Dear participant,

Welcome to the very first PhD retreat of the Amsterdam Infection & Immunity Institute! You have two days filled with interesting talks, a mind-blowing activity, fun poster sessions and an awesome party ahead of you. You can find further details on the program on page 7, while the program at a glance is at the back of this abstract book.

This retreat has been organized by seven of your fellow PhD students from the VUmc, AMC and Sanquin. Nicki is a second year PhD student in the field of Rheumatology and Nuclear Medicine. She investigates the role of PET imaging for early diagnostics and therapy monitoring in patients with rheumatic diseases. Maartje is a third year PhD student in the Host Defense group of professor Geijtenbeek. She investigates the role of Langerhans Cells in HIV-1 and HCV infection and transmission. Charlotte, also a third year PhD student, investigates differences and similarities between skin and oral mucosa immunology in human immune competent skin and oral mucosa equivalents in the VUmc dermatology lab of Sue Gibbs. Reza is a PhD student in the lab of prof. Reina Mebius at the department of molecular cell biology and immunology at the VUmc. His work focuses on the functional development of the immune system. The last three members are all fourth year PhD students. Abilash is from the Lung group headed by Rene Lutter in the Experimental Immunology department of the AMC. He investigates the role of bronchial epithelial cells in the pathophysiology of asthma and COPD. Anna works in the compliment lab of Dr. Diana Wouters at Sanquin, but also under supervision of prof. Taco Kuijpers. She studies complement factor H and factor H-related proteins in the context of bacterial infections in children. Saskia studies the role of secreted proteins on B and T cell differentiation through high-throughput screens in the group of Dr. Robbert Spaapen at Sanquin.

While we all work on very interesting projects, we will not be presenting our work during this retreat. But we will be present to answer your questions and guide you through this retreat. We hope you will enjoy the next few days!

The PhD retreat 2017 committee,
Abilash Ravi, Anna van Beek, Nicki Verweij, Charlotte Rodrigues Neves, Saskia van Asten, Maartje Nijmeijer and Reza Nadafi
Word of welcome

We hereby would like to welcome you to the first PhD retreat of the Amsterdam Infection & Immunity Institute (AI&II). Our young Institute was founded in 2017 as part of the Alliance between AMC and VUmc, and the need for a research institute on Infection & Immunity in Amsterdam. The institute focuses on three research programs: infectious diseases, inflammatory diseases and cancer immunology. As will become clear during these two days, these programs often share similar underlying principles. For example, immunosuppression occurs during viral infection but also cancers create an immunosuppressive environment and these mechanisms could be shared.

We therefore believe that it is important to create and support collaborations between the researchers within the three programs, and the AI&II PhD Retreat is one of our most important tools to not only stimulate interaction/collaborations, but also to learn from each other’s research and to have fun doing so. As PhD students, you belong to the largest group of researchers not only within our institute (over 500 PhD students) but also compared to other institutes within our AMC-VUmc Alliance. You are therefore crucial to seed future research within Infection & Immunity.

Our Institute brings together more than 1000 researchers with about 100 Principal Investigators and over 500 PhD students from not only AMC and VUmc but also from our strategic partners such as Sanquin, Reade, GGD and Amsterdam Institute of Global Health and Development. Together we cover the full spectrum from fundamental to clinical research, with a strong emphasis on multidisciplinary approaches and translational research.

As you will learn over the next days, we do research on topics that include the unravelling of normal immune responses, the epidemiology and immunopathology of infectious diseases such as tuberculosis and HIV, sepsis and respiratory infections, the immunopathology of various chronic inflammatory disorders (e.g. rheumatoid arthritis, asthma, allergy, inflammatory bowel disease), as well as the immunological processes that occur during cancer and are used to treat cancer.

We believe that our Institute will become very important for the national and also international outreach of Infection & Immunity research. AI&II offers many opportunities for you as young researchers for interacting with international excellent scientists at seminars and masterclasses, attending meetings and visiting international groups to learn techniques or collaborations. We will also start speed dating sessions between all researchers of the institute to stimulate interactions and collaborations. Thus, always visit our website to check out new opportunities!

