of Molecular Life Science (RIMLS), Radboud University Medical Center, Nijmegen, The Netherlands

Introduction. Hyperuricaemia was traditionally considered to be an inoffensive by-product in metabolic syndrome and the background necessary condition for gout. More recently, high urate levels are linked with proinflammatory regulation in mononuclear cells. Two SOCS3 intragenic CpG sites were found statistically associated to hyperuricemia in a previous study using whole genome DNA methylation analysis. CpG Islands (CGI) in promoter region of a gene are almost always unmethylated and hypermethylation of these islands is associated with the shutting down of the expression of a particular gene. On the other hand, CpGs encoded within gene bodies are mostly methylated, but their impact on gene expression has not been fully elucidated.

The SOCS3 gene codes for a protein which is responsible for the suppression of cytokine signaling, being important for the regulation of inflammation.

Material and methods. PBMCs from healthy donors were cultivated for 24 h with RPMI and different concentrations or uric acid solubilised in RPMI with 10% serum at 0.5 mg/ml and 0.1 mg/ml. DNA was isolated and treated with bisulfite sodium for conversion and we further performed methylation specific PCR with primers designed for the methylated region and the unmethylated region respectively. In parallel, the transcription rate of SOCS3 was investigated within cells treated with the following doses of uric acid: 0.5 mg/ml, 0.25 mg/ml, 0.125 mg/ml and 0.06 mg/ml. The capacity of the cells to be primed with urate was assessed using qPCR and ELISA for IL-1β and IL-1Ra.

Results. Cells pre-treated with urate showed higher levels of IL-1β and lower levels of IL-1Ra on both transcription and protein secretion. SOCS3 gene expression increased with at higher doses of uric acid Intragenic CG sites showed a basal level of methylation.

Conclusions. The observed differential SOCS3 regulation is in line with previous data and supports the hypothesis that SOCS3 could play roles in hyperuricemia induced inflammation. Several studies associate methylation at intragenic sites with increased gene transcription. However, using current methodology, we were not able to document variation in DNA methylation at the targeted sites, and bisulfite sequencing approaches are warranted for validation of these results.

Keywords: hyperuricaemia, DNA methylation, SOCS3

PLASMA PR3 LEVELS ARE ASSOCIATED WITH LIVER FAT CONTENT IN OBESE PATIENTS WITH AN INCREASED RISK TO DEVELOP NAFLD

ANDREEA-MANUELA MIREA[1], ERIK J.M. TOONEN[1,2], INGE VAN DEN MUNCKHOF[1], JOOST RUTTEN[1], MARINETTE VAN DER GRAAF[3], CEES TACK[1], TRIANTAFYLLOS CHAVAKIS[4], LEO A.B. JOOSTEN[1,5]

[1]Department of Internal Medicine, Radboud University Medical Centre, Nijmegen, The Netherlands
[2]R&D Department, Hycult Biotechnology, Uden, The Netherlands
[3]Department of Radiology and Nuclear Medicine, Radboud University Medical Center, Nijmegen, the Netherlands
[4]Institute for Clinical Chemistry and Laboratory Medicine, University Clinic Carl-Gustav-Carus, Technische Universität Dresden, Dresden, Germany
[5]Department of Medical Genetics, Iuliu Hatieganu University of Medicine and Pharmacy, 400349 Cluj-Napoca, Romania

Background. Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease worldwide. Its prevalence is increasing paralleling the increasing prevalence of obesity. In recent years, several mouse models of obesity-induced NAFLD showed that an imbalance between neutrophil serine proteases and alpha-1 antitrypsin play an important role in disease pathogenesis. The aim of this study was to establish the role of neutrophil serine proteases proteinase-3 (PR3) and neutrophil elastase (NE) in NAFLD in obese patients.

Material and methods. We recruited a cohort of 302 obese individuals. The amount of liver fat was quantified by Magnetic Resonance Spectroscopy (MRS). A liver fat content over 5.6% was considered diagnostic of NAFLD. Plasma levels of proteinase 3 (PR3), neutrophil elastase (NE), alpha-1 antitrypsin (AAT), several cytokines, adipokines and lipids were measured in all subjects by ELISA or Ella technology.

Results. After excluding subjects with significant alcohol consumption, 159 out of 302 subjects fulfilled the EASL-ASD-EASO diagnosis criteria for NAFLD. PR3 plasma levels were significantly higher in the liver steatosis group compared to the control group (60.68 ng/ml vs 45.89 ng/ml, $p<0.0001$). No difference between the groups were observed for NE and AAT plasma levels. Additionally, PR3 was correlated significantly with several markers and risk factors for NAFLD: liver fat percentage, neutrophil to lymphocyte ratio, hsCRP, uric acid, IL-1β, IL-6 and HbA1c.

Conclusion. Proteinase-3 is associated with the pathogenesis of NAFLD in our cohort. We suggest that inhibition of PR3 could have therapeutic benefits for patients with NAFLD.

INHIBITION OF IL-1β ACTIVATION PATHWAYS PREVENTS DEVELOPMENT OF NAFLD

ANDREEA-MANUELA MIREA[1], ERIK TOONEN[2], KANNEGANTI THIRUMALA-DEVI[3], CEES TACK[1], TRIANTAFYLLOS CHAVAKIS[4], RINKE STIENSTRA[1,5], JOOSTEN LEO[1,6]

[1]Department of Internal Medicine, Radboud University Medical Center, Nijmegen, The Netherlands
[2]R&D Department, Hycult Biotechnology, Uden, The Netherlands
[3]Department of Immunology, St Jude Children's Research Hospital , Memphis, Tennessee 38105, USA
[4]Institute for Clinical Chemistry and Laboratory Medicine, University Clinic Carl-Gustav-Carus, Technische Universität Dresden, Dresden, Germany
[5]Nutrition, Metabolism and Genomics Group, Division of Human Nutrition, Wageningen University, Wageningen, the Netherlands
[6]Department of Medical Genetics, Iuliu Hatieganu University of Medicine and Pharmacy, 400349 Cluj-Napoca, Romania

Non-alcoholic fatty liver is becoming a public health problem worldwide. Although the disease is frequent not much is known about the underlying mechanisms of development and treatment is not available yet. Inflammation plays an important role in the disease pathogenesis and activation of pro-inflammatory cytokines such as IL-1β is known to promote disease progression. In recent years there is evidence that IL-1β activation pathways such as caspase-1 and the neutrophil serine proteases neutrophil elasatse and proteinase 3 play an important role in promoting NAFLD as well.

In this study we propose to investigate the therapeutic potential of inhibiting IL-1β activation pathways in obesity – induced NAFLD. To do so, we generated a quadruple knock out mouse for caspase-1/caspase-11/neutrophil elastase/proteinase 3 and investigated the development of obesity induced NAFLD after a high fat diet intervention.

Our results show that the caspase-1/caspase-11/neutrophil elastase/proteinase 3 mice were protected from developing diet-induced obesity and liver steatosis when compared to wild type controls on the same diet. The quadruple knockout mice had less plasma lipid content, didn't developed liver steatosis and had less inflammatory markers in liver and adipose tissue when compared to wild type controls.

We conclude that targeting IL-1β activation pathways has a protective effect against development of obesity-induced NAFLD.

LIPIN-2 REGULATES SATURATED FATTY ACID-INDUCED NLRP3 INFLAMMASOME ACTIVATION

I. SANJUÁN-GARCÍA[1,2], J. BALSINDE[1,2], M.A. BALBOA[1,2]

[1]Institute of Molecular Biology and Genetics (IBGM), Spanish National Research Council (CSIC), University of Valladolid, 47003 Valladolid, Spain
[2]Biomedical Research Networking Center in Diabetes and Associated Metabolic Disorders (CIBERDEM), 28029 Madrid, Spain

Objectives. Lipin-2 is a phosphatidic acid phosphatase involved in *de novo* lipid biosynthesis. Mutations in *LPIN2* produce in humans an inflammatory-based disorder known as Majeed syndrome, whose clinical manifestations can be improved with IL-1β blocking strategies. IL-1β is a pro-inflammatory cytokine produced by the activation of the cytosolic multiprotein complex NLRP3 inflammasome. It has been reported that saturated fatty-acids (SFAs) such as palmitic acid (PA) can activate the NLRP3 inflammasome; however, the underlying mechanism remains obscure. In the present work, we have investigated the role of lipin-2 in SFAs-induced NLRP3 inflammasome activation in murine and human macrophages.

Methods. Mice and human macrophages were transfected with specific siRNAs for *Lpin2/LPIN2* and *Ern1* (Ire1a) silencing. We also perform experiments with WT and Lpin2-/- iBMDM and primary bone marrow-derived macrophages from WT, *Lpin2-/-*, *Nlrp3-/-*, *Asc-/-* and *Casp1/11-/-* mice. Inflammasome activation was examined by caspase-1 activation with FAM-FLICA staining and IL-1β release by ELISA.

Results. Here we show that lipin-2 can regulate the inflammasome activation at two levels. First, depletion of lipin-2 promotes an increased expression of *Il1b*, as well as inflammasome components, which relies on overstimulated NF-κB and MAPKs pathways. In addition, lipin-2 controls key events during inflammasome activation, such as caspase-1 activation and IL-1β processing and release. Macrophages lacking lipin-2 present a stronger endoplasmic reticulum (ER) stress in response to SFAs, which triggers the excessive IL-1β production in an Ire1α-dependent manner. The overloading of SFAs in the absence of lipin-2 may modify lipids in the ER membrane leading to a stronger activation of the Ire1α sensor.

Conclusions. Our studies demonstrate a protective role for lipin-2 in SFAs-induced NLRP3 inflammasome activation, and may open new avenues to control low-grade chronic inflammation produced by excess of SFAs in obesity and other metabolic disorders.

THE FUNCTIONAL GENOMIC ARCHITECTURE OF TRAINED IMMUNITY AND INNATE IMMUNE TOLERANCE

SIMONE J.C.F.M. MOORLAG[1,*], YANG LI[2,*], MARIJE OOSTING[1], RUTGER JAN RÖRING[1], VERA P. MOURITS[1], SANNE P. SMEEKENS[1], RAÚL AGUIRRE GAMBOA[2], VASILIKI MATZARAKI[2], NIELS P. RIKSEN[1], REINOUT VAN CREVEL[1], RAMNIK J. XAVIER[3], CISCA WIJMENGA[2,4], VINOD KUMAR[2], LEO A.B. JOOSTEN[1], MIHAI G. NETEA[1,5]

[1]Radboud University Medical Center, Nijmegen, The Netherlands
[2]University Medical Center Groningen, Groningen, The Netherlands
[3]The Broad Institute of MIT and Harvard, Cambridge, MA, USA
[4]K.G. Jebsen Coeliac Disease Research Centre, Oslo, Norway
[5]Life and Medical Sciences Institute (LIMES), Bonn, Germany
***equal contribution**

The innate immune system undergoes functional adaptation after infections or vaccinations, a *de facto* innate immune memory also called *trained immunity*. In a cohort of 113 healthy volunteers, we demonstrate that induction of trained immunity and innate immune tolerance in monocytes are different aspects of the same immunological process, but with a different genetic regulation compared to primary cytokine production. In a genome-wide association study of genetic polymorphisms influencing trained immunity, we identify key signaling pathways including mTOR-induced glycolysis and the GM-CSF pathway. In addition, genetic variation in epigenetic regulators had a strong impact on trained immunity and tolerance, and a crucial role for the histone demethylase KDM4 was found. Intersection of the trained immunity/tolerance QTLs (ttQTLs) with disease-associated SNPs revealed that ttQTLs are highly enriched for SNPs associated with infections, cancer and metabolic disorders. These data reveal the genetic architecture of trained immunity and tolerance and its role in human disease.

THE ROLE OF TOLL-LIKE RECEPTOR 10 IN TRAINED IMMUNITY

MOURITS VP[1], OOSTING M[1], NOVAKOVIC B[2], STUNNENBERG HG[2], JOOSTEN LAB[1], NETEA MG[1,3]

[1]Department of Internal Medicine and Radboud Center for Infectious Diseases, Radboud University Medical Center, Nijmegen, The Netherlands
[2]Department of Molecular Biology, Radboud University, Nijmegen, The Netherlands
[3]Department for Genomics & Immunoregulation, Life and Medical Sciences Institute (LIMES), University of Bonn, Bonn, Germany

Introduction. Toll-like receptor 10 (TLR10) is a Toll-like receptor family member with unique anti-inflammatory properties. Although the ligand, signaling pathway and biological function of TLR10 is not clear yet, it has been shown that TLR10 is able to inhibit production of pro-inflammatory cytokines and induces IL-1 Receptor antagonist (IL-1Ra) production. This modulation of the innate immune system could possibly also play a role in trained immunity, the process in which human innate immune cells can undergo extensive metabolic and epigenetic reprogramming upon certain vaccinations of infections, resulting in an enhanced immune response upon heterologous re-infection. Inappropriate activation of trained immunity can result in maladaptive immune responses. By modulating specific mechanisms of trained immunity, maladaptive innate immune responses could possibly be restored.

Objective. The goal of this study is to investigate whether TLR10 plays a role in trained immunity.

Methods. Peripheral blood mononuclear cells (PBMCs) were co-cultured with anti-TLR10 antibody and TLR2 ligands Pam3Cys and *Borrelia Burgdorferi*, or anti-TLR10 antibody was crosslinked to PBMCs. Cytokine levels in supernatant were quantified by ELISA after 24 hours. Trained immunity was induced in monocytes from healthy individuals with or without anti-TLR10 antibody, and restimulated after 6 days with Pam3Cys. TLR10 expression was determined by FACS and RNA sequencing.

Results. As shown before, we observed higher cytokine levels of IL-6, IL-1β and TNFα when PBMCs were co-cultured with anti-TLR10 antibody. Upon crosslinking of anti-TLR10 antibody with PBMCs, an increased level of IL-1Ra was produced. During β-glucan-induced trained immunity *in vitro* we observed low mRNA expression levels of TLR10 which did not alter. TLR10 protein levels were also comparable before and after inducing trained immunity *in vitro*. When monocytes were incubated with anti-TLR10 antibody the β-glucan-induced trained immunity response was slightly reduced, as shown by decreased IL-6 and TNFα production. Upon β-glucan-induced training in monocytes of healthy individuals homozygous for missense TLR10 single-nucleotide polymorphism (SNP) N241H (rs11096957), a slight decrease in IL-6 production and increase in IL-1Ra production compared to wild type individuals is seen.

Conclusion. These results suggest that TLR10 does not play an important role during trained immunity.

STAT1 GAIN-OF-FUNCTION COMPROMISES SKIN HOST DEFENSE IN THE CONTEXT OF INTERFERON-γ SIGNALING

HANNA NIEHUES, BERENICE RÖSLER, DANIQUE A. VAN DER KRIEKEN, IVONNE M.J.J. VAN VLIJMEN-WILLEMS, DIANA RODIJK-OLTHUIS, MALOU PEPPELMAN, JOOST SCHALKWIJK, ELLEN H.J. VAN DEN BOGAARD, PATRICK L.J.M. ZEEUWEN, FRANK L. VAN DE VEERDONK

Background. Defective mucosal and skin host defense mechanisms are the hallmarks of the primary immunodeficiency chronic mucocutaneous candidiasis (CMC). We previously reported that heterozygous mutations in the signal transducer and activator of transcription 1 (*STAT1*) gene are responsible for autosomal dominant CMC. Moreover, we demonstrated that gain-of-function (GOF) mutations of STAT1 lead to its hyperphosphorylation and subsequent impairment of Th17 responses, finally resulting in a severe mucocutaneous *Candida albicans* infection. Although CMC manifests itself at the level of epithelia (skin and oral mucosa), research has so far been limited to the study of immune cells.

Objective. Using genetically defined epidermal cells, either wild type or carrying STAT1 GOF mutations, we investigated their response to proinflammatory cytokines, with respect to skin barrier and host defense gene expression

Methods. We generated 3D epidermal equivalents from keratinocytes of healthy controls and CMC patients (STAT1 GOF), and stimulated these with IL-17, IL-22 or IFNγ. The cellular responses were evaluated by immunohistochemistry.

Results. Stimulation by IFNγ, but not by Th17 cytokines, caused abnormal epidermal morphology and a strongly reduced expression of Late Cornified Envelope 3 (LCE3) proteins. We found that, in addition to their known antibacterial activity, LCE3 proteins had antifungal activity against *Candida albicans*.

Conclusions. This study demonstrates that epithelia of patients with a STAT1 GOF mutation have a functional defect that becomes apparent when immune cell-derived IFNγ is present. This results in structural abnormality of the epidermis and compromises the innate anti-*Candida* activity of the tissue.

INNATE IMMUNE ACTIVATION IS ASSOCIATED WITH PROGRESSION OF CEREBRAL SMALL VESSEL DISEASE: AN EXPLORATORY STUDY

MARLIES P. NOZ[1], ANNEMIEKE TER TELGTE[2], KIM WIEGERTJES[2], LEO A. B. JOOSTEN[1], MIHAI G. NETEA[1,3], FRANK-ERIK DE LEEUW[2], NIELS P. RIKSEN[1],*

[1]Department of Internal Medicine, Radboud UMC, Nijmegen, the Netherlands
[2]Department of Neurology, Radboud UMC, Donders Institute for Brain, Cognition and Behaviour, Center for Medical Neuroscience, Nijmegen, the Netherlands
[3]Department for Genomics & Immunoregulation, Life and Medical Sciences Institute (LIMES), University of Bonn, Bonn, Germany
***shared senior authorship**
*Corresponding Author: *e-mail: marlies.noz@radboudumc.nl*

Aim. Cerebral small vessel disease (cSVD) is the major vascular cause of cognitive decline and dementia. cSVD is characterized by white matter hyperintensities (WMH), lacunes and microbleeds on MR imaging. The pathogenesis of cSVD however, remains largely unknown. While several studies implicate systemic inflammation, detailed examination of the innate immune system has not yet been performed. This study aims to explore the role of the innate immune system in the progression of cSVD.

Methods. Individuals with moderate to severe cSVD, diagnosed on previous collected MRI data in 2006 and 2015, participated in the study. Subjects with large vessel (cerebro)vascular disease, dementia or Parkinson's disease were excluded. Progression of cSVD was determined by the change in WMH volume (mL) between 2006-2015 (ΔWMH). Peripheral blood mononuclear cells and monocytes were isolated and cytokine production was assessed after in vitro stimulation. Additionally, monocyte subsets were identified by flow cytometry.

Results. Fifty-one subjects (70±6years, 60% men, 5±6.4ΔWMH) were included. Circulating high sensitivity interleukin 6 (hsIL-6) strongly correlated with baseline and ΔWMH (p=0.005, CC:0.40). The percentage of 'CD14++CD16+' intermediate monocytes was associated with baseline WMH and ΔWMH (p=0.018, CC:0.34. Moreover, subgroup analysis detected a correlation of IL-6 production after Pam3CysK4 stimulation with baseline and ΔWMH (p=0.008, CC:0.44).

Conclusion. The progression of cSVD is correlated with inflammatory characteristics of innate immune cells. Most interestingly, and comparable to patients with atherosclerosis, intermediate monocytes correlate with disease severity. These novel findings suggest that innate immune activation drives the development and progression of cSVD, which offers novel opportunities for future prognostic and therapeutic strategies.

VDR GENE POLYMORPHISMS IN GOUT PATIENTS COMPARED TO HYPERURICEMIC CONTROLS

ORSOLYA GAAL[1,*], CRISTIAN G. ANDRA[1,*], ALEXANDRU C. BOLUNDUȚ[1], MEDEEA BADII[1], TANIA O. CRIȘAN[1], MARIUS F. FARCAȘ[1], IOANA HOTEA[2], CRISTINA PAMFIL[2], SIMONA REDNIC[2], RADU A. POPP[1], LEO A.B. JOOSTEN[1,3] AND HINT CONSORTIUM

[1]Department of Medical Genetics, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania
[2]Department of Rheumatology, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania
[3]Department of Internal Medicine and Radboud Institute of Molecular Life Science (RIMLS), Radboud University Medical Center, Nijmegen, The Netherlands
***These authors contributed equally to the research**

Introduction. Gout is an important inflammatory disease with high prevalence in developed countries. The disease is triggered by the presence of monosodium urate (MSU) crystals in the synovium. Hyperuricemia is a vital precondition for the deposition of MSU crystals and genetic loci associated to gout so far have been mapped to urate transporter genes. We hypothesize that gene variations that induce inflammation could also contribute to risk of gout. The present study aims to determine if two common variations present in the vitamin D receptor gene (VDR) have different distributions in patients with gout in comparison to patients with hyperuricemia.