We would really appreciate your involvement in our Institute and your ideas on how we can help you in your development as a professional and to make a success of your research. Therefore we will set up a PhD student committee that will steer us directors and the board of program leaders in facilitating you in your careers and research. So if you are interested, let us know.

We hope that you will have an excellent and fun PhD Retreat in lovely Heemskerk.

Best wishes

Yvette van Kooyk & Theo Geijtenbeek

Directors of the Amsterdam Infection & Immunity Institute
Keynote speakers

Prof. Dr. Maria Yazdanbakhsh

Maria Yazdanbakhsh studied Medical Parasitology at the London School of Hygiene and Tropical Medicine and thereafter did her PhD at Amsterdam University on Immunobiology of eosinophils, which in part was based on studying parasites. After a post-doctoral fellowship, studying molecular biology of parasitic helminths at Imperial College London, she started her own laboratory at the Department of Parasitology, in Leiden University. She has spent sabbatical time at the London School of Hygiene and Tropical medicine taking short courses in epidemiology and statistics. After heading the Leiden Immunoparasitology Group, she became the head of the Department of Parasitology in 2015.

Maria Yazdanbakhsh is the president of Dutch Society for Parasitology. She has served in various national scientific committees including TOP/ZonMW, VICI, ALW and WOTRO. At the LUMC she is a member of the Scientific Advisory Board, the management team of medical profile area Immunity, Infection and Tolerance as well as committee on internationalisation. She has received the Leyton Science Research Award, Marie Curie fellowship in 1987, MSD Award in Human Parasitology in 1997 and will deliver the Leiden University Dies lecture in 2017.

Alongside regular curricular teaching, Maria Yazdanbakhsh has placed much emphasis into training biomedical and medical students not only from the Netherlands and Europe but also from developing countries. She has developed and coordinates the half minor/elective program for (bio)medical students at LUMC.

Prof. Dr. Frank Miedema

Frank Miedema studied biochemistry at the University of Groningen, specializing in immunology. As Divisional Manager at the Central Laboratory of the Blood Transfusion Service (CLB) he was responsible for such things as education and research, before going on to become Director of Sanquin Research. Miedema was affiliated with the University of Amsterdam as professor of Immunology of AIDS. In 2004 he became head of the Immunology department at UMC Utrecht. Miedema is a member of various national and international scientific organizations and advisory committees. He has published hundreds of articles in medical journals, including Nature, Science and Lancet, and is one of the initiators of www.scienceintransition.com. The initiators of Science in Transition believe that the scientific system is in need of fundamental reform. Science should be appreciated for the added value it contributes to society and stakeholders in society must participate in decisions regarding the production of knowledge.
Notes:
Program
Thursday 5th October

08:30 - 09:15
registration
bags can be stored in the bagage room

09:15 - 09:20
Opening
lecture hall 1

09:20 - 10:20
session 1
chairs: Saskia van Asten and Reza Nadafi

Molecular fingerprinting of clonal T- and B-cell responses: identifying, quantifying, monitoring and cross-correlating antigen-specific responses, in vivo and in vitro
Sabrina Pollastro
abstract at page 58

The contribution of carbohydrate-dependent interactions in glioblastoma immune escape: New targets for check-point inhibition?
Sophie Dusoswa
abstract at page 33

Autologous Vγ9Vδ2-T cells as effector T cells in CLL immunotherapy
Iris de Weerdt
abstract at page 31

Inefficient mucosal CD8+ effector T cells set the stage for increased susceptibility to viral infections during infancy
Renee Schreurs
abstract at page 61

Loss of RALDH1 expression in the gut is compensated by expression of alternative ALDH enzymes
Martje Erkelens
abstract at page 35