Materials and methods. Samples from gout patients originating from Romania were compared with a hyperuricemic control (HU) group of same ancestry. 114 HU controls and 143 gout patients were analyzed by Taqman Assay genotyping and Polymerase Chain Reaction - Restriction Fragment Length Polymorphism technique (RFLP) for the following SNPs: rs2228570 and rs4516035. Data analysis was carried out using the dominant or recessive risk models for the obtained genotypes.

Results. The variant allele frequency for rs2228570 was 0.36 in gout patients and 0.4 in HU controls, OR= 0.832 (95% CI 0.58-1.192), p = 0.31. For rs4516035 the variant allele frequency was 0.39 in the gout group vs. 0.52 in the HU controls, OR = 0.595 (95% CI; 0.351-1.012), p = 0.054. The dominant and recessive models of risk association showed similar results.

Conclusion. In the given population the distribution of the alleles and genotypes showed a similar pattern between the two study groups. The analyzed data shows that the two polymorphisms in the *VDR* gene are not associated to gout susceptibility or disease severity. However, a trend for the protective association of the promoter polymorphism with gout was observed, and further studies in larger study grops are needed in order to draw more relevant conclusions for the general population.

Keywords: VDR, gout, hyperuricemia, polymorphism

EXPRESSION OF THE CELLULAR ENERGY SENSOR LKB1 IS IMPORTANT FOR THE LOCAL HOST DEFENSE DURING GRAM-NEGATIVE PNEUMONIA

N.A. OTTO[1], A.F. DE VOS[1], J.W.J. VAN HEIJST[2] AND T. VAN DER POLL[1]

[1]Center of Experimental and Molecular Medicine (CEMM), Academic Medical Center (AMC), University of Amsterdam (UvA), Amsterdam, the Netherlands
[2]Experimental Immunology (EXIM), Academic Medical Center (AMC), University of Amsterdam (UvA), Amsterdam, the Netherlands

LKB1 (Liver Kinase B1; also known as Serine/Threonine Kinase 11 - STK11) is a member of the serine/threonine kinase family and is the primary upstream activator of adenosine monophosphate-activated protein kinase (AMPK), the key regulator of cellular metabolic homeostasis. LKB1 activates AMPK during nutrient deprivation or hypoxia, when intracellular ATP declines and AMP increases. When energy levels are low, AMPK acutely stimulates glucose uptake and glycolysis to recover the energy level in the cell. However it also activates long-term adaptations to transit to a more oxidative approach of ATP generation in the mitochondria instead of the glycolytic approach, mainly through mitochondrial biogenesis. In recent years, it has become evident that cellular metabolism plays an important role in immune cell function. We aimed to study the role of the LKB1-AMPK pathway in myeloid cells (macrophages and neutrophils) in the host response during bacterial pneumonia and sepsis. To this end, we crossed mice in which the Stk11 gene is flanked by loxP sites ($Stk11^{fl/fl}$ mice) with mice expressing Cre recombinase under a promoter with myeloid cell-restricted expression (LysM-Cre mice) to generate mice lacking LKB1 in myeloid cells. LysM-Cre x $Stk11^{fl/fl}$ mice and $Stk11^{fl/fl}$ controls were infected with the common human gram-negative pathogen Klebsiella pneumoniae via the airways to induce a pneumonia (6-12 hours post infection) and pneumosepsis (12-40 hours post infection). Mice were euthanized at predefined time points (12 and 40 hours after infection) for measurement of bacterial burdens and inflammatory responses. LysM-Cre x $Stk11^{fl/fl}$ mice showed higher bacterial loads in the lung during pneumonia and pneumosepsis, while the bacterial loads in distant organs were unaffected. In lung, the production of inflammatory cytokines such as TNF, IL-6 and IL-1β were increased corresponding to the bacterial loads. Our results indicate that myeloid cell LKB1 is important for local host defense during *Klebsiella pneumoniae* induced pneumonia while LKB1 deficiency does not impact the ability to produce proinflammatory cytokines.

HIF1α IN MACROPHAGES, BUT NOT NEUTROPHILS, IS IMPORTANT FOR THE HOST DEFENSE DURING *KLEBSIELLA PNEUMONIAE*-INDUCED PNEUMOSEPSIS

N.A. OTTO[1], A.F. DE VOS[1], J.W.J. VAN HEIJST[2] AND T. VAN DER POLL[1]

[1]Center of Experimental and Molecular Medicine (CEMM), Academic Medical Center (AMC), University of Amsterdam (UvA), Amsterdam, the Netherlands
[2]Experimental Immunology (EXIM), Academic Medical Center (AMC), University of Amsterdam (UvA), Amsterdam, the Netherlands

HIF1α is a ubiquitous, constitutively synthesized transcription factor known for upregulating the expression of genes involved in the cellular response to hypoxia. These gene products include proteins such as GLUT1, PGK1, ENO1, LDH and other glycolytic enzymes. In normoxia, HIF1α is rapidly degraded via the ubiquitin-proteasome pathway. Recently, it has been reported that HIF1α expression can be upregulated in a NFκB dependent manner, suggesting a role for HIF1α during the activation of immune cells. Indeed, in primary human monocytes (Mo) we found an increase in HIF1α expression when stimulated with the TLR4-ligand lipopolysaccharide (LPS). We aimed to study the role of HIF1α in myeloid cells (macrophages and neutrophils) in the host response during bacterial pneumonia and sepsis. To this end, we crossed mice in which the HIF1α gene is flanked by loxP sites (HIF1αfl/fl mice) with mice expressing Cre recombinase under a promoter with myeloid cell-restricted expression (LysM-Cre mice) and a promoter with neutrophil-restricted expression (MRP8-cre) to generate mice lacking HIF1α in myeloid cells or neutrophils only. We inoculate these mice and CRE-negative littermate controls intranasally with the common human gram-negative pathogen *Klebsiella pneumoniae* to induce a pneumonia (12 hours post infection) and pneumosepsis (40 hours post infection). During the early phase of infection, no differences were found between LysM-cre x HIF1α$^{fl/fl}$ and littermate controls, however in the late phase (40 hours post infection) the LysM-cre x HIF1α$^{fl/fl}$ mice showed higher bacterial loads in lung and in distant organs such as blood, liver and spleen. Mice lacking HIF1α in neutrophils only (MRP8-cre x HIF1α$^{fl/fl}$) did not show any differences in bacterial loads. Our results indicate that macrophage HIF1α is important for local host defense during *Klebsiella pneumoniae* induced pneumosepsis.

IDENTIFICATION OF THE UNIQUE FUNCTIONAL PHENOTYPE OF IMATE-DEFINING MONOCYTES THAT DRIVE HEPATIC T CELL PROLIFERATION

K PAWELKA[1], M. TUEFFERD[2], M HEIKENWÄLDER[1,3], J. AERSSENS[2], J. BOLLEKENS[2], B HÖCHST[1], P KNOLLE[1]

[1]Institute of Molecular Immunology, Klinikum rechts der Isar, München
[2]Janssen Pharmaceuticals, Beerse, Belgium
[3]DKFZ, – Chronic inflammation and cancer, Heidelberg

Intrahepatic myeloid-cell aggregates form in response to Toll-like receptor 9 (TLR9) signaling in a TNF-dependent fashion to provide a unique anatomic structure that drives local proliferation of cytotoxic CD8 T cells (iMATEs) and confers protection against viral infection. Yet, the identity of the iMATE-defining myeloid cell population remained elusive.

We systematically analyzed the phenotype of myeloid cells in the murine liver after TLR9 activation using a set of different methodologies. Initial flow cytometric phenotypic characterization and tSNE analysis revealed a complex composition of monocytes and newly differentiating macrophages that hinted towards a sequential replacement of liver-resident macrophages followed by repopulation through bone marrow derived inflammatory monocytes. We were able to put together a set of marker proteins that enable us to define a particular phenotype of monocyte-derived macrophages that is exclusively found in iMATEs but not elsewhere in the organism.

Functional assays of these iMATE-defining monocyte-derived macrophages revealed that these cells are highly potent in the induction of CD8 T cell proliferation, in the differentiation towards GzmB expression rendering them efficient killer cells and in cross-presenting soluble antigens to CD8 T cells resulting in even stronger proliferation. Liver macrophages, in contrast, failed to provide any support for T cell proliferation and did not show significant cross-presentation capacity. Furthermore, we attribute a metabolic phenotype characterized by potent glycolysis to iMATE-defining monocyte-derived macrophages.

The transient presence of iMATE-defining monocyte-derived macrophages in the liver indicates that protective hepatic T cell immunity is determined by the dynamics of the changes in inhibitory vs stimulatory macrophage populations, that do not fall into the conventional M1/M2 categories but are related to iMATE formation.

INFLUENCE OF THE CAUSATIVE PATHOGEN ON PRESENTATION, OUTCOME AND HOST RESPONSE OF SEVERE COMMUNITY-ACQUIRED PNEUMONIA

ELIZABETH PEREVERZEVA[1], FABRICE UHEL[1], OLAF L. CREMER[2], MARC J. BONTEN[3,4], JANNEKE HORN[5], MARCUS J. SCHULTZ[5,6], TOM VAN DER POLL[1,7]

[1]Center for Experimental and Molecular Medicine, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands
[2]Department of Intensive Care Medicine, University Medical Center Utrecht, Utrecht, the Netherlands
[3]Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, The Netherlands
[4]Department of Medical Microbiology, University Medical Center Utrecht, Utrecht, The Netherlands
[5]Department of Intensive Care, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands
[6]Mahidol Oxford Tropical Medicine Research Unit, Bangkok, Thailand
[7]Division of Infectious Diseases, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

Background. Severe community-acquired pneumonia (CAP) is the most common cause of septic shock and frequently requires admission to the intensive care unit (ICU). The objective of this study was to determine whether the clinical presentation, outcome and host immune response during severe CAP depend on the causative pathogen.

Methods. A prospective cohort study was conducted in the ICU of two tertiary hospitals. We evaluated patients with severe CAP caused by one of the six most common pathogens (*Streptococcus pneumoniae* (n=66), *Staphylococcus aureus* (n=34), *Haemophilus influenzae* (n=27), Influenza virus (n=24), *Escherichia coli* (n=21) and *Pseudomonas aeruginosa* (n=21)). Immune responses were determined at ICU admission (day 0), day 2 and 4 by measuring 24 plasma biomarkers reflecting systemic inflammatory and cytokine responses, and activation of the vascular endothelium.

Results. Demographics, comorbidities and severity of disease were not significantly different between CAP patients according to causative pathogens. Patients with CAP due to *E. coli* had a shorter length of stay and a higher in-hospital and 30-days mortality rate when compared with CAP patients with other causative pathogens. Most host response parameters were similar between groups. The plasma levels of the pro-inflammatory cytokine Granulocyte-Macrophage Colony-Stimulating Factor and the Angiopoietin-2/Angiopoietin-1 ratio (indicative for microvascular permeability) were increased in CAP caused by *E. coli*. Plasma levels of neutrophil activation marker matrix metalloproteinase-8 were raised in patients with pneumococcal CAP on admission as well as on day 2 and 4.

Conclusion. The presentation, outcome and host response of patients with severe CAP is largely independent of the causative pathogen.

THE EFFECT OF HUMAN ADIPOSE-DERIVED MESENCHYMAL STEM CELLS ON THE HOST RESPONSE TO INTRAVENOUS LIPOPOLYSACCHARIDE IN HUMANS

DESIREE PERLEE[1], LONNEKE A. VAN VUGHT[1], BRENDON P. SCICLUNA[1,2], ANJA MAAG[1], RENÉ LUTTER[3], ELLES M. KEMPER[4], CORNELIS VAN 'T VEER[1], MARIE A. PUNCHARD[6], JESÚS GONZÁLEZ[6], MARIE PAULE RICHARD[6], WILFRIED DALEMANS[7], ELEUTERIO LOMBARDO[6], ALEX F. DE VOS[1], TOM VAN DER POLL[1,5]

Academic Medical Center, University of Amsterdam
[1]Center of Experimental & Molecular Medicine
[2]Department of Clinical Epidemiology and Biostatistics
[3]Department of Experimental Immunology & Respiratory Medicine
[4]Department of Pharmacy
[5]Division of Infectious Diseases
[6]TiGenix SAU, Madrid, Spain
[7]TiGenix NV, Leuven, Belgium

Introduction. Adult mesenchymal stem cells (MSCs) can modulate various immune responses implicated in the pathogenesis of sepsis. Intravenous injection of lipopolysaccharide (LPS) into healthy subjects is a well-characterized model of human inflammation with relevance for the host response to sepsis.

Objective. To determine the effect of MSC infusion on the early inflammatory response to intravenous LPS in humans.

Methods. A phase I, randomized, single-blind, placebo controlled study was performed in 32 healthy subjects with 4 treatment arms (n=8 per group): placebo or adipocyte-derived MSCs intravenously at either 250.000, 1 million or 4 million cells/kg; all subjects received LPS intravenously (2 ng/kg) one hour after the end of MSC infusion. Endpoints included safety, vital signs and symptoms and laboratory measurements providing insight in innate immune responses.

Results. MSC infusion was not associated with serious adverse events. LPS induced fever, leukocytosis, release of multiple cytokines and chemokines, and activation of the vascular endothelium and the coagulation system. Most of these LPS effects were not influenced by MSC infusion. However, MSCs given at the highest dose increased the febrile response and exerted mixed pro-inflammatory (enhanced IL-8 release) and anti-inflammatory effects (increased IL-10 and TFG-β release) on activation of the cytokine network. In addition, the highest MSC dose enhanced coagulation activation (elevated plasma levels of thrombin-antithrombin complexes and D-dimer) and inhibited the fibrinolytic response (lower tissue-type plasminogen activator levels). Whole blood genome-wide transcriptome analyses showed that MSCs had a time-dependent effect on the LPS response: at 2 hours post LPS MSC infused subjects displayed higher expression of genes involved in various innate immune pathways (including NFκB signaling), whereas at 4 hours post LPS these subjects had lower expression of innate immune pathway genes (again including NFκB signaling). Infusion of MSCs did not modify the "ex vivo" responsiveness of whole blood to various bacterial agonists (including LPS), either before or after in vivo LPS injection.

Conclusion. Intravenous infusion of adipocyte-derived MSCs at a high dose (4 million cells/kg) has a variety of proinflammatory, anti-inflammatory and procoagulant effects during human endotoxemia.

HUMAN ADIPOCYTE-DERIVED MESENCHYMAL STEM CELLS AS TREATMENT IN GRAM-NEGATIVE PNEUMOSEPSIS IN MICE

DESIREE PERLEE[1], ALEX F. DE VOS[1], PABLO MANCHEÑO[3], ELEUTERIO LOMBARDO[3], TOM VAN DER POLL[1,2]

Academic Medical Center, University of Amsterdam
[1]Center of Experimental & Molecular Medicine
[2]Division of Infectious Diseases
[3]TiGenix SAU, Madrid, Spain

Introduction. *Klebsiella (K.) pneumoniae* is a common cause of gram-negative pneumonia and sepsis. Adult mesenchymal stem cells (MSCs) have immunomodulatory and antibacterial effects that might improve the host response during severe infections.

Objective. To study the effect of human adipocyte-derived MSC infusion on the host response during pneumosepsis caused by *K. pneumoniae* in mice.

Methods. C57BL/6 mice were infected with *K. pneumoniae* (104 colony forming units) via the airways. One or 6 hours (h) after infection, mice were infused intravenously with human MSCs (1 x 106 cells) or Ringers lactate (placebo), and euthanized after 16h (MSC infusion at +1h) or 48h (MSC infusion at +6h). The effects of freshly cultured of cryopreserved MSCs were compared, the latter formulation being more clinically relevant. In separate experiments postponed treatment with cryopreserved MSCs starting at 30h post-infection in combination with ceftriaxone (20 mg/kg) was evaluated (study endpoints at 72 or 96h).

Results. Intravenously administered MSCs were visualized in lung tissue by immunostaining at 1 and 3h, but not at 16h after infusion. While early after infection (16h) MSCs did not influence bacterial loads, infusion of freshly cultured or cryopreserved MSCs was associated with reduced bacterial burdens in lungs and distant organs at 48h. In addition, both freshly cultured and cryopreserved MSCs reduced the lung levels of proinflammatory cytokines (TNF-α, IL-1β and IL-6) and the chemokine MIP-2 at 16h and 48h, as well as pulmonary endothelial cell activation (E-selectin) at 48h after infection. These anti-inflammatory effects of MSC infusion were accompanied by transient formation of micro-thrombi in lung tissue (at 16h). Cryopreserved MSCs attenuated lung pathology at 48h, but did not influence survival. MSC infusion had no effect on distant organ injury. Postponed MSC infusion together with ceftriaxone did not influence bacterial loads or lung inflammation.

Conclusion. Infusion of freshly cultured or cryopreserved adipocyte-derived MSCs similarly reduces bacterial burdens and lung inflammation during *Klebsiella* induced pneumosepsis.

IS THERE A ROLE FOR HMGB1 IN THE CONTEXT OF TRAINED IMMUNITY OR TOLERANCE?

J.H. VAN PUFFELEN[1,2], L.S. HELDER[2], L.A.B. JOOSTEN[2], S.H.H.M VERMEULEN[1]

[1]Department for Health Evidence, Radboud university medical center and Radboud Institute for Health Sciences (RIHS), Nijmegen, The Netherlands
[2]Department of Internal Medicine, Radboud university medical center and Radboud Institute for Molecular Life Sciences (RIMLS), Nijmegen, The Netherlands

Background. Recently it was shown that the chromatin-binding protein high-mobility group nucleosome-binding protein 1 (HMGN1) is capable of inducing tolerance, similar to LPS-induced tolerance (Arts, Huang et al. 2018). Another similar chromatin-binding protein high mobility group box 1 (HMGB1) was shown to increase LPS-induced tolerance (Aneja, Tsung et al. 2008). HMGB1 may therefore be able to deteriorate the trained immunity phenotype induced by training agents such as BCG or β-glucan.

Purpose. The aim of this study is to assess whether simultaneous stimulation of monocytes with HMGB1 and BCG can decrease the induction of trained immunity phenotype induced by BCG or even induce tolerance. A second goal is to determine whether BCG- or β-glucan-trained macrophages produce more cytokines upon restimulation with HMGB1 compared to their non-trained counterparts.

Methods. Adherent monocytes from 9 donors were stimulated for 24 hours with HMGB1, BCG, β-glucan or a combination. After 24 hours the stimulus was removed and the adherent monocytes were cultured for 6 days. At day 6, the monocytes were stimulated for 24 hours with LPS, P3C or HMGB1. Subsequently, supernatants were collected and cytokine concentrations were measured using ELISA.

Results. HMGB1 exposure does not induce a tolerant phenotype in adherent monocytes, nor does it influence the trained phenotype induced by BCG or β-glucan. This was represented by no significant differences in cytokine production between trained macrophages and RPMI control macrophages. Using HMGB1 for the induction of a secondary immune response after 6 days of cell culture did not evoke a cytokine response in BCG or β-glucan trained macrophages.

Discussion. Our finding that HMGB1 does not induce tolerance, or influences trained immunity, does not match the finding that another chromatin-binding protein, HMGN1, is capable of inducing tolerance (Arts, Huang et al. 2018).

UNSATURATED FATTY ACID SYNTHESIS IS A METABOLIC FEATURE OF THYROID CANCER-ASSOCIATED MACROPHAGES

KATRIN RABOLD[1,2], YVETTE SLOOT[3], CHRISTOPH THIELE[4], JOACHIM L. SCHULTZE[4], JAN W.A. SMIT[3], GOSSE J. ADEMA[2], MIHAI G. NETEA[1,4], ROMANA T. NETEA-MAIER[3]

[1]Department of Internal Medicine, Radboud University Medical Center, Nijmegen, The Netherlands
[2]Radiotherapy and OncoImmunology Laboratory, Department of Radiation Oncology, Radboud University Medical Center, Nijmegen, The Netherlands
[3]Department of Internal Medicine, Division of Endocrinology, Radboud University Medical Center, Nijmegen, The Netherlands
[4]Department for Genomics & Immunoregulation, Life and Medical Sciences Institute (LIMES), University of Bonn, Germany

Introduction. Tumor associated macrophages (TAMs) are the most abundant innate immune cells in non-medullary thyroid carcinoma (TC) and have been associated with poor prognosis. An important feature of macrophages is their high plasticity, which enables them to adapt to environmental changes by adjusting their cellular metabolism and immunological phenotype. Therapeutic approaches to reprogram pro-tumoral TAMs towards an anti-tumoral phenotype by targeting cell metabolism may represent an important therapeutic target, yet little is known about the metabolic reprogramming in TC-associated macrophages.

Aim. To study the metabolic and functional changes in TC-induced macrophages.

Methods. Transcriptomics, qPCR, immunofluorescent staining of neutral lipids and mass spectrometry were performed on monocytes co-cultured with TC cell lines in a transwell model. The impact of fatty acid (FA) uptake and biosynthesis, cholesterol biosynthesis and the transcription factor SREBP was assessed using pharmacological inhibitors.

Results. Transcriptome analysis in TC-induced macrophages identified increased inflammatory characteristics and rewiring of cell metabolism as key functional changes. Next to an increase in aerobic glycolysis, FA synthesis and desaturation were upregulated. Furthermore, the intracellular concentration of the FA precursor Acetyl-CoA was increased. Immunofluorescent staining confirmed an increase of neutral intracellular lipids in TC-induced macrophages. Whereas inhibition of FA uptake and the transcription factor SREBP did not affect the inflammatory characteristics, inhibition of FA synthesis led to an decrease of the inflammatory response. The concept of an important change of FAs in TC-associated macrophages was supported by validation through mass spectrometry of the increase in unsaturated fatty acids in TC-induced TAMs.

Conclusions. Fatty acid synthesis of unsaturated FAs is upregulated in TC-induced macrophages. Furthermore, FA synthesis contributes to the inflammatory characteristics of TAMs.

INTERLEUKIN-33 IMPROVES HOST DEFENSE DURING GRAM-NEGATIVE PNEUMOSEPSIS

I. RAMIREZ-MORAL[1], D.C. BLOK1, S. KNAPP[2], L. BOON[3], A.F. DE VOS[1], T. VAN DER POLL[1,4]

[1]Academic Medical Center, Center for Experimental and Molecular Medicine, Amsterdam, The Netherlands
[2]Austrian Academy of Sciences, Research Center for Molecular Medicine, Vienna, Austria
[3]Bioceros, Utrecht, The Netherlands
[4]Academic Medical Center, Division of Infectious Diseases, Amsterdam, The Netherlands

Background. Interleukin (IL)-33 is a pleiotropic cytokine that activates cells via its unique receptor IL-1 receptor like-1 (ST2), which is expressed by multiple cell types. IL-33 administration has been reported to protect mice from mortality during polymicrobial abdominal sepsis. Pneumonia is the most common cause of sepsis and the effect of IL-33 herein is not known.

Aims. To assess the effect of recombinant IL-33 administration on host defense during pneumonia derived sepsis induced by the common human gram-negative pathogen *Klebsiella (K.) pneumoniae*.

Methods. Wild type and ST2$^{-/-}$ mice were treated with IL-33 or vehicle prior to (and in some experiments after) infection with K. pneumoniae via the airways and sacrificed at predefined time points. To decipher the effect of IL-33 during Klebsiella pneumonia, similar experiments were conducted in mice deficient for IL-13 (IL-13$^{-/-}$) and mice lacking B, T, natural killer (NK) and innate lymphoid cells (ILC) (Rag2$^{-/-}$ / il2gc$^{-/-}$). Additionally, mice were treated with IL-5 depleting antibody, monocyte and neutrophil depleting antibody a-Gr1 or neutrophil-specific depleting antibody a-Ly6G prior to infection.

Results. Administration of IL-33 strongly improved host defense against *K. pneumoniae*, as reflected by attenuated bacterial growth and dissemination, and a prolonged survival, effects that were dependent on ST2. Postponed treatment with IL-33 (3 hours post infection) also lowered bacterial burdens. While IL-33 strongly induced IL-13 and IL-5, its effect on bacterial loads was still present in IL-13-/- and anti-IL-5 treated mice. Moreover, IL-33 treated Rag2$^{-/-}$ / il2gc$^{-/-}$ mice, also showed decreased bacterial counts compared to vehicle-treated mice. While IL-33 enhanced recruitment of neutrophils to the lungs, it was still capable of limiting bacterial outgrowth in the lungs of neutrophil-depleted mice but not in mice depleted of both monocytes and neutrophils. The effect of IL-33 on Klebsiella outgrowth in other organs, however, was dependent on neutrophils.

Conclusions. These data indicate that IL-33 treatment enhances host defense during *Klebsiella* pneumonia via an ST2-dependent mechanism. The beneficial effect of IL-33 during infection is partially driven by neutrophils and inflammatory monocytes, whereas type-2 cytokines, B, T, NK or ILC are not crucially involved herein.

EFFECTS OF LACTATE ON METABOLISM AND CYTOKINE PRODUCTION OF HUMAN IMMUNE CELLS IN VITRO AND IN VIVO

JACQUELINE M. RATTER[1,2], HANNE M. M. ROOIJACKERS[1], ANNEKE G.M. HIJMANS[1], BASTIAAN DE GALAN[1], CEES J. TACK[1], RINKE STIENSTRA[1,2]

[1]Department of Internal Medicine, Radboud University Medical Center, Nijmegen, the Netherlands
[2]Nutrition, Metabolism and Genomics Group, Division of Human Nutrition and Health, Wageningen University, Wageningen, the Netherlands

Lactate, the end product of anaerobic glycolysis, is produced and secreted in high amounts by innate immune cells during inflammatory activation. Although immunomodulating effects of lactate have been reported, evidence from human studies is scarce. Here we show that extracellular lactate modulates metabolism of human monocytes, evidenced by reduced glycolytic and increased oxidative rates immediately after exposure to lactate *in vitro*. To assess short-term effects of lactate *in vivo* in humans, we analyzed *ex vivo* metabolism and cytokine production of immune cells after a 30min lactate infusion. Although the lactate infusion increased ex vivo glucose consumption of PBMCs, effects on metabolic rates and 24h cytokine production were limited. Interestingly, long-term treatment with lactate *ex vivo*, which may reflect pathophysiological conditions in local microenvironments, such as the tumor microenvironment or the adipose tissue, significantly modulated immune cell function. Lactate predominantly had anti-inflammatory effects, demonstrated by decreased production of IL-1β, IL-6, TNFα and IL-10, yet effects were dose- and stimuli-dependent. Whereas lactate clearly had anti-inflammatory effects in cells stimulated with the TLR4-ligand LPS, effects were less pronounced in cells stimulated with the TLR2-ligand Pam3Cys, and for some cytokines production was even increased. Together, our findings reveal lactate as a robust modulator of myeloid cell metabolism which reduces inflammation. Hence, extracellular lactate may function as a negative feedback signal to curtail inflammatory responses.

CONTROL OF IMMUNE RECEPTOR SIGNALLING BY ENDOCYTIC TRAFFICKING

LOREDANA SAVEANU

INSERM U1149, Centre de Recherche sur l'Inflammation, ATIP-AVENIR team Faculté de Médécine X. Bichat 16 rue H Huchard, 75018 Paris

Endocytosis of cell surface receptors might either terminate or sustain the receptor signalling. Among innate immune receptors, which can signal both from cell surface and endocytic vesicles are the important family of toll like receptors (TLRs). Unlike cell surface TLRs, TLR9 recognize pathogen-derived nucleic acids and can also be activated by host derived DNA. Therefore, activation of TLR9 must be subject to tight regulation. This regulation involves the retention of TLR9 in the endoplasmic reticulum, which prevents its activation under basal conditions. Upon cell activation, TLR9 traffics to specific endosomal/lysosomal compartments to encounter it ligand. We identified the insulin responsive aminopeptidase (IRAP) endosomes as major cellular compartments for the early steps of TLR9 activation in dendritic cells (DCs). Both TLR9 and its ligand CpG were found as cargo in IRAP endosomes. In the absence of IRAP, CpG and TLR9 trafficking to lysosomes and TLR9 signalling were enhanced in DCs and in mice following bacterial infection. IRAP stabilized CpG-containing endosomes by interacting with the actin nucleation factor FHOD4, slowing down TLR9 trafficking in lysosomes. In conclusion, endosome retention of TLR9 and CpG via IRAP interaction with the actin cytoskeleton is a mechanism that prevents TLR9 hyper-activation in DCs (Babdor J et al., *Nat. Immunology 2017*). In addition, our recent preliminary data support the hypothesis that IRAP+ endosomes modulate also the activation of adaptive immune receptors, such as the T cell receptor (Evnouchidou I et al., in preparation).

Keywords: Endosome, Toll-Like-Receptors, T-Cell-Receptor, Signalling, Inflammation

OPTIMIZED SINGLE-CELL TRANSCRIPTOMICS WORKFLOW FOR THE CLINICAL ASSESSMENT OF THE INNATE IMMUNE SYSTEM IN CHRONIC INFLAMMATORY DISEASES

JONAS SCHULTE-SCHREPPING[1], KEVIN BASSLER[1], STEFANIE HERRESTHAL[1], COLLINS OSEI-SARPONG[2], WATARU FUJII[1], THEODOROS KAPELLOS[1], CLAUS SCHOLZ[1], MATTHIAS BECKER[1], PAWEŁ BIERNAT[1], KATHRIN KLEE[1], KRISTIAN HÄNDLER[1,3], MARC BEYER[2], JOACHIM L. SCHULTZE[1,3]

[1]Genomics and Immunoregulation, Life & Medical Sciences (LIMES) Institute, University of Bonn, Bonn, Germany
[2]Molecular Immunology in Neurodegeneration, German Center for Neurodegenerative Diseases (DZNE), Bonn
[3]Platform for Single Cell Genomics and Epigenomics at the German Center for Neurodegenerative Diseases and the University of Bonn, Bonn, Germany

One of the most promising technological developments in the last years has been the introduction of single-cell genomics technologies with single-cell RNA-sequencing (scRNA-seq) leading the revolution. Numerous studies have been described assessing the immune compartment of several organisms and the Human Cell Atlas consortium as one of the largest international efforts in the life sciences leads the way to apply this technology to human cells, tissues and organs.

While several scRNA-seq methods including droplet-based methods seem to be straight forward, their application to clinical samples is not as clear-cut. Issues concerning sample procurement procedures, cell isolation and purification, sample multiplexing, library production and sequencing, not to forget about analytical approaches, have not been assessed in a systematic fashion. Particularly the adaptation of single cell genomics to clinical routine procedures is far from being satisfactory.

We have therefore engaged in optimizing each step of the procedure to obtain near-optimal clinical scRNA-seq data. Using COPD and IBD as model diseases, we have developed and are continuously optimizing the complete workflow from clinical procedures for sample procurement and processing via optimization of single-cell sequencing procedures up to standardized data analysis within a web-based analysis portal (FASTGenomics).

This setup allows us to analyze patient samples derived from any hospital in the world in a standardized fashion. Together with our colleagues at the clinical centers, we have started to assess disease-related alterations in distributions of peripheral blood cells in patients and controls and to determine changes in gene expression on the single-cell level between all major immune cell classes in the blood. From these initial steps, it becomes clear that optimized single-cell genomics procedures not only allow higher resolution transcriptomics, but will enable the analysis of numerous disease-relevant parameters, including cell type distributions in diseased tissues, cell type specific changes in gene expression, proliferation and cell cycle, identification of new cell types as well as novel biomarkers for disease.

Similarly important, for future scRNA-seq projects, much more emphasis has to be placed at the interphase between clinicians, pathologists and experts in single-cell genomics to obtain best possible single-cell data quality.

CREATING A LIBRARY OF TRAINED IMMUNITY NANOTHERAPEUTICS

ABRAHAM J. P. TEUNISSEN, ALEXANDRE BERCHOUCHI, CARLOS PÉREZ-MEDINA, WILLEM J. M. MULDER

In recent years, many drugs targeting trained immunity mechanism have been discovered and used to treat malignancies. However, these drugs are generally administered systemically, which lowers their efficacy and often leads to side-effects. A well-established way to control the biodistribution of drugs is to incorporate them into nanocarriers. Surprisingly, such an approach has remained relatively uninvestigated for drugs targeting trained immunity mechanisms. Furthermore, the effect of drug derivatization on the efficacy of trained immunity nanotherapeutics has not been investigated in a systematic manner.

In this study we created a library of 12 trained immunity (pro-)drugs and used these to established a robust but flexible nanotherapeutic platform (Figure 1). First, we developed a microfluidic-based approach to create reconstituted high-density lipoprotein (HDL) nanoparticles, which are well studied in our group and known to specifically target the innate immune system. Secondly, the influence of the nanocarrier's size on its biodistribution was examined by analyzing four sizes of HDL particles (15, 35, 65, 135 nm) in WT mice. It was found that particles with a size of 35 nm have the highest preference for bone marrow progenitor cells, which we chose as an initial target of our study due to their important role in the innate immune system. Subsequently, a library of 12 trained immunity (pro-)drugs was synthesized, consisting of 5 bare drugs and - where possible - cholesterol and C18 aliphatic chain functionalized derivatives. Finally, the (pro-) drugs were incorporated in the nanocarrier to create a library of nanotherapeutics specially aimed at targeting trained immunity mechanisms through the bone marrow.

After creating the nanotherapeutic library, the nanoparticles were fully characterized for their suitability as therapeutic agent. Amongst other factors, we examined the efficiency with which the (pro-)drugs get incorporated, (pro-) drug hydrolysis rates, the charge and size of the particles, and the rate with which the drugs leak out of the particles under *in vivo* conditions. Preliminary results show that our drug functionalization strategy significantly increases both the drug's incorporation efficiency, as well as its retainment in the nanocarrier. By using this approach, the amount of drug that gets delivered at the target side is expected to increase more than 10-fold compared to the administration of nanotherapeutics containing the bare drugs. Furthermore, the difference in functionalization strategy (either with cholesterol or an aliphatic chain), will likely influence the kinetics of *in vivo* drug activation.

Combined, these characteristics will allow control over the nanotherapeutic's biodistribution (by changing their size) and the *in vivo* release and activation of the trained immunity drugs (by functionalizing the drugs). In this manner, our nanotherapeutic library represents a versatile platform which can be used to target multiple malignancies related to trained immunity. Currently, our library is being tested *in vitro* on both mice and human cells, the results of which will be used to guide subsequent *in vivo* studies.

HYPERGLYCEMIA MEMORY OF INNATE IMMUNE CELLS PROMOTES PRO-INFLAMMATORY RESPONSES IN VITRO

KATHRIN THIEM, RINKE STIENSTRA, SAM T. KEATING, MIHAI G. NETEA, CEES J. TACK, JANNA A. VAN DIEPEN

Radboudumc Nijmegen

Background. Hyperglycemia in patients with diabetes is a well-known risk factor for the development of cardiovascular diseases (CVD). Even in patients that have returned to normoglycemia, the increased risk for CVD persist, a phenomenon termed 'glycemic memory'. Atherosclerosis, the major cause of CVD, is a chronic inflammatory disease partly mediated by innate immune cells. We hypothesize that hyperglycemic memory exists in monocytes/macrophages and changes their epigenetic state translating into a pro inflammatory phenotype and accelerating atherosclerosis in diabetic subjects.

Methods. To explore the relevance of glycemic memory in innate immune cells, monocytes from healthy subjects were cultured in the presence of normal (5 mM) or high glucose (25 mM) concentrations for 24 hours. Thereafter, medium was removed and cells were kept in RPMI medium containing 6mM of glucose for 6 days. In addition, cells were exposed to oxidized LDL (oxLDL) in the first 24 hours. After 6 days, inflammatory properties of the cells were tested using TLR2/4 ligands for 24 hours. To explore the relevance of epigenetic changes, Set7 and mixed lineage leukaemia (MLL) methyltransferases were inhibited using CPH (100 μM) and MM 102 (50 μM) or MI-2 (50 μM). These methyltransferases have been implicated in inflammatory regulation in diabetic subjects.

Results. Exposure to high glucose in the first 24 hours of incubation, led to higher TNFα cytokine secretion (11.1%, p=ns) 6 days later compared to cells culture in normal glucose continuously. Cytokine secretion of TNFα was enhanced after simulation with oxLDL in normal glucose conditions (33.6%, $P<0.0001$) and was further increased in high glucose conditions (37.1%, $P<0.0001$). These effects were reversed by inhibition of epigenetic changes using the specific inhibitors.

Conclusion. Hyperglycemia primes innate immune cells towards a pro inflammatory phenotype. The underlying mechanisms may involve epigenetic reprogramming. These results contribute to unraveling the underlying molecular mechanism for the enhanced risk of atherosclerosis in patients with diabetes.

USE OF CRISPR/CAS9 SYSTEM TO EXPLORE THE INFLUENCE OF DEFICIENCIES IN METABOLIC ENZYMES TO INFLAMMATION

LAMBERTO TORRALBA-RAGA, SAEED ESTHAD, YENAN T. BRYCESON

Center for Hematology and Regenerative Medicine, NEO Building, Department of Medicine Huddinge, Karolinska Institutet, Stockholm, Sweden

Several studies have highlighted how metabolism regulates the differentiation and function of immune cells. A therapeutically relevant pathway is the cross talk between cholesterol synthesis and inflammation. Mevalonate kinase deficiency results in an autoinflammatory disease characterized by loss-of-function mutations in mevalonate kinase, an enzyme that participates in the production of endogenously synthesized cholesterol, isoprenoids and other end products required for cell energetics. By whole exome sequencing, we have identified a novel homozygous mutation in another gene in the mevalonate pathway in three patients suffering from recurrent fevers, cytopenia and hepatosplenomegaly. Experiments confirmed excessive production of the pro-inflammatory cytokine IL-1β. Given the scarce knowledge on the enzyme-specific influence to disease, we seek to unravel and quantify the contribution of key enzymes in the mevalonate pathway to immune cell differentiation and production of pro-inflammatory cytokines. To achieve this goal, we are specifically modifying human monocytic THP-1 cells by the CRISPR/Cas9 system. Generated knock-outs and knock-ins, together with reconstitution experiments will provide information on their contribution to inflammation. Complementation of results with human primary cells, both hematopoietic and non-hematopoietic cells, will be performed. A better understanding of the molecular mechanism underlying the impact of cholesterol enzymes to inflammation can lead to new therapeutic targets for patients. Results and conclusions will be presented and discussed.

A FAVORABLE CLINICAL OUTCOME TO INFLUENZA INFECTION IN NEONATES IS LINKED TO REPROGRAMMING OF MONOCYTES TOWARDS A LESS INFLAMMATORY PHENOTYPE WITH METABOLIC SHIFT

ULAS T.[1,*], PIRR S.[2,*], MASS E.[3], SCHULTZE J. L.[1,4], VIEMANN D.[2]

[1]Genomics and Immunoregulation, LIMES-Institute, University of Bonn, 53115 Bonn, Germany
[2]Department of Pediatric Pneumology, Allergy and Neonatology, Hannover Medical School, 30625 Hannover, Germany
[3]Developmental Biology of the Innate Immune System, LIMES-Institute, University of Bonn, 53115 Bonn, Germany
[4]PRECISE Platform for Single Cell Genomics and Epigenomics at the German Center for Neurodegenerative Diseases and the University of Bonn
***these authors contributed equally**
*Corresponding Author: *e-mail: t.ulas@uni-bonn.de*

Background. The morbidity and mortality from influenza infections are surprisingly low in neonates compared to older children and adults. Interactions of the virus with host cells, particularly monocytes, are associated with a poor patient outcome. Blood monocytes are involved in the immune responses to influenza mainly by RIG-I-signaling. RIG-I is a cytosolic pattern recognition receptor that senses viral nucleic acids and induces innate immune activation and secretion of type I interferons. The different outcomes in neonates and adults due to viral infection and the molecular mechanisms behind it is still entirely unclear.