10:20 - 10:40
coffee break
<table>
<thead>
<tr>
<th>Time</th>
<th>Session 2A</th>
<th>Session 2B</th>
</tr>
</thead>
</table>
| 10:40 - 11:40| **Expanded B-cell receptor clones in blood samples of patients with Chronic Inflammatory Demyelinating Polyneuropathy**  
Gwen van Lieverloo  
abstract at page 74 | **mTOR inhibition enhances the immune response of bronchial epithelial cells in response to Gram-negative pathogens but not LPS**  
Ivan Ramirez Moral  
abstract at page 59 |
|              | **NFkB1 haploinsufficient patients show impaired proliferation, plasmablast formation and immunoglobulin production ex vivo**  
Paul Tuijnenburg  
abstract at page 68 | **Role of the mycosin proteases in Type VII secretion systems of pathogenic mycobacteria**  
Vincent van Winden  
abstract at page 76 |
|              | **regulation of plasma cell formation by the dynamic interplay between specific B cells and follicular T helper cells**  
Casper Marsman  
abstract at page 51 | **Metagenomic sequencing to replace semi-quantitative urine culture for detection of urinary tract infections: a proof of concept**  
Victoria Janes  
abstract at page 42 |
|              | **Dissecting the Rheumatoid Factor response**  
Willem Falkenburg  
abstract at page 36 | **RNA helicase DDX3 is a novel sensor for HIV-1**  
Melissa Stunnenberg  
abstract at page 65 |
|              | **How do glycans affect immune cells in RA?**  
Anoushka Molhoek  
abstract at page 53 | **isolation and characterization of monoclonal antibodies from HIV envelope immunized rabbits**  
Marlies van Haaren  
abstract at page 72 |
|              | **TNF in RA during biological treatment**  
Lea Berkhout  
abstract at page 21 | **The Netherlands Chlamydia Cohort Study (NECCST): Risks of Long-term Complications Following Chlamydia Trachomatis Infections in Women**  
Bernice Hoenderboom  
abstract at page 38 |

<table>
<thead>
<tr>
<th>Time</th>
<th>Lunch</th>
</tr>
</thead>
<tbody>
<tr>
<td>11:40 - 12:40</td>
<td>lunch</td>
</tr>
</tbody>
</table>


### 12:40 - 13:30

#### Session 3A

**Chairs:** Marieke Heineke and Willem Falkenburg  
**Omaima El Tahir**  
*Integrating host genetic variants in clinical prediction rule for hearing loss after childhood bacterial meningitis: a model renewing study*  
*Abstract at page 34*

#### Session 3B

**Chairs:** Sophie Horrevorts and Dieke van Rees  
**Tom Hofland**  
*Phenotype and function of Natural Killer cells in CLL*  
*Abstract at page 40*

### 13:30 - 14:00

**Coffee break**
14:00 - 15:00

session 4a
chairs: Eveline Li and Hina Naz Khan

A novel ILC/NK-like cell may be involved in Inflammatory Bowel Disease
Lisette Krabbendam
abstract at page 47

The effect of complement inhibition on erythrocyte destruction in AIHA
Inge Baas
abstract at page 19

Cross-talk of dendritic cells with neutrophils that have been activated with immunoglobulin A
Annelot Breedveld
abstract at page 24

Identifying the T cell receptor sequences of pathogenic T cells of origin in psoriasis
Tiago Matos
abstract at page 52

Radiological and Immunological markers of disease activity in juvenile idiopathic arthritis
Anouk Barendregt
abstract at page 20

TLR-FcγR cross-talk induces inflammation by human myeloid antigen-presenting cells through IRF5 dependent gene transcription
Willianne Hoepel
abstract at page 39

Ultrasound Abnormalities Predict Arthritis Development in ACPA and/or RF Positive Arthralgia Patients
Annies Blanken
abstract at page 22

IL-17D is expressed by stromal cells during bone formation
Sijia Chen
abstract at page 29

Pregnancy outcome in women with systemic lupus erythematosus, a multicenter cohort-study
Birgit Schutte-Blomjous
abstract at page 62

The contribution of non-canonical NF-κB signalling in endothelial cells to pathological bone formation in spondyloarthritis
Merlijn Kaaij
abstract at page 44

NLRX1 deficiency prevents diet-induced hepatic steatosis by promoting mitochondrial oxidative phosphorylation in hepatocytes
Lotte Kors
abstract at page 46

Personisation of Intravenous Immunoglobulin Therapy for Childhood Immune Thrombocytopenia
David Schmidt
abstract at page 60

15:00 - 15:30
get dressed for the activity!