Methods. In order to identify the developmental program that influences the age-dependency of the host's immune responses, we challenged neonatal and adult human monocytes ex vivo with a human Influenza A virus (IAV) isolate (H1N1/California/04/2009) and performed a global transcriptomic analysis using a variety of state-of-the-art computational methods.

Results. Since IAV cause contrary immune responses in neonates and adults, we first identified gene modules with opposing but also common patterns in response to IAV by employing a combination of transcriptomic, immunological, co-expression network analysis and functional predictions. We found a clear reduction of RIG-I signaling and thereby less induction of interferon-induced genes in neonatal monocytes infected with IAV. In contrast, there was a significant increase in RNA processing and RNA transport in neonatal compared to adult monocytes, which was also the case in steady-state. Similarly, we saw higher basal expression levels of genes that are associated with FOXO signaling and metabolic processes. Pathway enrichment analysis revealed that these metabolic processes could be associated with central carbon metabolism (CCM) and oxidative phosphorylation. Their expression was significantly underrepresented in neonatal monocytes compared to adult monocytes.

Conclusions. Our current computational model hints towards a reduced RIG-I and interferon mediated inflammatory response of neonatal monocytes while at the same time metabolic changes seem to allow the cell to better deal with the influenza virus itself. Together, these molecular mechanisms might be directly linked to the more favorable clinical outcome of neonatal in response to IAV infection. These findings might be used to molecularly modify innate immune cells in adult IAV infection as well.

THE INTER-INDIVIDUAL VARIATION AND FUNCTIONAL RELEVANCE OF GLYCOLYSIS IN HUMAN IMMUNE CELLS

XANTHE VAN DIERENDONCK[1,2], ROB TER HORST[1], MARTIN JAEGER[1], MIHAI NETEA[1], RINKE STIENSTRA[1,2]

[1]Department of Medicine, Radboud University Nijmegen Medical Centre, Nijmegen
[2]Nutrition, Metabolism, and Genomics Group, Division of Human Nutrition, Wageningen University, Wageningen
*Corresponding Author: *e-mail: xanthe.vandierendonck@radboudumc.nl*

Introduction. Changes in cellular metabolism are crucial for effective immune cell function and go beyond the sole supply of energy. It is been recently established that different immune cell activators can induce complex metabolic patterns determining functional output of the immune cell including the release of cytokines. Although the metabolic patterns provoked by different immunological stimuli are found to be unique, the induction of glycolysis seems a general metabolic trait of immune cell activation accompanied by increased release of its end-product lactate. Although it is known that host-related factors influence inter-individual differences in immune responses, it is currently unknown whether differences in basal glycolytic rate or after specific stimulation of the cells impact on the inter-individual variation in immune function in human subjects. Using glycolysis as a general marker for immune cell metabolism, we set out to investigate the differences in glycolytic activation upon different types of pathogenic stimuli and provide insight in its functional relevance for human immune cells on a larger scale.

Methods. PBMCs of approximately 500 healthy volunteers (500FG study) were isolated and treated with different types of pathogenic stimuli (RPMI, LPS 1ng, LPS 100ng, Pam3Cys, C. Albicans and S. Aureus) for 24 hours. Afterwards, the production of IL6, IL1β, TNFα, IL10, Il8, IL1ra and lactate were measured. A follow-up study was done in 50 healthy volunteers selected from the 500FG cohort (50FG study), where the volunteers donated blood in four different time points within one year.

Results. In comparison to unstimulated PBMCs (RPMI), all stimuli lead to an increased inflammatory and glycolytic response as determined by the amount of lactate and cytokines produced by the cells.

The magnitude of the glycolytic response showed great variation between the subjects whereas intra- individual variation was relatively low. Based on the inter-individual variation, both a group of high- responders and a group of low-responders could be identified, which could be categorised in the same highest or lowest percentile respectively across all stimuli.

Using advanced multi-channel fluorescence flow cytometry, it was determined that the percentage of monocytes part of the PBMCs positively correlated with the amount of lactate being produced both by unstimulated cells (p=2.2E-07) and even more profoundly after pathogenic stimulation (p-value ranges from E-04 to E-13).

On a functional level, the relative increase in lactate production in PBMCs served as a predictor for the production of several cytokines. Interestingly, despite the fact that lactate production and cytokine release were enhanced after all pathogenic stimulations, the glycolytic rate served as a stimuli-and cytokine-specific predictor. Whereas after LPS stimulation, lactate could be used to predict the amount of IL-6 (p=5.03E-14), IL-1β (p=5.03E-14) and IL-8 (p=1.15E-06) being produced, the glycolytic response after S. Aureus stimulation served to predict the amount of IL-1β (p=2.0E-04) and TNFα (p=1.2E-03) released by the cells.

Conclusion. Although all stimuli appear to increase glycolysis in human PBMCs, inter-individual variation in basal and activated glycolysis exists, while the intra-individual glycolytic response to different immunological stimuli is relatively low. Groups of subjects identified as either high- or low-producers could be categorised into the same highest or lowest percentile across all stimuli. Importantly, the functional relevance of glycolysis appears to be stimulus and cytokine-specific.

MTOR-S6K1 SIGNALING REGULATES MACROPHAGE METABOLISM AND PROLIFERATION IN ATHEROSCLEROSIS

VAN LEENT MMT[1,2,*], LAMEIJER MA[1,2,*], ZHOU X[3], MALKUS J[1], FAY F[1], SANCHEZ-GAYTAN BL[1], PÉREZ-MEDINA C[1], YE Y[4], WOJTKIEWICZ G[4], STROES ESG[5], LUTGENS E[2], FISCHER EA[6], NETEA MG[7], SWIRSKI FK[4], NAHRENDORF M[4], FAYAD ZA[1], ZHANG B[3], MULDER WJM[1,2], DUIVENVOORDEN R[1,5,8]

[1]Translational and Molecular Imaging Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA
[2]Department of Medical Biochemistry, Academic Medical Center, Amsterdam, The Netherlands
[3]Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, USA
[4]Center for Systems Biology, Massachusetts General Hospital, Harvard Medical School, Boston, MA
[5]Department of Vascular Medicine, Amsterdam Cardiovascular Sciences, Academic Medical Center, Amsterdam, The Netherlands
[6]Department of Medicine (Cardiology) and Cell Biology, Marc and Ruti Bell Program in Vascular Biology, NYU School of Medicine, New York, USA
[7]Department of Internal Medicine and Radboud Center for Infectious Diseases, Radboud University Medical Center, Nijmegen, the Netherlands
[8]Department of Nephrology, Academic Medical Center, Amsterdam, The Netherlands
***Both authors contributed equally**

Introduction. Macrophages play a pivotal role in setting off and perpetuating chronic inflammation in atherosclerosis. Activated by lipids, macrophages become highly metabolically active. The mTOR - S6K1 signaling pathway is at the center of regulating cellular metabolism in mammalian cells, however little is known about its role in regulating the activity of plaque macrophages.

Methods. Apoe-/- mice on a high cholesterol diet for 12 weeks were used as a model for advanced atherosclerosis. Nanobiologics were formulated with an mTOR- and S6K1-inhibitor incorporated in high-density lipoprotein (mTORi-HDL and S6K1i-HDL), to facilitate specific targeting of plaque macrophages. Mice were treated during one week with four intravenous injections of mTORi-HDL (5 mg/kg), S6K1i-HDL (5 mg/kg), HDL or control. In a subset of mice osmotic pumps were implanted for continuous release of BrdU during the week of treatment, to enable quantification of macrophage proliferation in vivo.

Results. Flow cytometry analysis of whole aortas showed a 62% ($p<0.001$) and an 82% ($p<0.001$) decrease in macrophages in mTORi-HDL and S6K1i-HDL treated mice, as compared to controls (fig. 1a). This rapid decrease in plaque inflammation was confirmed by histology analysis (fig. 1b). Subsequently, we investigate the molecular mechanism by which mTOR and S6K1 inhibition affects plaque inflammation, by isolating plaque macrophage RNA for whole transcriptome analysis. By performing a Weighted Gene Co-Expression Network Analysis, we observed that processes involved in RNA translation, protein synthesis and protein transport were affected (fig. 1c). This was accompanied by suppression of genes involved in oxidative phosphorylation (fig. 1d), which was confirmed in vitro (fig. 1e). Protein synthesis and mitochondrial respiration are essential for cell growth and proliferation. Therefore, we investigated whether mTor and S6K1 inhibition affected local self-renewal of plaque macrophages. mTORi-HDL and S6K1i-HDL treatment resulted in a reduction of proliferating macrophages of 63% ($p<0.01$) and 76% ($p<0.001$), respectively, as compared to control (fig. 1f), and was confirmed by an in vitro analysis (fig. 1g). Hub genes - highly connected differentially expressed genes in the network - were identified and revealed that PSAP, which encodes for the prosaposin protein, was suppressed in plaque macrophages of both the mTORi-HDL and S6K1i-HDL treated mice (fig. 1h). This suggests that prosaposin plays an important role in mediating the effects of mTOR-S6K1 signaling on plaque macrophage proliferation. Prosaposin plays a central role in sphingolipid metabolism.

Conclusion. This study indicates that inhibition of mTOR-S6K1 signaling is involved in regulating macrophage proliferation in atherosclerosis, that it impaired protein synthesis and mitochondrial respiration, and that PSAP expression plays an important role in regulating these effects.

ALDOSTERONE INDUCES LONG-TERM PRO-ATHEROGENIC CHANGES IN HUMAN MONOCYTE-DERIVED MACROPHAGES

CHARLOTTE D.C.C. VAN DER HEIJDEN[1], LASZLO GROH[1], SAMUEL T. KEATING[1], LEO A.B. JOOSTEN[1], MIHAI G. NETEA[1,2], NIELS P. RIKSEN[1]

[1]Department of Internal Medicine, Radboud University Medical Center, Geert Grooteplein Zuid 10, 6525 GA Nijmegen, The Netherlands
[2]Department for Genomics & Immunoregulation, Life and Medical Sciences 12 Institute (LIMES), University of Bonn, 53115 Bonn, Germany

Aim. Primary hyperaldosteronism is associated with an increased cardiovascular risk in humans, and with accelerated atherosclerosis in animal models. Atherosclerosis is a low-grade inflammatory disorder, with monocyte-derived macrophages as major drivers of plaque formation. Monocytes can adopt a long-term pro-inflammatory phenotype after brief stimulation, which has recently been termed 'trained immunity'. Therefore, we investigated whether aldosterone induces trained immunity and explored pivotal mediating intracellular pathways.

Methods. Human monocytes were exposed to aldosterone (10nM) or serum obtained from patients with primary hyperaldosteronism (PA) or essential hypertension (ET) for 24 hours or 6 days, differentiated to macrophages, and restimulated with toll-like receptor agonists on day 7. Cytokine production was measured with ELISA. In addition, an RNA microarray was performed in aldosterone exposed monocyte-derived macrophages of three donors, and the results were validated with real-time PCR.

Results. Both 24 hour and 6 day exposure to aldosterone augmented the IL-6 and TNF-α response to restimulation. In addition, ROS production on day 7 was increased. This phenotype was prevented by co-incubation with the mineralocorticoid receptor antagonist spironolactone. Incubation with serum of PA patients induced a similar pro-inflammatory phenotype. We identified enhanced fatty acid synthesis as potential pathway that could modify the pro-inflammatory changes upon aldosterone exposure.

Conclusion. Stimulation of human monocytes to relevant concentrations of aldosterone induces a persistent pro-atherogenic macrophage phenotype via stimulation of the MR. Further elucidation of intracellular mediating pathways could offer novel therapeutic targets to prevent atherosclerotic cardiovascular disease.

THE DIFFERENTIAL ROLE OF IL-1α FROM COLON EPITHELIAL AND MYELOID CELLS IN ACUTE COLON INFLAMMATION

ELENA VORONOV[1], MARINA BERSUDSKY[1], IRENA KAPLANOV[1], ALEX BRAIMAN[1], DANIEL FISHMAN[2], CHARLES A. DINARELLO[3], RON N. APTE[1]

[1]The Shraga Segal Department of Microbiology,Immunology and Genetics, Ben-Gurion University of the Negev, Beer-Sheva, Israel
[2]The Department of Morphology, Ben-Gurion University of the Negev, Beer-Sheva, Israel
[3]Department of Medicine, University of Colorado Denver, Aurora, CO 80045 USA

The relationship between IL-1 and the development of IBD has been reported previously. In WT mice, IL 1α has been found by us mainly in intestine epithelial cells (IECs), both under homeostatic conditions and after treatment with DSS. In areas of severe tissue damage, IL-1α was also detected in myeloid infiltrating cells. To better understand the effects of IL-1α in acute colon inflammation, we used mice with a complete deficiency of IL-1α (IL-1α KO mice) or mice with a specific deletion of IL-1α in IECs (IL-1$\alpha^{int/\Delta}$) or myeloid cells (IL-1$\alpha^{Lyz/\Delta}$).

IL-1α KO mice and IL-1$\alpha^{int/\Delta}$ mice were more resistant to DSS-induced colitis and showed less mortality compared to control and IL-1$\alpha^{Lyz/\Delta}$ mice. Mice with conditional depletion of IL-1α in myeloid cells showed a more severe form of the disease with poor prognosis and high lethality compared to control mice. The increased influx of T cells, especially CD8 and Treg-positive cells, which are known to be important for colon homeostasis and are correlating with a good prognosis, was observed in mice with deficiency of IL-1α in epithelial cells. In mice deficient in IL-1α in myeloid cells, we observed an increased number of pro-inflammatory $CD11b^{+}Ly6C^{+}CD11c^{+}$ cells in the lamina propria.

Thus, our results show that IL-1α from colon epithelial cells is an important molecule in the progression of colon inflammation, whereas IL-1α from myeloid cells is necessary for colon protection.

VENDOR EFFECTS ON MURINE GUT MICROBIOTA AND ITS INFLUENCE ON LIPOPOLYSACCHARIDE INDUCED LUNG INFLAMMATION AND GRAM-NEGATIVE PNEUMONIA

N.S. WOLFF[1], M.C. JACOBS[1], R.B. VAN DER LEE[1], B.W. HAAK[1], F. HUGENHOLTZ[1], W.J. WIERSINGA[1,2]

[1]Center for Experimental and Molecular Medicine, Academic Medical Center, University of Amsterdam, Amsterdam, Meibergdreef 9, 1105 AZ, The Netherlands
[2]Department of Medicine, Division of Infectious Diseases, Academic Medical Center, University of Amsterdam, Amsterdam, Meibergdreef 9, 1105 AZ, The Netherlands

Background. Limited reproducibility of experimental animal models of pulmonary inflammation and pneumonia across different laboratories is a concern. We hypothesized that differences in microbiota composition across vendors can explain a part of this observed variation in phenotypes.

Methods. In order to address this question and to study the effect of different bacterial gut microbiota, a multi-vendor approach was used with genetically similar mice derived from three different vendors (Janvier (Jan), Envigo (Env), and Charles River (CR)). This model was employed to study the effect on the host response to a pulmonary lipopolysaccharide (LPS) challenge (1μg *K. pneumoniae* LPS intranasal), as well as experimental *Klebsiella pneumoniae* infection (ATCC43816, 1x10e4 CFU intranasal). Gut microbiota was analysed using 16s sequencing.

Results. Gut microbiota analysis revealed profound intervendor differences in bacterial composition, shown by alpha diversity and at various taxonomic levels. In a first set of experiments, tumor necrosis factor (TNF)-α and interleukin (IL)-6 release in lung and bronchoalveolar lavage fluid (BALF) was determined 6 and 24 hours after intranasal treatment with LPS. No differences were found between the groups, with the exception for Envigo, showing a slightly higher level of TNF-α in lung and BALF at 6 hours. In a second set of experiments, mice from different vendors were subjected to a clinically relevant model of Gram-negative pneumonia (*K. pneumoniae*). At 12 and 36 hours post infection, no intervendor differences were found in bacterial dissemination, or TNF-α and IL-6 levels in the lungs.

Conclusion. Although there is a marked variation in the gut microbiota composition of mice from different vendors, we could not demonstrate a vendor effect during experimental *K. pneumoniae* pneumonia and LPS induced lung inflammation, except for an early TNF-α response.

C1-ESTERASE INHIBITOR DOES NOT INFLUENCE EOSINOPHILIC INFLAMMATION IN MILD ASTHMA PATIENTS: A RANDOMIZED TRIAL

J. YANG[1], T.S.R. VAN ENGELEN[1], C. VAN 'T VEER[1], P.I. BONTA[2], C.J. MAJOOR[2], E.M. KEMPER[4], S.S. ZEERLEDER[3,6], E.H.D. BEL[2], T. VAN DER POLL[1,5]

Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands
[1]Center of Experimental and Molecular Medicine
[2]Department of Respiratory Medicine
[3]Department of Haematology
[4]Department of Pharmacy
[5]Division of Infectious disease
[6]Sanquin Research, Amsterdam, the Netherlands: Department of Immunopathology.

Background. Allergic asthma is characterized by chronic eosinophilic airway inflammation, chest tightness and airway hyperresponsiveness (AHR). Allergic asthma is most often evoked by contact with house dust mite (HDM). HDM derived proteins together with lipopolysaccharide (LPS) induce an enhanced inflammatory response in the airways of sensitized asthmatic patients. Complement system activation occurs after allergen challenge in the airways of asthmatic patients. Preclinical data suggest that inhibiting complement activation could attenuate the inflammatory response in asthma. The goal of this study is to determine whether complement system inhibition attenuates airway inflammation in asthmatic patients, using the capacity of C1-esterase inhibitor (C1-inh) to inhibit complement activation.

Methods. In this randomized, double-blind, placebo-controlled trial 24 patients with mild asthma and HDM allergy were challenged with HDM and LPS in one lung subsegment and with saline (control) in a contralateral lung segment using a bronchoscope. 12 patients received intravenous C1-inh (100U/kg) prior to HDM/LPS challenge; 12 patients received placebo. Bronchoalveolar lavage fluid (BALF) was harvested seven hours after the challenge from both lung segments for measurements of inflammatory responses. In addition, blood samples were taken before and after C1-inh infusion and the challenge. The primary outcome of this study is eosinophil numbers in BALF, reflecting eosinophil recruitment into the airways.

Results. Administration of C1-inh resulted in elevated C1-inh levels in both plasma and BALF. HDM/LPS challenge elicited strong inflammatory responses in the airways, including enhanced influx of eosinophils and neutrophils, release of fifteen measured cytokines and chemokines, and protein leakage. No difference in the primary outcome ($p=0.29$) nor other inflammatory markers was found between the C1-inh and control group following HDM/LPS challenge.

Conclusion: Intravenous C1-inh does not alter bronchial eosinophil recruitment and cytokine production in mild asthmatic patients following intrabronchial challenge with HDM and LPS.

SEPSIS
SESSION I

SURVIVING SEPSIS CAMPAIGN 2016 - WHY NEW DEFINITIONS?

MICLAUS DIANA

Intensive Care Department, Clinical Hospital of Infectious Diseases, Cluj-Napoca, Romania
*Corresponding author: *e-mail: miclaus1diana@gmail.com*

Objectives. Sepsis and septic shock represent a major public health problem, costing the USA 20 millions dollars in 2011. Septic shock, the classic form of distributive shock, is the most common form of shock admitted to ICU – 62%.

In 2016, due to controversy regarding the exact definitions, epidemiology and mortality, a working group set the scene of The 3rd International Consensus Definitions for Sepsis and Septic Shock (Sepsis – 3).

Materials and methods. A retrospective view over the definitions and criteria starting with 1992, when the term of "sepsis" was first mentioned, until 2016 .

Results

- Screening and management of infections
- The acknowledgement of organ dysfunction
- ¨3 hours bundle¨

Conclusions. The Surviving Sepsis Campaign promoters belief is that like in the case for stroke and acute myocardial infarction, the early implementation of the bundles of care could make an important difference in the outcome.

APPROACHES TO ANTIBIOTIC TREATMENT OF SEPSIS: ANTIMICROBIAL STEWARDSHIP

SIMIN-AYSEL FLORESCU

University of Medicine and Pharmacy Carol Davila, Bucharest, Romania

The proper antibiotic treatment is the cornerstone of survival in sepsis, and it takes into account in equal proportions the type of antibiotic, the dose, the right antibiotic association and the safest moment to start. At the same time, we must consider the possible etiology, resistance, outcome and comorbidities.

In the last years, all these types of interventions were grouped under the name of antimicrobial stewardship, as coordinated interventions designed to improve and measure the appropriate use of antimicrobials by promoting the selection of the optimal antimicrobial drug regimen, dose, duration of therapy, and route of administration. Besides the direct effect on the patient, as achieving optimal clinical outcomes, minimize toxicity and other adverse events, it supports the need to reduce the costs of health care for infections, and limit the selection for antimicrobial resistant strains [1].

In order to start a proper antibiotic treatment, we need an efficient way to recognize the first signs. The most recent definition of sepsis considers this clinical entity as a life-threatening organ dysfunction caused by a disturbed host response to infection. Organ dysfunction is quantified by an increase in the Sequential [Sepsis-related] Organ Failure Assessment (SOFA) score of 2 points or more, or as a rapid identification of patients with possibly poor outcomes typical of sepsis with the quick SOFA (qSOFA) score: respiratory rate of 22/min or greater, altered mentation, or systolic blood pressure of 100 mm Hg or less [2].

Antibiotic stewardship is not nationally or otherwise regulated, being a local or regional approach. During the last 20 years since this concept was first introduced, some parts of antibiotic stewardship were largely discussed, like antibiotic de-escalation. There are many observational studies regarding de-escalation in a variety of severe infectious diseases, mainly sepsis, septic shock, pneumonia, urinary tract infections, but less randomized controlled trials in this particular area. Moreover, the studies addressed mainly to clinical outcome of de-escalation, and less to particular effect on antibiotic resistance or on the ecologic environment of the patient. The results of the reviews and meta-analysis were contradictory, depending on the types of studies examined (observational or randomized controlled studies) [3,4].

Other parts of antibiotic stewardship in sepsis have more coordinated results, mainly the rapidity of sepsis recognition and the shortening of antibiotic starting interval. There are newly developed sepsis diagnosis tools, new algorithms for diagnosis and empirical treatment, which have good results on clinical outcome [5].

However, there are many other aspects, like local pattern of antibiotic resistance, economical aspects, access to new drugs, which must be taken into account as variable components of antimicrobial stewardship.

Considering all these aspects, there is a need for antimicrobial stewardship guided sepsis treatment, which must be adapted continuously to local patterns.

Keywords: sepsis, antibiotic, antimicrobial stewardship, results

HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS IN THE CLINICAL HOSPITAL OF INFECTIOUS DISEASES CLUJ-NAPOCA

LUCIA HERBEL[1], LAURA DAMIAN[2], LAURA ALBU[3], MIHAELA LUPSE[4]

[1]Clinical Hospital of Infectious Diseases Cluj-Napoca, ICU, Cluj-Napoca, Romania
[2]Rheumatology Department, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania
[3]Clinical Hospital of Infectious Diseases, Cluj-Napoca, Romania
[4]Infectious Diseases Department, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania
*Corresponding author: *e-mail: herbellucia@yahoo.com*

Objectives. In this study, we assessed the evolution of patients with secondary HLH/MAS (Macrophage Activation Syndrome), discharged from our hospital.

Materials and methods. Observational, retrospective study - the data were gathered from the clinical observation charts and electronic medical records of patients discharged with the diagnosis of HLH/MAS between the years 2012-2018.

Results. 13 patients: -2 AIDS -5 rheumatic disease -5 unknown etiology (possibly septic) -1 Coxiella and Chalmydia infection Age: 21-77 years, average 50.69 years, median 45 years. Sex: 8 males -9 of 13 patients admitted to ICU with a diagnosis of etiologically unspecified sepsis with organ dysfunction -5/13 deceased. Diagnostic criteria for HLH/MAS distribution: -100% fever -61.5% serum feritin>500 ng/dl -69.23% splenomegaly -100% cytopenia (53.84% pancytopenia, 46.15% bicytopenia/leucopenia) -79.92% high serum triglicerides/low fibrinogen -92,3% hemophagocytosis on bone marrow biopsy -15.38% positive phenotyping -84.61% with 5/8 diagnostic criteria (2 patients with 4/8 criteria with intense hepophagocytosis on bone marrow biopsy). All patients were treated with iv corticosteroids (pulse therapy) plus etoposide in 3 cases, cyclosporine in 2 cases.

Conclusions. HLH/MAS should be considered in the differential diagnosis of patients with persistent fever of unknown origin. The mortality rate is high even with appropriate treatment.

ATTRIBUTABLE MORTALITY OF HEALTHCARE-ASSOCIATED INFECTIONS IN THE MEDICAL INTENSIVE CARE UNIT, THE TEACHING HOSPITAL OF INFECTIOUS DISEASES, CLUJ-NAPOCA

AMANDA RADULESCU[1], LUCIA HERBEL[2], MIRELA FLONTA[3], ARIANA ALMAS[3], ADRIANA SLAVCOVICI[4]

[1]Epidemiology Department, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania
[2]SCBI, ICU, Cluj-Napoca, Romania
[3]SCBI, Laborator, Cluj-Napoca, Romania
[4]Infectious Diseases Department, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania
*Corresponding Author: *e-mail: aradulescu@umfcluj.ro*

Objectives. The purpose of this study was to quantify the effect of healthcare-associated infections (HAIs) on mortality in a medical intensive care unit (ICU).

Materials and methods. We conducted a retrospective study of patients primarily admitted for sepsis and other severe infections in the ICU, University Hospital of Infectious Diseases, Cluj-Napoca, Romania, between January 1, 2010 and December 31, 2016. HAIs were diagnosed according to ECDC surveillance case definitions and clinicians' judgment. We used StatSoft Statistica version 8.0 for statistical analysis.

Results. Among 2396 patients (average annual number 342) we evaluated 329 patients with at least one HAI selected by surveillance requirements, severity and treatment: probable ventilator-associated infections (VAI), bloodstream and central line-associated bloodstream infections (CLABSI). Catheter-associated urinary tract infections without bacteremia and other infections were recorded only when being unique. Overall, the incidence rate was 15.3/1000 patients-days in ICU and the prevalence 13.7%. Median age was 70 years (IQR range 68 to 72), Charlson index 7 (IQR range 4 to 9). Probable VAIs were the most frequent events with an incidence of 7/1000 ventilator-days and bloodstream infections (including CLABSI) 5/1000 central venous catheter-days. Gram-negative bacteria (200 strains) were dominant, 60.5% being extensively drug resistant, followed by Gram-positive methicillin-resistant staphylococci (28/34, 82%), vancomycin resistant Enterococcus faecium (6/27 strains), Candida spp. (33) and Clostridium difficile (28). The risk of death was associated with age over 65 years (OR 1.7; 95%CI 1.08 to 2.69), Charlson index (OR 1.9; 95%CI 1.21 to 2.96), mechanical ventilation (OR 3.87; 95%CI, 2.37-6.3), extensively resistant/panresistant bacteria from invasive and noninvasive cultures which were possibly colonizations (OR 2.25; 95%CI, 1.44-3.53), length of stay (P

Conclusions. Our results show that older age, severity index, device-associated HAIs and multidrug resistant bacteria are related to higher mortality rates in medical ICU.

INFECTIONS IN THE ELDERLY

VIOLETA BRICIU

Infectious Diseases Department, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania
*Corresponding Author: *e-mail: violeta.briciu@gmail.com*

Objectives. Ageing seems to be the most important social transformation in the 21st century. Despite advances in antibiotic therapy, infectious diseases continue to be a major cause of mortality in the older adults. The aim of the study was to evaluate hospitalization trend and fatality rate due to infectious diseases in older patients. Secondary objectives were to present the common infectious diseases and co-morbidities associated with increased need for in-hospital care.

Materials and methods. A retrospective, descriptive study was performed, using the hospital information system, in patients aged more than 65 years old, hospitalized for an infectious disease from 01.01.2007 to 31.08.2017 in the Clinical Hospital of Infectious Diseases, Cluj-Napoca, Romania. Hospitalization trend and fatality rate were evaluated in two age groups: older patients (more than 65 years old) and oldest old patients (more than 85 years old). The diagnostic and co-morbidities like dementia, pressure ulcers and stroke sequelae were evaluated in patients hospitalized from 01.01.2016 to 31.08.2017.

Results. An ascending trend of hospitalization was described, up to a 1.5 increase in 2015 compared to 2007 in older patients, and 3.44 increase in 2015 compared to 2007 in the oldest of elderly patients. Fatality rate raised from 1.88% in 2007 to 7.89% in 2017 in older patients, and from 6.15% to 17% the same interval in the oldest old group. 2932 old patients were hospitalized from 01.01.2016 to 31.08.2017: 23% for pneumonia, 12% for urinary tract infections, 9.5% for enterocolitis and 6.8% for Clostridium difficile associated colitis. Sepsis was present in 499 patients (17%). 366 oldest elderly patients were hospitalized from 01.01.2016 to 31.08.2017: 32% for pneumonia, 10.3% for urinary tract infection, 9.2% for enterocolitis, 6% for Clostridium difficile associated colitis. Sepsis was present in 70 patients (19.1%). Dementia was present in 31.9% of the oldest elderly patients, 16% associated stroke sequelae and 7.9% a pressure ulcer.

Conclusions. The study underlines the ascending trend of hospitalization in the last 10 years in elderly patients with infectious diseases. The increase of the fatality rate underlines the severity of infections in this age group.

RE-EMERGING INFECTIONS
SESSION II

THE IMPACT OF MIGRATION ON TUBERCULOSIS EPIDEMIOLOGY IN EUROPE AND SCREENING FOR TUBERCULOSIS AMONG MIGRANTS

ADRIANA HRISTEA, RALUCA JIPA

National Institute for Infectious Diseases Prof Dr Matei Bals, University of Medicine and Pharmacy, Infectious Diseases, Bucharest, Romania
*Corresponding author: *e-mail: adriana_hristea@yahoo.com*

Objectives. Migration is not a risk for tuberculosis (TB) by itself. Nevertheless, TB continues to be a public health concern in countries with low TB burden, since immigrants from TB endemic countries account for a significant proportion of TB cases. Our aim was to review epidemiology data regarding TB among migrants and current practices in Europe for screening.

Materials and methods. We reviewed data published on the screening for TB among migrants.

Results. Reactivations of previously acquired latent tuberculous infection (LTBI), which occurs months to years after settlement in the host country, accounts for most of the cases. The risk of LTBI reactivation is higher within the first two to four years and decreases slowly over time but remains higher than in the host population. Most of the data on the prevalence of LTBI are from cross sectional studies, and longitudinal data on reactivation are very limited. Second-generation migrants, who often keep a link with their country of origin, or international travelers including visiting friends and relatives, especially children, are known to represent high-risk groups for TB. In most short-term travelers the absolute risk for acquiring TB is low, but in long-term travelers or military personnel the tuberculin skin test conversion rate might be substantial. The screening for active TB can be done pre-arrival (pre-entry) or post entry and the screening for LTBI is performed post-entry with a continuing identification of active TB and contact tracing (targeting community transmission). Most developed countries screen for active TB, but screening for LTBI is less commonly performed. The aim of the screening for LTBI is the prevention of progression to active TB via preventive therapy. There are discrepancies between policies and implementation of migrants screening. A review of ten studies published between 2000 and 2014 showed that detecting and treating LTBI in immigrants was associated with substantial health and economic benefits. The strategy for LTBI screening in migrants might depend on the country-specific treatment procedures and costs. Targeting young migrants from countries at higher incidence of TB increases the cost-effectiveness of screening.

Conclusions. Screening migrants from high TB incidence countries for active TB or LTBI is a critical component of the TB elimination strategies in low-incidence countries.

EMERGING INFECTIOUS DISEASES – A NEW THREAT?

SIMIN-AYSEL FLORESCU

University of Medicine and Pharmacy Carol Davila, Bucharest, Romania
*Corresponding author: *e-mail:*

New infectious diseases are being described constantly, despite major advances in the development of diagnostic, therapeutic methods, vaccines. However, in the last years we have been witnessing increasing numbers of newly emerging and reemerging infectious diseases throughout the world, including Europe.

The determinants of this are dependent on human host, human environment and the characteristics of the microbes.

During the last years, large human movements and climate changes seem to have accelerated the changes in the infectious diseases aspects throughout the world, affecting both the health and the economic stability of societies.

There are evidences of vector-borne diseases imported in areas of Europe which have not been signaled before, or were eliminated many years ago (malaria in Greece, France, Dengue fever in France, Croatia, visceral leishmaniasis in the same areas); at the same time, new vectors are being found in new areas, like Aedes albopictus in Romania, opening the way for transmitting new diseases;

New viruses, like pandemic influenza, and new resistance profiles of known germs are appearing more frequently, generating increasingly harder challenges for containment and treatment.

It becomes evident that the battle against infectious diseases is a continuous process, which needs constant awareness.

Keywords: emergent, infectious, determinants

CHARACTERISTICS OF URINARY TRACT INFECTIONS DUE TO CARBAPENEM-RESISTANT KLEBSIELLA PNEUMONIAE

GIULIA STAN[1], ADRIANA HRISTEA[1], DANIELA TALAPAN[2], COSMINA GINGARAS[1], CORINA CIONT[1], CRISTINA CHERAN[1]

[1]INBI Matei Bals, Infectious Diseases, Bucharest, Romania
[2]INBI Matei Bals, Laboratory Medicine, Bucharest, Romania
*Corresponding author: *e-mail: giulia.stan@yahoo.com*

Objectives. Carbapenem-resistant Enterobacteriaceae is of increasing concern worldwide and Klebsiella pneumoniae is most likely to develop resistance. Our aim was to compare the patient characteristics associated with carbapenem-resistant K.pneumoniae (CRKP) vs carbapenem-susceptible K.pneumoniae (CSKP) isolated from urine and to describe the carbapenemases responsible for carbapenem-resistance.

Materials and methods. We retrospectively studied patients over 14 years old hospitalized in our institution between January and December 2015, in which K.pneumoniae was isolated from urine. For non-susceptible strains (according to EUCAST 2014 clinical breakpoints, determined using microdilution MicroScan Panels - Siemens Healthcare Diagnostics) we performed the modified Hodge test (MHT) as phenotypic confirmatory test for carbapenemase production according to CLSI guidelines and the combination disk test (KPC, MBL, OXA-48 Confirm kit, Rosco Diagnostica) according to EUCAST guidelines.

Results. We identified a total of 72 patients, 25 (34.7%) with CRKP and 47 (65.3%) with CSKP. There was a female predominance in both groups, 13 (52%) patients in CRKP group vs 29 (62%) in CSKP group (p=0.22, OR[95%CI] 0.67[0.24-1.83]). The median age was 69 (IQR 59-85) years in CRKP group vs 63 (IQR 38-77) years in CSKP group (p=0.02). Among patients from CRKP group, 12 (48%) patients presented urinary tract infections (UTI) and 13 (52%) asymptomatic bacteriuria (AB) vs 28 (60%) UTI (including eight patients with urosepsis) and 19 (40%) AS in CSKP group (p=0.18, OR [95%CI] 0.63[0.23-1.69]). The in-hospital mortality in CRKP group was 16% vs 6 % mortality in CSKP group (p=0.11, OR [95%CI] 2.75[0.52-15.94]). However, in all cases in CRKP group death was associated with C. difficile infection in patients with coexisting morbidities and three out of four patients had AB. The in-hospital mortality of patients with UTI in CRKP group was 8% vs 7% in CSKP group (p=0.43, OR[95%CI] 1.17[0.03-16.87]). The most frequent carbapenemase was the OXA-type detected in 15 (60%) patients, followed by NDM-type carbapenemase in 5 (20%) patients, both OXA+NDM or OXA+KPC type carbapenemase in 5 (20%) patients.

Conclusions. Patients in CRKP group were older. In both groups AB represented an important percentage. Mortality in patients with UTI was similar in both groups and most patients who died had coexisting infections and morbidities. OXA-type was the most frequently identified carbapenemase.

CYTOKINES PROFILE IN DIFFERENT INFECTIONS SESSION III

SERUM CYTOKINE PATTERN INVOLVED IN THE INFLAMMATORY RESPONSE IN ACUTE VIRAL HEPATITIS

MELINDA MILENA HORVAT[1], CRISTINA CISMARU[1], MONICA MUNTEAN[1], MADALINA VALEANU[2], MIHAELA LUPSE[1]

[1]Infectious Diseases Department, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania
[2]Medical Informatics and Biostatistics Department, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania
*Corresponding author: *e-mail: horvatmelinda@yahoo.com*

Objectives. Hepatitis A virus (HAV) and hepatitis B virus (HBV) infections are currently an important public health problem at international level, producing a wide spectrum of clinical manifestations, which vary from asymptomatic, subclinical form of the disease to moderate, severe or even fulminant evolution forms, with acute hepatic failure. The interaction between hepatitis viruses and the host entails the evolution of acute viral hepatitis, an essential role being played by the immune system, through the local and systemic production of cytokines, along with innate and adaptive immune responses. To analyze the cytokine's profile in adult patients with non-fulminant acute hepatitis A and B versus a control group, and to compare the cytokine's serum concentrations between the two groups of hepatitic patients. Another aim was to analyze the correlations between the cytokines levels and the biochemical parameters at admittance.

Materials and methods. The serum levels of cytokine IL-10, IL-1Ra, IL-6, tumor necrosis factor alpha (TNF-alpha) and interferon gamma (IFN-gamma) were determined by mul¬tiplex xMAP technology (Luminex) in 52 adult pacients with non-fulminant acute viral hepatitis A and B admitted over a 3 years period and healthy volunteers. The ROC curve was used and the area under the curve (AUC) was calculated to determine the sensitivity (Sn) and specificity (Sp) of these biomarkers. The AUC values close to 1 indicated a high diagnostic accuracy.

Results. The serum concentration of IL-10, TNF-alpha, IL-6, IL-1Ra were significantly higher ($p<0.05$)

Conclusions. This study proved that serum levels of cytokines in acute hepatitis A and B may constitute sensitive markers upon the extension of liver tissue injury with prognostic value. Key words: hepatitis, cytokines, IL-10.

THE CYTOKINE PROFILE IN HIV PATIENTS FROM CLUJ REGIONAL CENTRE

IRINA FILIPESCU[1], CRISTIAN JIANU[1], CORINA ITU-MURESAN[1], MIHAELA LUPSE[2]

[1]Infectious Diseases Department, Infectious Diseases Teaching Hospital of Cluj-Napoca, Romania
[2]Infectious Diseases Department, Iuliu Hatieganu University of Medicine and Pharmacy, Romania
*Corresponding author: *e-mail: irina20bobby2000@yahoo.com*

Objectives. To perform an evaluation of pro and anti-inflammatory cytokines in HIV patients in Cluj regional centre.

Materials and methods. Pro and ant inflammatory cytokines were measured in 122 HIV patients that presented for evaluation in the HIV department of Infectious Disease Teaching Hospital of Cluj-Napoca between the 1st of March 2016 and 21st of April 2017. IL-6, IL-8, IL-18, IL-1Ra, IL-18BP and high sensitive CRP were assessed using Elisa assay. The patients were also evaluated for CD4 count and viral load. Medical history, stage of infection and antiretroviral therapy were taken in consideration.