15:30 - 18:30
activity

10
18:30 - 19:30
dinner

19:30 - 20:30
poster making

Poster sessions: look up your abstract in this booklet and check whether you have an even or uneven page number

20:30 - 21:00
poster viewing session even numbers

21:00 - 21:30
poster viewing session uneven numbers

21:30 - 01:00
Party!
Friday 6th October

08:00 - 09:00
Breakfast

09:00 - 09:30
check-out

09:30 - 10:30
session 5
chairs: Anna van Beek and Abilash Ravi

*Exploration of Human Lung CD4+ T cell Heterogeneity*
Anna Oja
abstract at page 56

*Human B cells internalize pathogens through BCR-Nck-PI3K signaling without CD19 co-receptor involvement*
Niels Verstegen
abstract at page 77

*Immunophenotyping macrophages via Folate receptor beta and other macrophage markers in Rheumatoid Arthritis*
Durga Chandrupatla
abstract at page 28

*IgG opsonization of viruses functions as an endogenous suppressor of type I and III IFN-related anti-viral immunity by human myeloid cells through FcγRIIa*
Melissa Newling
abstract at page 55

*On the mechanism of IVIG-associated hemolysis by antibodies against A and B blood group antigens in IVIG products*
Christine Bruggeman
abstract at page 26

10:30 - 11:00
coffee break

11:00 - 12:00
Keynote speaker: Maria Yazdanbakhsh

12:00 - 13:00
lunch
### 13:00 - 14:00

**session 6a**

chairs: Christine Bruggeman and Tom Hofland

- **Chronic lymphocytic leukemia impairs metabolic fitness in CD8 T cells**
  Armando van Bruggen
  abstract at page 70

- **Innate immunity in allogeneic hematopoietic stem cell transplantation**
  Yannouck van Lier
  abstract at page 73

- **Immunity against measles before and after allogeneic hematopoietic stem cell transplantation**
  Mariëlle van Aalst
  abstract at page 69

- **Immune-mediated mechanisms of maladaptive renal repair**
  Alessandra Tammaro
  abstract at page 66

- **Palmitoylated antigens efficiently target skin-resident dendritic cells for treatment of cancer.**
  Sophie Horrevorts
  abstract at page 41

- **SF3B1 mutations in cancer lead to an altered DNA damage response, and splice products that are substrates for nonsense-mediated decay**
  Alexander Leeksma
  abstract at page 49

**Session 6b**

chairs: Lotte Kors and Alexander Leeksma

- **Antibody-dependent destruction of B lymphoma cells by neutrophils.**
  Dieke van Rees
  abstract at page 75

- **HIV-1 infection in macrophages reduces expression of pro-inflammatory genes.**
  Zita Kruize
  abstract at page 48

- **Exploration of crosstalk between C-type Lectin Receptors and Toll-like Receptors using single molecule vaccine modalities**
  Eveline Li
  abstract at page 50

- **iPSC-derived granulocytes as a source to model human disease**
  Cathelijn Aarts
  abstract at page 18

- **Fc alpha receptor I: the extraordinary receptor for IgA**
  Marieke Heineke
  abstract at page 37

- **The Endothelium in Control to Reduce the Risk of Transfusion Related Acute Lung Injury (TRALI)**
  Sofia Morsing
  abstract at page 54