Results. 98 (71.3%) of the patients were men. 21 patients were naïve, at their first evaluation. 111 (91%) of the patients had a CD4 count higher then 200 cells/cmm and 59.8% had a detectable viral load. There was statist there was a statistically significant difference in CD4 count (p=0.005) between patients with detectable and undetectable viral load. The cytokines levels between patients with CD4200 cells/cmm were statistically significant higher for IL-1R, IL-18, IL-18BP in the CD4.

Conclusions. Our study is the first to analyze the cytokine profile in HIV patients in Cluj regional centre. The correlation between the CD4 count and the IL-1Ra, IL-18 and IL-18BP levels are similar with other literature data, but further studies are necessary to establish the importance of the cytokine profile in our clinical practice.

INFLAMMATION MARKERS IN ROMANIAN PEOPLE WHO INJECT DRUGS INFECTED WITH HIV

BANICA LEONTINA[1], OVIDIU VLAICU[2], IONELIA NICOLAE[2], EMIL NEAGA[2], DAN OTELEA[2], SIMONA PARASCHIV[2]

[1]Institute for Infectious Diseases "Prof. Dr. Matei Bals", Molecular Diagnostics Laboratory, Bucharest, Romania
[2]National Institute for Infectious Diseases "Prof. Dr. Matei Bals", Molecular Diagnostics Laboratory, Bucharest, Romania
Corresponding author: *e-mail: leo_mirela@yahoo.com

Background. Inflammation and immune activation are hallmarks of HIV infection, being associated with disease progression and non-AIDS defining co-morbidities. Microbial translocation as a consequence of destruction of the gut associated lymphoid tissue persists despite efficient antiretroviral therapy. High levels of inflammation markers (hsCRP, IL-6, TNF-α), coagulation (D-dimer) and microbial translocation (sCD14) were observed in HIV infected patients. Romania has faced an HIV outbreak among people who inject drugs (PWID) starting 2011, affecting mainly young men with low socio-economic status and present or past detentions. Molecular clock analysis indicated for most of the cases a recent acquired HIV infection.

Materials and methods. Plasma samples were collected from 26 PWID recently diagnosed with HIV infection and 8 healthy controls. All the HIV patients were infected with F1 subtype strains. They were further stratified based on CD4 counts, 13 with CD4350 cells/µl. The level of CRP, IL-6, TNF-α and sCD14 was evaluated by ELISA using commercial kits and following manufacturer instructions. For all HIV patients viral loads (VL) and CD4 counts were evaluated using IVD kits. Statistical analysis of the data was performed with GraphPad Prism 6.

Results. High level of inflammation markers (CRP, IL-6, TNF-α, sCD14) was observed in HIV infected PWID as compared with the control group. Furthermore, the patients with CD4.

Conclusions. HIV infection induces inflammation and this might contribute to the CD4 loss. HIV infected patients with low CD4 counts had an increased level of microbial translocation, suggesting that early treatment when circulating CD4 cells depletion is still limited, might reduce microbial translocation levels and consequent inflammation.

UP-TO-DATE
SESSION IV

UP TO DATE IN INFECTIOUS DISEASES

MONICA MUNTEAN

Infectious Diseases Department, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania
*Corresponding author: *e-mail: turdean_monica@yahoo.com*

Objectives. This review emphasizes the most important changes that are taking place in the field of infectious diseases over the year 2018.

Materials and methods. The significant specialty literature published between 07/2017-07/2018 was reviewed.

Results. Clostridium difficile infection (CDI) is the most common health-care-associated infection worldwide. Most of the excitement in the field of management is the assessement of the safety and efficacy of new therapeutic agents such as ridinilazole - a targeted-spectrum antimicrobial that shows potential in treatment of initial CDI and in providing sustained benefit through reduction in disease recurrence. Another interesting study find out that dietary disaccharide trehalose enhances virulence of two epidemic Clostridium difficile ribotypes (RT027 and RT078). Gram-negative bacteraemia (GNB) is a major cause of morbidity and mortality in hospitalized patients. Data to guide the duration of antibiotic therapy are limited and no randomized controlled trials have been conducted. Conclusions of a number of trials were published noting that in patients hospitalised with GNB and sepsis resolution before day 7, a course of 7 antibiotic days was non-inferior to 14 days, reduced antibiotic days and resulted in a more rapid return to baseline activity. EASL published this year the Clinical Practice Guidelines on hepatitis E virus (HEV) infection. The understanding of HEV has changed completely over the past decade. Previously, HEV was thought to be limited to certain developing countries but we now know that HEV is endemic in most high-income countries and is largely a zoonotic infection. Of note, chronic HEV infection in immunosuppressed individuals seems to be restricted to genotype 3 infection, while excess mortality during pregnancy is a unique feature of genotypes 1 and 2 infection. Extrahepatic manifestations of HEV infection are increasingly recognised, the most important being neurological. Ribavirin monotherapy has been more extensively studied in the treatment of chronic HEV infection in solid-organ transplant recipients with a number of case series reporting high sustained virological response rates after a three-month course of therapy.

Conclusions. Infectious diseases are constantly moving targets which raise new challenges or bring old problems as new challenges.

GLOBAL THREATS IN 2017-2018

AMANDA RADULESCU

Epidemiology Department, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania
*Corresponding author: *e-mail:aradulescu@umfcluj.ro*

Objectives. A new outbreak of Ebola in the Democratic Republic of Congo was declared in May 2018 and was over in the end of July at two viral incubation periods, after the last victim recovered. There were 54 cases including 33 deaths. What was different compared to the biggest Ebola fever epidemic 2014-2016 that took more than 11,000 lives? Beyond rapid action, the vaccine developed during the last epidemic, a recombinant vesicular stomatitis virus–Zaire Ebola virus (rVSV-ZEBOV) vaccine was under a compassionate use trial protocol. A ring vaccination involved the most likely to be infected and showed 100% protection among more than 10,000 vaccinated people. Experimental treatments as ZMapp – a monoclonal antibody cocktail and Favipiravir, a small-molecule antiviral were tested under an ethical protocol developed by WHO.

Materials and methods. Yellow fever was a source of terror for centuries yet mass vaccination campaigns led to a dramatic drop in cases worldwide, still 40 countries are considered at highest risk. Recently, Brazil was confronted with more than 1,218 human cases of yellow fever virus infection, including 364 deaths (fatality rate of 30%). Cases occurred in densely populated metropolitan areas, such as Rio de Janeiro and Sao Paulo, encompassing a population of over 32 million inhabitants and up until April 2017, these areas were not deemed to be at risk for yellow fever virus transmission. Mass vaccination was started and total population coverage is esteemed till 2020. Cases ending in death occurred in travelers despite yellow fever immunization recommendation.

Results. Addressing Zika virus health threat is a difficult task but it seems that sofosbuvir, approved and marketed to treat and cure hepatitis C infections works against Zika virus (both viruses belong to the same family) and the preliminary work is promising mainly arresting the transmission from mother to fetus. Beyond the neurological complications related to Zika virus' tropism for brain cells, the current trend is of using genetically engineered oncolytic pathogens as a safe way to eliminate tumors is under development.

Conclusions. Genetically engineered ZIKV might be a potential new strategy for neural cancer management through the induction of endogenous digoxin synthesis in glioblastoma cells.

UP-TO-DATE IN HIV. TRANSMISSION, TREATMENT AND CURE

CORINA ITU-MURESAN

Clinic Hospital for Infectious Diseases, Infectious diseases/Immunodepression, Cluj-Napoca, Romania
*Corresponding Author: *e-mail: corinaitu@yahoo.com*

TRANSMISSION Final results from PARTNER 2, the second phase, which only recruited gay couples confirm the results originally announced in 2014 from the first phase, PARTNER 1, that "Undetectable equals Untransmittable" (U=U). The results indicate, as expressed by the researchers, "A precise rate of within-couple transmission of zero" for gay men as well as for heterosexuals.

ANTIRETROVIRAL THERAPY (ART) The Panel on Antiretroviral Guidelines for Adults and Adolescents in 2018 doesn't recommend the use Dolutegravir in pregnant women. The Tsepamo surveillance in Botswana showed that Dolutegravir treatment at the time of conception is associated with a higher risk of neural tube defects in infants when compared to efavirenz. The Panel no longer prohibits the use of efavirenz during the first trimester of pregnancy.

Bictegravir (BIC) is a new HIV-1 integrase strand transfer inhibitor (INSTI) that has been approved in 2018 by the U.S. Food and Drug Administration for initial therapy in adults with HIV as part of a single-tablet, once-daily regimen that includes tenofovir alafenamide and emtricitabine (BIC/TAF/FTC). On the basis of clinical trial results, the Panel recommends the use of BIC/TAF/FTC 50/25/200 mg once daily as one of the Recommended Initial Regimens for Most People with HIV (AI). A two-drug combination of dolutegravir (Tivicay) and lamivudine suppressed viral load as well as a standard three-drug antiretroviral regimen for people with HIV starting treatment for the first time, according to results from the GEMINI studies. On 16th May 2018, the European Commission (EC) approved Juluca (a combination pill containing dolutegravir and rilpivirine) for the treatment of human immunodeficiency virus type 1 (HIV-1) infection in adults but only as a switch option for people with suppressed viral load.

CURE The researchers' findings regarding the ,shock and kill' approach to curing HIV are disappointing. They discovered that a remarkably small number (less than 5%) of latently infected cells are reactivated using LRAs (latency-reversing agents). There is a need to explore other strategies to control the latent HIV reservoir.

HIV INFECTION
SESSION V

20 YEARS OF HIV INFECTION IN AIDS CENTER CLUJ

CRISTIAN JIANU[1], IRINA FILIPESCU[2], ADRIANA TOPAN[3], CORINA IT-MURESAN[2], SORANA BOLBOACA[4]

[1]Clinical Hospital of Infectious Diseases, Medical Informatics and Biostatistics, Department 12 – Medical Education, Faculty of Medicine, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Immunodepresion, Cluj-Napoca, Romania
[2]Clinical Hospital of Infectious Diseases, Immunodepresion, Cluj-Napoca, Romania
[3]Iuliu Hatieganu University of Medicine and Pharmacy Cluj-Napoca, Immunodepresion, Cluj-Napoca, Romania
[4]Medical Informatics and Biostatistics, Department 12 – Medical Education, Faculty of Medicine, Iuliu Haţieganu University of Medicine and Pharmacy, Medical Informatics and Biostatistics, Cluj Napoca, Romania
*Corresponding author: *e-mail:cristian_jianu@yahoo.co.uk*

Objectives. In Romania, on 31st December 2017 there were 15009 patients living with HIV infection [1]. I studied the characteristics of patients with HIV infection from the AIDS Center Cluj between 1997-2017.

Materials and methods. I realized a retrospective cohort study of 704 patients which were monitored in our hospital, invastigating for age, gender, way of transmission and antiretroviral treatment.

Results. AIDS Center Cluj enrolled patients from 5 counties: Cluj, Salaj, Maramures, Satu-Mare and Bihor. From 704 patients included in this study, 565 are in active follow-up, 95 were deceased and 44 were lost from follow-up. From the 565 patients in active monitoring, 213 (37.69%) are female and 352 (62.31%) are male. The medium age is 37.08 years old. The main way of transmission was the sexual one (226- 40% heterosexual, 321- 56.81% MSM). 3.19% belongs to Romanian Cohort. 538 (95.22%) patients are treated, 27 (4.78%) refused antiretroviral treatment. From 44 patients were lost from evidence, 6 were referred to other AIDS Centers or established in other countries. We had 95 (13.49%) deceased patients: 72 (75.78%) had diseases associated with HIV infection and 23 (24.22%) had other causes of death.

Conclusions. The number of patients increased in the last years due to increase the addressability. The way of transmission is the sexual one. Over 90% of patients are in treatment with antiretroviral drugs. The main cause of death in these patents is HIV related.

MORTALITY BY CAUSES IN HIV INFECTED PATIENTS: A TWENTY YEARS TIME EVOLUTION (1998-2017) IN THE CLUJ HIV/AIDS REGIONAL CENTRE, ROMANIA

ADRIANA TOPAN[1], CORINA ITU-MURESAN[2], CRISTIAN JIANU[2], IRINA FILIPESCU[2], MIHAI CALIN[3], MIHAELA LUPSE[1]

[1]Infectious Diseases/Medical Specialties Department, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania
[2]Hospital of Infectious Diseases, Infectious Diseases, Cluj-Napoca, Romania
[3]Hospital of Infectious Diseases, Social worker/Psychosocial Care, Cluj-Napoca, Romania
*Corresponding author: *e-mail: topanadriana@yahoo.com*

Objectives. The knowledge of the causes of death in HIV infected patients is important for targeted interventions. In the late HAART era, non AIDS related diseases tend to replace AIDS related deaths due to the increased life expectancy and ageing. The aim of this study is to evaluate the evolution of mortality causes in HIV patients during the last 20 years.

Materials and methods. Data were collected from the medical death records of HIV infected patients from the Cluj HIV/AIDS Regional Centre. We compared mortality causes in HIV infected patients between two decades 1998-2007 and 2008-2017. Fisher exact test was used with statistical significance p.

Results. We recorded 127 deaths, 54 cases in the first decade and 73 cases in the second decade. The probable route of transmission was sexual in 89 cases, parenteral from Romanian cohort in 33 cases, vertical in 3 cases, IDU in 1 case and unknown in 1 case. The average age at death time was 32.18 years for the first decade vs.35.68 years in the second decade, (p=0.10). CD4 at baseline was available in 103 cases (81.10%), with late presentation (CD4).

Conclusions. The average survival time from diagnosis to death has increased in the last decade. There is a significant increasing trend in non AIDS related deaths due to comorbidities in the last decade. Unfortunately, in our area, deaths due to opportunistic infections and tuberculosis still remain important.

Keywords: HIV, mortality, trend

MANAGEMENT OF THE HIV PERINATALLY EXPOSED CHILDREN IN ROMANIA

MARIANA MARDARESCU[1], CRISTINA PETRE[1], CARINA MATEI[1], ALEXANDRA MARDARESCU[2]

[1]National Institute for Infectious Diseases "Prof. Dr. Matei Bals", Pediatric and Adolescents' Immunodepression Department
[2]National Institute for Infectious Diseases "Prof. Dr. Matei Bals", Romanian HIV/AIDS Center
*Corresponding author: *e-mail: mardarescum@yahoo.com*

Mother to child transmission of HIV can be reduced by applying preventive strategies in both mothers and newborns. In Romania, since the early 2000s the rate of vertical transmission has decreased significantly, the latest percentages, at 31 December 2017 being 2.2% [1]. This value is due to the implementation of a national mother to child transmission programme.

National intervention methods for the reduction of perinatal acquired HIV are based on a few basic principles, that contain among others: universal testing of all pregnant women, ARV prophylaxis during pregnancy and birth, the newborn's ART prophylaxis, scheduled caesarean section, formula feeding, assessment and monitoring of the child for rapid determination of the HIV status, assessment of mothers' co-infections and the newborns' monitoring for long and short term toxicities due to ART exposure, evaluation of the mother's or family's social status. The methods are applied by a multidisciplinary team, namely: infectious diseases specialist, paediatrician, laboratory, psychologist, social worker, general practitioner.

Management of the HIV perinatally exposed newborn includes rapid virological assay in order to obtain an HIV status, based on the recommendations of national and international guidelines. Children with a confirmed HIV status will start immediately ART treatment and will be monitored periodically. In terms of feeding, all newborns will receive only artificial formula, given that breastfeeding represents an essential risk factor of HIV transmission.

All newborns with perinatal exposure to HIV benefit from prophylaxis as a measure of reduction in the rate of vertical transmission. Prophylaxis is administered from the first 6-12 hours of life (in maternity), the selection of the prophylactic regime depending on the risk factors associated to both mother and child, in accordance with the national therapeutic algorithms. In terms of monitoring, the newborn undergoes regular assessment for possible toxicities associated to prophylaxis. Furthermore, the child is evaluated for maternal co-infections.

Vaccination of the perinatally exposed newborn follows the national vaccination plan, approved by Ministry of Health, except for BCG vaccination whose administration is postponed until the 2 months' virological assay.

Given that the main objective is to maintain a low rate of perinatal HIV transmission in our country, it is essential to integrate all clinical, social and counseling parties by applying as coherently as possible, the necessary prophylactic measures.

OUTCOME OF NEWLY DIAGNOSED HIV PATIENTS: RETROSPECTIVE COHORT STUDY IN A SINGLE TERTIARY CARE CENTER

ANCA CRISTINA DAMALAN, RALUCA JIPA, ELIZA MANEA, ANA MARIA CIOTI, CRISTIAN NICULAE, ADRIANA HRISTEA

National Institute of Infectious Diseases ,"Prof. Dr. Matei Bals", Infectious Diseases, Bucharest, Romania
*Corresponding author: *e-mail: scoicaru.anca@gmail.com*

Objectives. Since the introduction of the highly active antiretroviral therapy (HAART) HIV morbidity and mortality have decreased substantially. Between 2012-2016, in Romania, 997 people died from HIV-related causes [1]. The aim of the study was to evaluate the epidemiological and clinical characteristics of newly diagnosed HIV infected patients and to assess risk factors for death.

Materials and methods. We retrospectively studied patients over 18-year-old notified in our institution between January 2012 and December 2013, and found 499 out of 727 newly diagnosed patients in Bucharest. Late-presenters (LP) were defined as patients presenting with CD4 T-cell count below 350 cells/mm3 or with an AIDS defining event. Patients with advanced HIV disease (AHD) were defined as persons with a CD4-T cell count bellow 200 cells/mm3. Virological failure (VF) was defined as HIV viral load (VL) >200 copies/ml at 6 months after starting therapy in people not previously on HAART [2]. Differences between groups were analyzed using the Mann-Whitney U test for continuous variables and the chi-square test for dichotomous variables.

Results. Twenty-one patients were lost from the follow up. Out of 478 patients included, 350 (73%) were male. The median age was 30 (IQR 26-36). Two hundred ninety-three (61%) were LP and 177 (37%) were patients with AHD. The median CD4 and CD8 count were 291 cells/mm3 (IQR 125-485) and 845 cells/mm3 (IQR 523-1275), with a median CD4/CD8 ratio of 0.304 (0.153-0.507). The median HIV viral load was 99829 copies/ml (IQR 33982-275667). HAART was started in 382 (80%) patients. Fifty-five (18%) patients died during the study period. VL result between 6 – 24 months after starting HAART was available in 291 (60%) patients. Seventy-nine (27%) of these patients had VF and eight (11%) of them died. No statistically significant difference was found between patients who died vs patients who survived, except for CD4-T cell count.

Conclusions. More than half of newly HIV diagnosed patients in our hospital were LP. Death in HIV infected patients was associated with a worse clinical and immunological status, as well as the lack of HAART.

HIV INFECTION IN ROMANIA- 33 YEARS SINCE THE FIRST REPORTED CASE

MARIANA MARDARESCU

Pediatric and Adolescents' Immunodepression Department, National Institute for Infectious Diseases "Prof. Dr. Matei Bals", Bucharest, Romania
*Corresponding Author: *e-mail: mardarescum@yahoo.com*

ECDC's annual report on the status of HIV epidemic in Europe, released in November 2017 states that during 2006-2016 the trend of diagnoses was stable with reports from 6.8 and 6.9/100.000 in the earlier period of surveillance to 5.9/100.000 in 2016 [1]. In this context, besides interventions and financial investment in the National HIV/AIDS programme, Romania has also adapted and implemented ECDC's monitoring tool - Cascade of Care.

This monitoring system helps identify the inconsistencies between the numbers of diagnosed and undiagnosed persons, access to public medical services, success and gaps in the specific national interventions using the Continuum of Care monitoring tool.

Each stage of the continuum was adapted and applied to the statistical data centralized annually in the National HIV/AIDS Data Base. Given our country's specific epidemic, the essential factors that help perform a correct evaluation of the HIV cascade of cares in Romania are: the Romanian Cohort infected in the late 1980s and early 1990s, with multiple ART schemes (33% more than three therapeutic schemes) and therapeutic fatigue; new HIV cases detected in young people in their fertile age with low CD4 count at the time of diagnoses (<350 cel/mm3) and who represent approximately 50% of the total number of cases; men who have sex with men (20.08% of the new cases in 2017), injecting drug users (13.18% from the total new cases diagnosed in 2017) and young mothers [2].

Thus the numbers reflected by the continuum stages in Romania are: 15009 people living with HIV/AIDS (83% from UNAIDS estimates of 16.000-18,000) registered in the national HIV/AIDS Data Base, of these 12806 (85%) persons are in active records, of these 11570 (92%) are under ART and finally from the latter- 7386 (68%) are virally suppressed (<50 copies/mL) [4]. From the overall 15009 patients in life, 5500 (37%) come from the Romanian cohort, non-vertically infected.

Given the desirable percentage of 74% virally suppressed of all people living with HIV [1], Romania's interventions should be adapted to current epidemic status focusing on: the correct management of long term survivors, pre-exposure prophylaxis as means to prevent HIV transmission (which has the potential to reduce the risk of HIV to seronegative partners), pregnant women and perinatally children exposed, optimal therapeutic regimens for children, correct assessment of drug-drug interactions, multidisciplinary teams and maybe the most important one- universal HIV testing for all vulnerable groups.

Keywords: HIV, cascade of care, cohort

IMMUNOTHERAPY
SESSION VI

ORAL AND TOPICAL IMMUNOTHERAPY IN VARIOUS INFECTIONS

DUMITRU CARSTINA[1], CRISTIAN JIANU[2], SERBAN TOMESCU[2]

[1]Infectious Diseases Department, Clinical Hospital of Infectious Diseases, Cluj-Napoca, Romania
[2]Immunodepression Department, Clinical Hospital of Infectious Diseases, Cluj-Napoca, Romania
*Corresponding Author: *e-mail: dcarstina@personal.ro*

Objectives. In an age when microbial resistance to antibiotics is steadily increasing, it becomes very important to find alternative or adjunctive therapy. Under these conditions, immunotherapy returns as a complementary component or even as replacement of the antibiotic treatment. In this paper, there are arguments in favor of the beneficial effect of immunotherapy.

Materials and methods. Antibodies are obtained by vaccination from the serum of animals as well as from their products (colostrum from cows and IgY from the egg yolk of vaccinated hens). Antibodies obtained in colostrum and egg yolk have similar effects to those obtained traditionally. Colostrum obtained from vaccinated cows during pregnancy contains a large amount of specific IgG (hyperimmune colostrum). These antibodies are gastro-resistant and maintain their effectiveness throughout the digestive tract. They can be pharmaceutically formulated into tablets, capsules, powders. They have been used successfully in the treatment of diseases such as: dental infections (including dental caries), helicobacter pylori infections, rotavirus infections, and favorable results in clostridium diffusion infection in the case of animal experiment. Antibodies obtained from egg yolk - IgY (by vaccination of hens) have proven remarkable qualities and efficacy. They have been successfully applied in diseases such as: pseudomonas infections (IgY anti-pseudomonas), in pulmonary cystic fibrosis, lingering skin infections and infected burns, including cosmetic treatments, dental issues (IgY antistreptococcus mutans), helicobacter pylori infections (gastritis, ulcer), E. coli enteric infections, salmonella, and particularly rotavirus infection, with excellent results.

Results. The results reported in the literature recommend immunotherapy with these antibodies as an adjuvant or alternative to antibiotics.

Conclusions. Immunotherapy in infections comes back to topicality especially by new preparations containing specific antibodies.

INFECTION – A MULTISPECIALITY APPROACH SESSION VIII

CLINICAL MANIFESTATIONS OF MENINGOCOCCAL INFECTION: IS IT ALWAYS NEUROTROPIC?

NICULAE CRISTIAN-MIHAIL[1], CORINA CIONT[1], GIULIA STAN[1], ANCA DAMALAN[1], RUXANDRA MOROTI[2], ADRIANA HRISTEA[2]

[1]National Institute for Infectious Diseases Matei Bals, Infectious Diseases, Bucharest, Romania
[2]National Institute for Infectious Diseases Matei Bals, University of Medicine and Pharmacy "Carol Davila", Infectious Diseases, Bucharest, Romania
*Corresponding author: *e-mail: niculae_cristian87@yahoo.com*

Objectives. Neisseria meningitidis infection (NMI) is a significant cause of morbidity and mortality especially in children/young adults worldwide due to sporadic or epidemic meningitis and/or fulminant meningococcemia. In Europe, most cases of invasive disease are caused by serogroup B. The aim of our study was to describe the clinical characteristics of meningococcal infection.

Materials and methods. This is a retrospective analysis based on reviewing data of adult patients diagnosed with NMI between January 2012-April 2018 in our hospital. We included patients with clinical signs of invasive infection and a positive microbiological diagnosis from blood/CSF or CSF PCR (PLEX-ID, IRIDICA system).

Results. We included 21 patients with invasive NMI, six males and 15 females, median age of 47 (17-87) years. We identified 14 cases of acute meningoencephalitis and seven cases with non-neurotropic infection: four patients with acute benign febrile purpura and three patients with septic shock (including two cases of fatal Waterhouse-Friderichsen syndrome). Neisseria meningitidis serogroups were B (7), C (2), D (1), A (1) and W-135 (1). Onset symptoms were ranging from 5-36 h, fever being present in 89% of patients. Patients with non-neurotropic vs neurotropic infection were younger healthy adults (median age of 38 vs 50 years, $p=0.2$), had a mean leukocyte count of 21.000 vs 19.000/mm3, mean platelet count of 119.000 vs 142.000 mm3 and mean procalcitonin values of 86 vs 17 ng/mL ($p<0.05$). All patients with febrile purpura had a preceding flu-like illness a few days before, during the cold season (November-March).

Conclusions. Meningococcal infection is a serious clinical challenge. Acute invasive disease, caused especially by serogroup B meningococcus is manifested mainly as meningitis, but meningococcemia is another threat. Febrile purpura even in otherwise young healthy adults should raise the suspicion of Neisseria meningitidis invasive infection and prompt diagnosis and urgent antibiotic therapy is needed.

CLINICAL OBSERVATIONS AND RESISTANCE PROFILE FOR INFECTIONS WITH *ACINETOBACTER BAUMANNII*

I. DIACONESCU, L. GIUBELAN, ADINA TURCU, IRINA NICULESCU, MIRELA CIUCU

Clinical Hospital of Infectious Diseases Craiova, Romania

Nosocomial infections and resistant multidrug bacteria are major health problems and challenges for current medicine. Their knowledge through permanent local analyzes is fundamental to an informal and responsible attitude.

We proposed to analyze the types of infections and the resistance profile for *Ainetobacter baumannii.*

Material and method. We analyzed the isolates from the clinical hospital of infectious diseases in Craiova between 2017-2018. Identification and antibiogram were performed using Vitek technology.

Results. 19 strains of Acinetobacter baumannii were isolated. The majority were from sputum (665); from urine 13%, hemocultures 13%, wound 7%.

The sensitivity was:

Colistin 83%, Rifampicin: 75%, Minocycline: 83%, Levofloxacin: 35%, Ciprofloxacin: 21%, Gentamicin: 31%, Tobramycin: 68%, Imipenem: 25%, Ceftazidim: 10% :Cefepime: 26%, Aztreonam: 8%, Ticarcillin: 5%, Cotrimoxazole: 22%.

Ampicillin, cefazolin, ceftriaxone, nitrofurantoin: 0%.

For one of the two strains isolated by haemoculture, resistance was for all antibiotics tested (including colistin imipenem, rifampicin, aminoglycosides)

Conclusions. The resistance to *Acinetobacter baumannii* was major for most antibiotics. The only active antibiotics (but not totally!) are colistin, minocycline and rifampicin.

ASPECTS OF CYTOMEGALOVIRUS INFECTIONS IN PATIENTS WITH CARDIAC TRANSPLANT

BRINDUSA TILEA[1], MARIUS POPOVICI[2], SIMONA HORHAT[2], MIHAELA ISPAS[3], HORATIU SUCIU[4], IOAN ANDREI TILEA[5]

[1]University of Medicine and Pharmacy Tirgu Mures, 2nd Infectious Diseases Clinic, Mures County Clinical Hospital, Infectious Diseases, M4 Department, Tirgu Mures, Romania
[2]2nd Infectious Diseases Clinic, Mures County Clinical Hospital, Infectious Diseases, Tirgu Mures, Romania
[3]Emergency Institute of Cardio-Vascular Diseases and Transplant, Cardiology, Tirgu Mures, Romania
[4]Emergency Institute of Cardio-Vascular Diseases and Transplant, Cardio-Vascular Surgery, Tirgu Mures, Romania
[5]University of Medicine and Pharmacy Tirgu Mures, Tirgu Mures, Romania
*Corresponding author: *e-mail: brindusa_tilea@yahoo.com*

Objectives. Evaluation of prevalence of infections with cytomegalovirus (CMV) and correlation between the virus and the complications post-transplantation, appearance of the acute organ rejection and of the allograft vasculopathy and long term survival of this patients.

Materials and methods. Over a period of 5 years (2013 - 2018) 37 patients were followed up who had undergone orthotopic cardiac transplantation. The receivers were found with positive IgG-CMV antibodies. Were followed the infectious episodes at 1, 6, 12 months and the evolution based on the positivity or negativity of the screening for cytomegalovirus in correlation with infection, acute rejection, and allograft vasculopathy. Patients followed an immunosuppressive treatment with Micophenolate mofetil, Ciclosporin or Tacrolismus, Prednisone and prophylactic treatment with Valganciclovir for 12 months and in the periods where the IgM-CMV were positive. For diagnosis we used bacteriologic and virological techniques, endomiocardic biopsy, echocardiography, radiologic and computed tomography examinations.

Results. The mean age of the patients was 38.5 years. In the period of 1-6 months we identified 50 infectious episodes, with an appearance rate of 1.35 infections per patient. After 6 months, the rate was 1.83 infections per patient, with 68 infectious episodes and after 1 year appeared 22 infectious episodes with a rate of 0.59 infections per patient. Observing the correlation between IgG-CMV antibodies and bacterial infections, we discovered 2 pulmonary infections, 4 cases with upper respiratory tract infections, 3 cases with urinary tract infections, a single case of sepsis. 76.9 % of the patients with IgG CMV positive antibodies did not present infectious episodes. Regarding viral infections, of the 26 patients with IgG-CMV positive antibodies, 16 did not develop infection. There were six patients with IgM positive antibodies, 3 were diagnosed with viral pneumonia, 2 cases with Varicella-Zoster Virus and one with Herpes simplex virus. Eleven patients with IgG CMV negative antibodies did not develop any infection. Here we followed the correlation between the presence or the absence of IgG CMV and the acute organ rejection score. Nine patients with positive IgG antibodies had a rejection score of 1, 15 with rejection score of 0. The patients with negative CMV serology had 0 rejection score. Four vasculopathies were registered in the patients who had positive IgG CMV antibodies.

Conclusions. No reduction of survival was found in relation to the chronic infection with CMV, but the role of the infection in the appearance of the allograft vasculopathy was confirmed.

HIV-RELATED RENAL DISORDERS

CARMEN CHIRIAC, ANCA GEORGESCU, IRINGO ZAHARIA KEZDI , ANDREA INCZE , CRISTINA GÎRBOVAN, CRISTINA MĂNĂȘTUREAN

Infectious Diseases Department, University of Medicine and Pharmacy, Tg. Mures, Romania
*Corresponding author: *e-mail: carmen.l.chiriac@gmail.com*

Objectives. In the post HAART (Highly Active Antiretroviral Therapy) era HIV infected patients (PLHIV – Patients Living with HIV) face complex kidney damage which can compromise the quality of life, increase the risk of chronic kidney failure and mortality.

Materials and methods. Standard laboratory analysis was performed including urea, creatinine, proteinuria, lipid profile. eGFR (estimated glomerular filtration rate) was calculated. These were correlated with the immune status, the length of HIV infection and antiretroviral therapy.

Results. 95 PLHIV followed up in the 1st Infectious Diseases Clinic of Tg. Mureș were included in the study, 52 (54.73%) of them were females. The mean age was 26 years, the mean duration of HIV infection was >20 years in 16 patients (16.84%), 10-20 years in the majority of patients. 34.73% of patients had dyslipidemia, who also had associated kidney affection. 17.2% of patients were diagnosed with eGFR < 60 ml/min/1.73 mm2, 25% of patients were diagnosed with eGFR between 89-60 ml/min/1.73 mm2. Proteinuria was present in 10% of patients, it was correlated with low eGFR (p 0.021).

Conclusions. The majority of patients had stage II chronic kidney disease (CKD), correlated with dyslipidemia, proteinuria. It is important to follow up the kidney status of PLHIV using simple laboratory tests.

Keywords: HIV, chronic kidney disease (CKD), eGFR

COLONIZATION WITH MULTIDRUG RESISTANT BACTERIA IN AN INFECTIOUS DISEASES INTENSIVE CARE UNIT

TIMEA SZILAGYI, LUCIA HERBEL, DIANA MICLAUS, ADRIAN MUNTEAN, ADRIAN DICEA, MIRELA FLONTA, MIHAELA LUPSE

The University Hospital of Infectious Diseases Cluj-Napoca, Romania

Antimicrobial-resistant bacteria have become more widespread worldwide. Multidrug resistant (MDR) nosocomial infections are of increasing importance, especially in intensive care units, significantly raising mortality rates. Colonization with multi-drug resistant pathogens could precede nosocomial infections.

This study had two purposes. The first aim was to monitor colonization with antimicrobial-resistant microorganisms and the persistence of carriage of such pathogens among patients admitted to the Intensive Care Unit of the Hospital for Infectious Diseases in Cluj-Napoca. The second aim was to determine the incidence and the risk factors for nosocomial infections associated with these MDR pathogens. In order to assess the relation with nosocomial infections, we examined colonization with Extended-spectrum beta-lactamase producing Enterobacteriaceae (EN-ESBL), Carbapenemase-producing Gram negative bacteria (BGN-CBP), Vancomycin resistant Enterococci (VRE), Methicillin-resistant S. aureus (MRSA).

This study suggests that colonization could represent a risk factor for increased rates of infection. An active surveillance program may help in the identification of MDR bacteria colonized patients. Infection control strategies targeting these groups should be implemented to combat the rising incidence of nosocomial infections.

POSTER SESSION

SOCIAL AND DEMOGRAPHIC ASPECTS OF CARE ENGAGEMENT IN PEOPLE LIVING WITH HIV

CALIN GEORGE MIHAI[1], ADRIANA TOPAN[2], IRINA FILIPESCU[2], CRISTIAN JIANU[2], CORINA ITU MURESAN[2]

[1]Clinical Hospital for Infectious Diseases, Social Worker, Cluj-Napoca, Romania
[2]Immunodeficiency Department, Clinical Hospital for Infectious Diseases, Cluj-Napoca, Romania
*Corresponding author: *e-mail: mihai_calinn@yahoo.com*

Objectives. Patients commitment with the care provider is a vital condition in achieving optimal health status and in reducing disparities to health care services for people living with HIV [1,2]. The study had a cross-sectional design and its goal was to highlight the relationship between the level of care engagement of HIV patients and their social and demographic characteristics.

Materials and methods. We included in the analysis data about 538 patients reported on ART at the end of 2017. The study variables were gender, age, route of HIV acquiring, residence, occupational status, and education. Care engagement was measured by counting the number of visits to the hospital, patients with more than two visits per year were considered as engaged in care [3]. Variables were treated as dichotomous and the chi-square test was performed in order to find statistical significance.

Results. The study group consisted of 373 males and 165 females; distribution of patients after HIV acquiring route was 264 (50%) heterosexual, 161 (30%) MSM, 97 (18%) Romanian cohort, 16 (2%) vertical. Most of the patients were from urban communities, 394 (73%), 419 (77%) patients achieved at least a high school education and 297 (55%) patients were employed. 397 (73%) patients showed optimal engagement in care with more than two visits and 144 (27%) patients displayed low engagement to care with less than two visits. Contingency tables pointed out that 51 (35%) out of patients from rural areas had less than two hospital visits (p=.006); 44 (36%) out of patients with low education status were found in the group with low care engagement (p=.004); 68 (55%) out of heterosexual men had less than two hospital visits (p =.002).

Conclusions. The most significant difference found was between MSM patients and heterosexual male patients, the former group showed higher levels of care engagement, 85% vs 67%. This finding is related to the social profile of MSM patients, most of these patients having urban residence and at least high school education. On the other hand, heterosexual male patients are more likely to come from small communities where HIV people are stigmatized, which may prevent these patients from accessing medical services on a regular basis.

EVOLUTIONARY PARTICLE CHARACTERISTICS OF FUNGAL INFECTIONS IN A PATIENT WITH MULTIPLE CHRONIC CONDITIONS

MIRANDOLINA PRIȘCĂ[1], VALENTINA AVRAM[1], LENUȚA-VALERUȚA TIMIȘ[2], ELVIRA BLAJOVAN[1], IULIANA BOLOGAN[1]

[1]Infectious Diseases Unit, County Emergency Hospital, Arad, Romania
[2]Epidemiology, County Emergency Hospital, Arad, Romania
*Corresponding author: *e-mail: avamvalentinaelena@gmail.com*

Objectives. Presentation of a clinical case with the evolutionary features of fungal infections in a patient with multiple chronic conditions.

Materials and methods. A male patient, M.I, 64 years old, married, coming from the rural area was admitted between 16.03-08.05.2018 to the Medical II in Arad. On admission he complained of: mixed dysparea, dry cough, fatigue at small efforts, wheezing with onset about 2 weeks before and accentuation in the last 2 days. The patient becomes comatose, tachypneic, spontaneously breathing O2 on facial mask SpO2 = 84%, diminished vesicular murmur bilateral, bronchoalveolar bronchial, cyanotic extremities, BP = 130/75 mmHg, HR = 110 b / min Medical II.

Results. The laboratory tests evidenced: 13.300 WBC/mm3, RBC 6.55 mill/mm³, Hb 16.40 g% (10,10 g%) Ht 52,40% (32.70%), Thrombocytes 198.0 mii / mm³ (134.0), CHEM 31.30 (26.10), HEM 25.0 (30.90), Creatinine 0.97 mg / dl (0.54), Ureea 36.0 mg / dL (60.90). Uroculture - between 10,000-100,000 UFC / mL, fungi culture- Candida albicans, Hemoculture- Acinetobacter baumanni, Tracheo-bronchial Secretion-Enterobacter aerogenes. After hydroelectrolytic, acido-basal rebalancing and antibiotic therapy, according to antibiogram and antifungal, corticotherapy, bronchodilators, diuretics, gastric antisecretory, anticoagulants, antihypertensive clinical-biological evolution was slow, but favorable.

Conclusions. Patients with multiple chronic conditions represent a risk group for the occurrence of complications, superinfections, fungal infections, requiring rigorous clinical and biological monitoring, long-term and costly hospitalization, together with a complex and effective therapy.

CANDIDA ALBICANS INFECTION IN A PATIENT WITH SECONDARY PULMONARY TB AND CACHEXIA

LENUȚA-VALERUȚA TIMIȘ[1], MIRANDOLINA PRIȘCĂ[2], VALENTINA AVRAM[2], IULIANA BOLOGAN[2], ELVIRA BLAJOVAN[2]

[1]Epidemiology, County Emergency Hospital, Arad, Romania
[2]Infectious Diseases Unit, County Emergency Hospital, Arad, Romania
*Corresponding author: *e-mail: avamvalentinaelena@gmail.com*

Objectives. Presentation of a clinical case of Candida albicans infection in a patient with secondary pulmonary TB and cachexia.

Materials and methods. A male patient, B.P, 57 years old, coming from the rural area was admitted between 30.04-22.05.2018 County Hospital-section TB Arad . On admission he complained of: dry cough, weight loss, asthenia, weakness, low grade fever. On 17.05.2018 the patient's condition is altered, becoming comatose, vesicular murmur present with bilateral disseminated broncho-alveolar rallies, BP = 90/60 mmHg, HR = 140 b / min, cold skin, marbled, are passed on Intensive Therapy.