### 14:00 - 14:20

coffee break

### 14:20 - 15:20

Keynote speaker: Frank Miedema

### 15:20 - 15:40

coffee break
| 15:40 - 16:40 | session 7a | Harnessing intracellular mycobacteria for therapy against cancer and infectious disease  
Ran Troost  
abstract at page 67 |
|---|---|---|
| session 7b | chairs: Alessandra Tammaro and Vincent van Winden | The composition of the vaginal microbiomes and the relation to fertility and In Vitro Fertilization techniques – a systematic review and meta-analysis  
Martin Singer  
abstract at page 63 |

| Improving BCG vaccination and bladder cancer treatment by heterologous secretion: using LipY as a carrier  
Maroeska Burggraaf  
abstract at page 27 |
|---|---|---|
| Depletion of high molecular weight kininogen attenuates airway hyperresponsiveness independent of allergic lung inflammation in a house dust mite induced model  
Jack Yang  
abstract at page 78 |

| The effect of leukocyte DNA methylation on host defense mechanisms during community-acquired pneumonia  
Xanthe Brands  
abstract at page 23 |
|---|---|---|
| Identification of blood transcriptional networks dependent on lipopolysaccharide dose in human endotoxemia  
Hina Naz Khan  
abstract at page 45 |

| 16:40 - 17:00 | award ceremony |
## Evaluation form presentations

<table>
<thead>
<tr>
<th>Name</th>
<th>Research</th>
<th>Presentation</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sabrina Pollastro</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sophie Dusoswa</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iris de Weerdt</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renee Schreurs</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Martje Erkelens</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gwen van Lieverloo</td>
<td>2a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paul Tuijtenburg</td>
<td>2a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Casper Marsman</td>
<td>2a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Willem Falkenburg</td>
<td>2a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anoushka Molhoek</td>
<td>2a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lea Berkhout</td>
<td>2a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ivan Ramirez Moral</td>
<td>2b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vincent van Winden</td>
<td>2b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Victoria Janes</td>
<td>2b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melissa Stunnenberg</td>
<td>2b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marlies van Haaren</td>
<td>2b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bernice Hoenderboom</td>
<td>2b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Omaima El Tahir</td>
<td>3a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Natasja Otto</td>
<td>3a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Judith Zandstra</td>
<td>3a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Philip Brouwer</td>
<td>3a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eleanne van Ess</td>
<td>3a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tom Hofland</td>
<td>3b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sanne Duinkerken</td>
<td>3b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dorian Stolk</td>
<td>3b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kim Jeucken</td>
<td>3b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ammarina Chuwonpad</td>
<td>3b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lisette Krabbendam</td>
<td>4a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Annelot Breedveld</td>
<td>4a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anouk Barendregt</td>
<td>4a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Annelies Blanken</td>
<td>4a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Name</td>
<td>Research Group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------------------</td>
<td>----------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birgit Schutte-Blomjous</td>
<td>4a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lotte Kors</td>
<td>4a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inge Baas</td>
<td>4b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tiago Matos</td>
<td>4b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Willianne Hoepel</td>
<td>4b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sijia Chen</td>
<td>4b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Merlijn Kaaij</td>
<td>4b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>David Schmidt</td>
<td>4b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anna Oja</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Niels Verstegen</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Durga Chandrupatla</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melissa Newling</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Christine Bruggeman</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Armando van Bruggen</td>
<td>6a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yannouck van Lier</td>
<td>6a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mariëlle van Aalst</td>
<td>6a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alessandra Tammaro</td>
<td>6a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sophie Horrevorts</td>
<td>6a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alexander Leeksma</td>
<td>6a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dieke van Rees</td>
<td>6b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zita Kruize</td>
<td>6b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eveline Li</td>
<td>6b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cathelijn Aarts</td>
<td>6b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marieke Heineke</td>
<td>6b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sofia Morsing</td>
<td>6b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ran Troost</td>
<td>7a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maroëska Burggraaf</td>
<td>7a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xanthe Brands</td>
<td>7a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Martin Singer</td>
<td>7b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jack Yang</td>
<td>7b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hina Naz Khan</td>
<td>7b</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Abstracts
Oral presentations
iPSC-derived granulocytes as a source to model human disease

Aarts C.E.M., Kuijpers, T.W. Akker, van den E., Lindern, von M., Berg, van den T.K.
Blood Cell Research, Sanquin

Over the past decade, induced pluripotent stem cells (iPSCs) have emerged as an attractive source for disease modelling. It is a source of unlimited supplies of hematopoietic stem and progenitor cells, which have the potential to give rise to multiple different cell types. iPSCs were firstly generated from human fibroblasts by the introduction of four transcription factors (Oct3/4, Sox2, Klf4 and c-Myc) by the group of Shinya Yamanaka in 2007. Nowadays, iPSCs can be generated from only 5 ml of blood, creating an immortal source of (patient) cells. The aim of our research is to optimize the expansion and differentiation of iPSC lines into granulocytes and apply this to various disease models.