Results. 8.85 WBC/mm3 mm³ (22.43), RBC 2.66 mil / mm³ (3.46), Hb 8 g% (10.50 g%) Ht 25.00% (29.60%), Thrombocyte 457.0 mii / mm³, Neutrofile 69.70% (89.60), Lymphocyte 16.30% (4.70), VSH 84, LDH 237, Creatinine 1.43 mg / dl (1.25), Ureea 29.20 mg / dl (16.02), TGO 43 U / L TGP 29 U / L (64), K 2.4 mmol / L (2.6), Amylase 56 U / I (169), CPK 448 U / I (778), fungi culture-Candida albicans. Sput BK-POSITIVE. Despite supportive treatment with hydro-electrolytic rebalancing solutions, vasopressors, inotropes, antibiotics, anticoagulants, prokinetics, bronchodilators, the patient presents heart attack, the patient is not responding, declaring death.

Conclusions. Patients with chronic conditions are prone to complications, superinfections or fungal infections. They require rigorous clinical and biological monitoring and complex and effective therapy. Evolution is not always favorable, therefore a Candida albicans infection should be considered more often in the differential diagnosis.

GROWING OLD WITH HIV INFECTION

SIMONA MANUELA ERSCOIU[1], IONUT CRISTIAN POPA[1], CRISTINA PATRU[1], GRATIELA TARDEI[1], PETRE CALISTRU[2], EMANOIL CEAUSU[3]

[1]Clinical Hospital of Infectious and Tropical Diseases Dr.Victor Babes, HIV/AIDS, Bucharest, Romania
[2]Infectious Disease Department, University of Medicine and Pharmacy Carol Davila, Bucharest, Romania
[3]Clinical Hospital of Infectious and Tropical Diseases Dr.Victor Babes, B4, Bucharest, Romania
*Corresponding author: *e-mail: serscoiu@yahoo.com*

Objectives. According to the Center for Disease Control and Prevention (CDC), in 2014, an estimated 45% of Americans living with diagnosed HIV were aged 50 and older. Until now, limited information exists regarding HIV infection among elderly. While older persons are at significant risk for HIV infection, they are less included in prevention programs. The present paper analyzed clinical, immunological and viral status of HIV infection in the elderly diagnosed and followed up in our clinic during 1990 and 2010 in first period and until May 2018 in the second period. We studied the incidence of opportunistic infections (OI), cardiovascular, metabolic, neurology, malignancies and bone disorders, in connection with the older patients, treated by HAART. We assessed the survival duration and the cause of death.

Materials and methods. The retrospective study consists of patients diagnosed with HIV infection at the age of 55 for women and 60 for men, age of the beginning of retirement in 2010, in Romania. All investigations used in HIV diagnose and those characteristic of the elderly diseases.

Results. There were diagnosed 11 women and 18 men, mean age of 61 for women respectively 70 for men. The route of transmission of HIV infection was transfusion in 5 women and sexual in the others patients. The clinical and immunological stage at the moment of HIV diagnosis was C3 in the majority. HAART consisting of 2 to 5 regimens received all patients, on average 96 weeks after confirmation of seropositive status. The average survival was 144 months. OI were the cause of death in 13 of 21 dead patients. Cardiovascular disease had 22 patients, diabetes mellitus 15 patients, neurological disorders 5 patients, bone diseases 15 patients and others 7 had malignancy.

Conclusions. HAART management at an older age must be better studied. The interdisciplinary collaboration and a better social assistance must be implemented in this category of patients.

REZI-CASE SESSION

ETIOLOGY OF RECURRENT BACTERIAL MENINGITIS ASSOCIATED WITH CSF LEAK

CRISTINA-ALEXANDRA CHERAN[1], DOINA CLEMENTINA ANTONICA[1], RALUCA ELENA JIPA[2], SERBAN BENEA[2], ADRIANA HRISTEA[2]

[1]National Institute for Infectious Diseases „Prof. Dr. Matei Balş", Infectious Diseases, Bucharest, Romania
[2]National Institute for Infectious Diseases „Prof. Dr. Matei Balş", University of Medicine and Pharmacy „Carol Davila", Infectious Diseases, Bucharest, Romania
*Corresponding author: *e-mail: cristinacheran11@gmail.com*

Objectives. Recurrent bacterial meningitis is defined as two episodes of meningitis separated by full recovery. It may be caused by the same germ or different germs and it is a rare condition in the general population. It is frequently associated with anatomical defects, CSF leaks or immunological deficiencies. We aimed to discuss the predisposing condition and review the etiology of recurrent bacterial meningitis.

Materials and methods. We present the case of a 43-year patient, having a rhinopharyngeal tumor, stage IV A, for the last two years, who has undergone chemotherapy and radiotherapy, presenting with recurrent meningitis.

Results. The patient was transferred from oncology for fever, headaches, nausea, vomiting that started 2 weeks before, for which she has been treated with antibiotics. On admission, clinical examination showed meningeal syndrome. Laboratory tests showed an inflammatory syndrome and lumbar puncture showed 450 cells/mm3, 60% neutrophils, glucose level of 20 mg/dl (serum glucose 96 mg/dl), CSF protein level of 328 mg/dl, Latex-agglutination positive for Streptococcus pneumoniae. The patient was started on Ceftriaxone and Vancomycin, corticosteroids and mannitol, with a favourable outcome. On day seven of treatment the patient presented abundant rhinorrhea from the left nostril. Rhinoliquorrhea was suspected and the CT scan confirmed the diagnosis of CSF fistula as a result of the local tumour invasion of the sphenoidal sinus. When pneumococcal meningitis treatment was completed, the patient was sent in an otorhinolaryngology clinic for the CSF leak repair. After a month the patient returned to our clinic for headaches, fever, vomiting and abundant rhinoliquorrhea. The CSF examination showed a new episode of bacterial meningitis, but due to Haemophilus influenzae. The immunological deficiency associated with neoplasia may increase the susceptibility of the patient to encapsulated germs, although H. influenzae is rarely associated with recurrent meningitis secondary to CSF leaks.

Conclusions. CSF leak is the most incriminated condition associated with bacterial recurrent meningitis and is caused by anatomical defects, trauma and rarely by tumour invasion in the base of the skull. Although the most commonly involved bacteria in recurrent bacterial meningitis associated with CSF leak is Streptococcus pneumoniae other germs from the nasopharynx need to be considered.

SALMONELLA SPONDYLODISCITIS IN AN IMMUNOCOMPETENT PATIENT WITH SEPSIS - CASE REPORT

CRISTINA MANASTUREAN[1], CARMEN CHIRIAC[2], CRISTINA GIRBOVAN[2], CRISTINA BRUCKNER[1], ANCA GEORGESCU[2]

[1]Infectious Diseases Department, Mures Clinical County Hospital, Targu-Mures, Romania
[2]Mures Clinical County Hospital, University of Medicine and Pharmacy, Infectious Diseases, Targu-Mures, Romania
*Corresponding author: *e-mail: cris.mitre@yahoo.com*

Objectives. Salmonella spondylodiscitis is an uncommon complication of salmonella infection that occurs secondary to hematogenous spread after the bacteremia episode.

Materials and methods. We present the case of a 52 year old male patient with prolongued febrile illness and severe pain in his right upper quadrant, initially diagnosed with renal colic, urinary tract infection followed by an intestinal obstruction syndrome. Blood tests revealed positive inflammatory markers and procalcitonin level over 10 ng/ml. A series of blood cultures identified the pathogen-group B Salmonella. Antibiotherapy was administered along with symptomatic treatment, but the patient developed severe lumbar pain, thus an MRI was performed revealing spondylodiscitis, retrocrural abcesses and reactive retroperitoneal lymphadenopathy.

Results. After more than thirty days of intravenous antibiotherapy, our patient had a favorable evolution, no fever and partial remission of the lumbar pain. He was discharged and scheduled for a second MRI scan and a neurosurgical consult.

Conclusions. Even though prodromal gastrointestinal symptoms are not usually present, immunocompetent patients can also develop sepsis with Salmonella, and in rare cases it can be followed by complications such as spondylodiscitis and abscesses.

Keywords: Salmonella, sepsis, spondylodiscitis

ACUTE THROMBOTIC INFECTIVE ENDOCARDITIS AS COMPLICATION OF RECURRENT SEPSIS IN HEMODIALYSED PATIENT REQUIRING COMPLEX SURGICAL MANAGEMENT

CRISTINA BRUKNER, CRISTINA MANASTUREAN

Infectious Diseases Department, Mures Clinical County Hospital, Targu-Mures, Romania
*Corresponding author: *e-mail: cristina.brukner@yahoo.com*

Background. Infective endocarditis in haemodyalised patients with dysfunctional/ infected arterio-venous fistulas / catether, with persistent bacteremia can be a severe form of sepsis, witch imposes the surgical intervention and removal of the prosthetic material immediately, because the antibiotic treatment alone is not effective.

Case presentation. A 39 years old woman, with chronic haemodyalisis, right nephrectomy, coraliform lithiasis, 2 episodes of sepsis with Staphylococcus aureus methicilino-resistant, in the last 2 years, after axilo-brachial graft thrombosis, unfunctional left arterio-venous fistula, confirmed with mitral valve infective endocarditis with Staphylococcus aureus and persistent bacteremia despite etiologic treatment. After 3 weeks of treatment the patient had severe abdominal pain and was diagnosed echocardiographicaly with the detachment of the vegetation from the mitral valve and with thrombosed aneurysm of the superior mesenteric artery and secondary hepatic and splenic abscesses.

Results. In this context the removal of the left arterio-venous fistula graft was mandatory, with favorable clinical and biological outcome (negative blood cultures and graft cultures). After 1 month the patient was admitted again with the same symptoms and diagnosed with partial stenosis of superior mesenteric artery and had an aorto-mesenteric by-pass with Gore-tex prosthesis.

Conclusions. Patients with chronic haemodyalisis are predisposed for catheter or graft infection and bacteremia. One of the consequences is infective endocarditis, persistent bacteremia and subsequent complications despite antibiotic treatment. In this case surgery is mandatory. Particularly for this case was the unpredictable outcome, persistent bacteremia under treatment, the detachment of the vegetations, the secondary septic hepatic and splenic abscesses and the severe clinical manifestation with reserved prognosis. Despite the clinical suspicion of infectious outbreak at graft site, the graft cultures were negative, still after surgery the clinical outcome was favorable.

Keywords: acute infective endocarditis, haemodialysed patient, vascular graft thrombosis

MULTIPLE HEALTH CARE ASSOCIATED INFECTIONS IN A TYPE 2 DIABETIC PATIENT – CASE REVIEW

GABRIELA RAMONA SCÂNTEIANU[1], ANDREI VATA[2], IRINA TEODORESCU[1], CATALINA MIHAELA LUCA[2], FLORIN ROȘU[2], CARMEN DOROBĂȚ[1]

[1]Infectious Diseases Hospital, Iasi, Romania
[2]University of Medicine and Pharmacy, Iasi, Romania
*Corresponding author: *e-mail: gaby_ramona_costan@yahoo.com*

Objectives. Health care associated infections (HCAI) are a concern in hospitals from Romania and are part of a list of priority issues through the consequences they might generate.

Case presentation. We present the case of a 77-year-old female patient with a significant history of type 2 Diabetes, cardiovascular comorbidities, who is found at home with altered mental state, fever, vomiting and diarrheal stools. After she is seen by the neurosurgeon, internal medicine and diabetic specialist she is admitted in our hospital. Broad spectrum antibiotic treatment is started and the adjusted after a Group D Salmonella strain is found in his blood cultures. She develops acute urine retention and a vesical catheter is inserted. After a week she develops a watery diarrhea from which Cl. difficile toxins are identified and treatment with oral Vancomycin started. 10 days later the fever returns and Candida spp. and Enterococcus faecalis are isolated from the urine cultures and the treatment is adjusted accordingly. The clinical evolution is favorable, but the patient's family refuses to take her home. 7 days later she becomes febrile again, the clinical state deteriorates and Pseudomonas aeruginosa is isolated the hemocultures. She is retreated with antibiotics, the urinary catheter is removed and she is discharged home after 50 days in our hospital.

Conclusions. The patient had multiple HCAI probably due to an impaired immune system, antibiotic use, prolonged hospitalization and invasive procedures which were early recognized and successfully treated.

RECURRENT ENDOCARDITIS DUE TO CANDIDA PARAPSILOSIS

MIHAI-ARONEL RUS, AMANDA RADULESCU, MIHAELA LUPSE

Teaching Hospital of Infectious Diseases, Cluj-Napoca, Romania

Candida parapsilosis was first described in 1928, initially named *Monilia parapsilosis*. It resides on human skin and was initially considered a non-pathogenic yeast. However, in the past 30 years, there has been a significant increase in non-*C. albicans* blood stream infections, with *C. parapsilosis* having the most important incidence increase. *C. parapsilosis* has capacity to grow on biofilms, indwelling catheters and prosthetic devices, and also in total parenteral nutrition environment. Its spectrum of clinical manifestations includes endocarditis, fungemia, arthritis, peritonitis, endophthalmitis.

We report the case of a 69 years old patient with diabetes, who developed endocarditis with *Candida parapsilosis* on biologic prosthetic aortic valve. Appropriate antifungal treatment was administered and surgery was performed, replacing the biologic valve with a mechanical one. Four years later, the patient returns to the clinic with endocarditis of the mechanical prosthesis with the same etiology. The case, as it shows an increased time span between the two endocarditis episodes, underlines the capacity of *C. parapsilosis*, to produce torpid infections of prosthetic materials.

CYTOMEGALOVIRUS AND PNEUMOCYSTIS JIROVECI CO-INFECTION IN A PATIENT DIAGNOSED WITH EARLY HIV INFECTION

ANA-MARIA CIOTI, IRINA STEFANIA LAPADAT, ELIZA DANIELA MANEA, COSMINA GINGARAS, ADRIANA HRISTEA

National Institute for Infectious Diseases "Prof. Dr. Matei Balş", Bucharest, Infectious Diseases, Bucharest, Romania
*Corresponding author: *e-mail: ciotiana_maria@yahoo.com*

Objectives. Presenting a case of Cytomegalovirus and Pneumocystis jiroveci co-infection in a patient diagnosed with early HIV infection Emphasizing the importance of close clinical monitoring during the first weeks after ART initiation in patients with low CD4 cell count.

Case presentation. Case report of a 27-year-old male diagnosed with early HIV infection.

Results. The patient was hospitalized for left paracardiac pneumonia and minimal pleural effusion and HIV-testing revealed early HIV infection (stage VI). The patient was started on antiretroviral therapy (ART) (Emtricitabine/Tenofovir/Raltegravir), while treating the lower tract respiratory infection with Ceftriaxone 2g iv. At diagnosis CD4 count was 138 cells/mm3 and viral load 260000 UI/mL). The fever continued and the chest X-ray performed nine days later revealed an abscess in the left lung and new lesions in the right lung. A bronchoscopy was performed and the polymerase chain reaction for tuberculosis as well as the cultures for usual germs were negative. The fever persisted despite the antibiotic treatment and the X-ray showed the expansion of the right lung processes as multiple foci of bronchopneumonia. The bronchoscopy was repeated and bronchoalveolar lavage cytology showed CMV cytopathic effects and P.jirovecii in the trophozoite stage. The patient was started on intravenous ganciclovir, oral trimethoprim-sulfamethoxazole, corticosteroids and he gradually improved. Unmasking immune reconstitution inflammatory syndrome (IRIS) could be discussed to explain the worsening of pulmonary lesions at two weeks after ART initiation, although IRIS commonly appears in patients with advanced immunosuppression.

Conclusions. The concomitant P.jirovecii and CMV infections are rare, especially in early HIV infection. Close clinical monitoring during the first weeks after ART initiation in patients with low CD4 cell count is needed.

FAMILIAL Q FEVER CLUSTERING

CRINA DIACONU, ANDREEA CIOARA, MIHAELA LUPSE

University Hospital of Infectious Diseases Cluj-Napoca, Romania

Q fever is a zoonosis caused by Coxiella burnetii. The main reservoirs are cattle, sheep and goats. Human infections occur after the inhalation of infected aerosols, but also after the consumption of dairy products from infected animals or by tick bites; transmission between humans is rare, although it can happen. Primary infection can be asymptomatic or usually manifested as febrile syndrome, pneumonia, hepatitis, which can progress to a chronic disease, most frequently endocarditis or vasculitis.

A 23 years old man from Gilau was consulted by a doctor for high fever, chills, sweating, headaches, myalgia. He lives at the country-side with his aunt and uncle, where they raise goats and they all consume raw goat milk. Considering that the uncle and the aunt had similar symptomatology, we hospitalized all the family members for investigations and adequate treatment. Even though the phase II Coxiella burnetii IgM antibodies were not detectable during hospitalization, the cases were interpreted as a possible familiar Q fever clustering, with immediate initiation of appropriate antibiotic therapy. After 2-3 weeks, the phase II Coxiella burnetii IgM antibodies were detectable in all three patients.

This case illustrates the importance of establishing the diagnosis of Coxiella burnetii infection when there is a high clinical and epidemiological suspicion even though the Coxiella burnetii antibodies are not detectable at the moment of examination, in order to treat and prevent progression to chronic disease.

IS HBS ANTIGEN EVIDENCE ALWAYS NECESSARY IN THE DIAGNOSIS ACUTE HEPATITIS B?

EMOKE ANTAL, ANDREEA CIOARA, MIHAELA LUPSE

The University Hospital of Infectious Diseases Cluj-Napoca, Romania
*Corresponding author: *e-mail: adrianamuntean77@gmail.com*

In the last decades the problematic of acute and chronic Hepatitis B infection was subject of several medical studies written by Romanian researchers and fellow colleagues from other countries. Studying the complex problematic of Hepatitis B screening – an ambitious, albeit difficult undertaking – offers a deeper understanding why high clinical suspicion is important, emphasises the reason for vigilance in screening and diagnosing various forms of Hepatitis B infection.

The aim of this presentation is to describe the process of diagnosing a case of a patient with severe acute hepatitis of unknown etiology, transferred and treated in The University Hospital of Infectious Diseases Cluj-Napoca. It is also an opportunity to discuss questions linked to screening of the disease, specially focused on detecting further familiar contacts and debating the need for vaccination or not in these cases.

This case study could provide a major signal for practitioners that they should reconsider to attempt diagnosing Hepatitis B infection if high clinical suspicion exists.

DIFFERENTIAL DIAGNOSIS BETWEEN STEVENS JOHNSON SYNDROME AND MEASLES DURING MEASLES OUTBREAK

LAURA ALBU, MIHAELA SABOU, LUCIA HERBEL, MIHAELA LUPSE

Infectious Diseases Clinical Hospital of Cluj-Napoca, Romania

Stevens Johnson Syndrome is a rare severe adverse cutaneous drug reaction. Rarely it could be caused by infections. There are to date no reported cases of Stevens Johnson Syndrome associated with measles. We presented the case of a 32 year old female who presented, during a measles outbreak, with fever, upper respiratory tract symptoms, followed by a generalized skin rash with bullae formation and epidermal detachment, erythema and ulcerations of the mucous membranes, after consumption of acetaminophen, sodium metamizole and drotaverine. Specific IgM serum antibodies for measles were positive. This case underlines the similarities between these two pathologies at their onset.

CRYPTOCOCCUS NEOFORMANS MENINGITIS IN A NON-HIV- INFECTED PATIENT

MADALINA TUNS, CRISTINA CISMARU, ADRIAN DICEA, MIHAELA LUPSE

Infectious Diseases Clinical Hospital of Cluj-Napoca, Romania

Cryptococcus neoformans continues to be an important cause of morbidity and mortality and despite of being the most frequent central nervous system mycosis in immunocompromised patients, there are an important number of cases in those who are phenotypically normal.

We presented the case of a young man 25 years old, with no medical history, who developed a meningitis with *Cryptococcus neoformans*. He had no HIV infection and no immunosuppression could be found. He was treated with Amphotericin B and fluconazole with good outcome. We are bringing news about this opportunistic fungal infection, in a non-HIV, non-transplant patient, emphasizing on the diagnosis, the complications that come along with the disease and treatment.