We reprogrammed human megakaryocytes to produce an iPSC line, using a polycistronic lentiviral vector containing the ‘Yamanaka-factors’ for cellular reprogramming by the aforementioned transcription factors. Differentiation of the iPSC line to mature granulocytes was performed using a feeder-free monolayer hematopoietic culture system, which resulted in a wave of granulopoiesis after 12 days of differentiation and subsequent stimulation for 7 more days with specific granulocyte growth factors (i.e. G-CSF). The iPSC-derived neutrophils showed the typical segmented nucleus and expressed myeloid and granulocytic surface markers. Furthermore, the iPSC-derived neutrophils produced reactive oxygen species (ROS) upon cell activation and showed the presence of MPO, elastase and lactoferrin, which are major constituents present in the azurophil and specific granules, respectively. However, the cell population also showed expression of surface markers of eosinophils and basophils.

By further optimization the protocol we are now able to generate non-dividing CD11b+CD16+ human neutrophils with proteolytic activity of the granule enzymes, which show phagocytosis of microbes and ROS production upon activation. Current efforts are directed to the generation of iPSC-derived neutrophils from patients with different well-identified granular defects to unravel aberrant developmental pathways in neutrophils.

Notes:
Autoimmune hemolytic anemia (AIHA) is a rare disease characterized by autoantibodies against erythrocytes. These autoantibodies may activate the classical complement pathway leading to opsonization by complement proteins C3b and C4b, resulting in increased clearance of erythrocytes by phagocytes, called extravascular hemolysis. Occasionally, complement activation results in formation of the membrane attack complex (MAC) resulting in intravascular hemolysis. C3-inhibitor compstatin prevents C3b deposition and thus it is expected that C3 inhibition will inhibit formation of the MAC. However no effect on C4b deposition is expected, thus this inhibitor can be used as a tool to distinguish the roles of C3b and C4b on clearance of erythrocytes by phagocytes. The current aim of this research is to investigate in vitro whether compstatin cp40 would be a suitable drug for AIHA treatment. Healthy donor erythrocytes were incubated with AIHA patient serum and opsonization was analyzed by FACS using anti-C3-FITC and anti-C4-APC antibodies. Compstatin completely inhibited C3 deposition on erythrocytes, while unexpectedly C4 deposition appeared to be increased. Inhibition of C3 deposition prevented formation of the MAC. To assess the effect of compstatin on uptake of erythrocytes by phagocytes, erythrocytes were fluorescently labeled before opsonization. Opsonized erythrocytes were then incubated with healthy monocyte derived macrophages and phagocytosis of erythrocytes was measured with ImageStream after lysing the non-phagocytosed erythrocytes. Results from these experiments showed that phagocytic uptake of erythrocytes by macrophages was decreased by compstatin. However, when erythrocytes were opsonized with both complement and IgG there was less inhibition of phagocytosis compared to erythrocytes opsonized with only complement. Since compstatin inhibits both intravascular and extravascular hemolysis it is an interesting candidate to consider for treatment of AIHA. However, it is important not to overlook the role of IgG mediated clearance. 

Notes:
Radiological and Immunological markers of disease activity in juvenile idiopathic arthritis

Barendregt AM, E.C. van Gulik, J.M. van den Berg, D. Schonenberg-Meinema, A. Nassar, T.W. Kuipers, R. Hemke, M. Maas,
Radiology/Pediatric rheumatology, AMC

Purpose
To compare two imaging biomarkers, the already established dynamic-contrast-enhanced MRI (DCE) and the new non-invasive diffusion-weighted imaging (DWI), in quantitatively assessing synovial inflammation in patients with juvenile idiopathic arthritis (JIA).

Methods
35 JIA patients underwent MRI of the knee. In addition to standard sequences an axial T1W DCE and axial T2W DWI were acquired. DWI was post-processed into apparent diffusion coefficient (ADC)50-600 map to eliminate signal intensity from vascular flow. To quantify signal from the synovium, regions of interest (ROI) were manually. Collected DCE perfusion parameters include maximum enhancement (ME), slope of enhancement (slope), time-to-peak (TTP) and % of time-intensity curves (TIC) 2-5. A subset of patients (n=5) was measured twice to check consistency of ROI drawing. Patients were subdivided based on the validated JIA MRI score (JAMRIS), a score of 0 for synovial hypertrophy corresponds to inactive disease (n=16), a score ≥1 to active disease (n=19). Mann-Whitney U was used for testing DCE and DWI parameters between patients with active and inactive disease, the intraclass correlation coefficient (ICC) was used to assess reliability of ROI drawing and Spearman’s rank for correlation between DCE and DWI parameters.

Results
High correlations for all parameters (ICCs 0.89-0.99, p<0.05) were found when assessing the twofold ROI placements. Correlation between ME and ADC was good (r 0.62, p=0.000), other correlations were not significant. ME, slope, TTP, % TIC 2-5 (all p<0.05) as well as ADC were significantly different in the active vs. the inactive patients with median ADCactive 1.49x10-3 mm2/sec, median ADCinactive 1.26 x10-3 mm2/sec, p=0.003.

Conclusion
Similar to DCE parameters, non-invasive DWI-derived ADC can differentiate active JIA from inactive JIA in the knee using a ROI drawing method that proved to be uniform. Diffusion in inflamed synovium is increased compared to non-inflamed synovium.

Notes:
Although therapeutic monoclonal antibodies (biologics) targeting TNF are widely used to treat inflammatory diseases, such as ankylosing spondylitis (AS) and rheumatoid arthritis (RA), little is known about the fate of TNF during anti-TNF treatment. This lack of knowledge can be explained by the lack of assays that efficiently quantify TNF while bound to the TNF inhibitor.

In the present study we developed a drug-tolerant assay allowing accurate measurement of TNF in the presence of large amounts of TNF inhibitor. We used this assay for the quantification of TNF in adalimumab-treated RA and AS patients. As expected, we observed TNF levels close to LOD at baseline, but levels become substantially increased upon start of adalimumab treatment, since half-life of TNF in complex with adalimumab is suggested to be substantially prolonged. However, using a functional assay we showed that TNF in complex with adalimumab is inactive. There is considerable variation in the levels of TNF across patients. Furthermore, several studies showed that a proportion of patients in remission can taper or even discontinue adalimumab treatment. This suggests that blocking TNF is no longer needed in those patients. Therefore, we hypothesized TNF levels to gradually decrease in patients who do respond to anti-TNF treatment. However, unexpectedly, we observed that after the initial rise in TNF, TNF levels continued to increase for prolonged periods of time.

Finally, we observed a more gradual increase in TNF in patients on adalimumab monotherapy, compared to patients that concomitantly used methotrexate. This was also observed for adalimumab-treated AS patients, who mainly are on adalimumab monotherapy.

Overall, the dynamics of TNF production and clearance during anti-TNF treatment appears more complex than anticipated. Monitoring TNF levels during anti-TNF treatment might prove a useful tool to personalize treatment and to identify (non-) responders in the early phase of treatment.
Ultrasound Abnormalities Predict Arthritis Development in ACPA and/or RF Positive Arthralgia Patients
Blanken A.B., M. van Beers-Tas, D. van Schaardenburg
Rheumatology, Reade/VUmc/AMC

Early diagnosis of rheumatoid arthritis (RA) is important for controlling disease activity and preventing joint damage. Individuals positive for anticitrullinated protein antibodies (ACPA) and/or rheumatoid factor (RF) are at risk of developing RA. In this study we investigated whether ultrasound (US) can predict development of arthritis in seropositive arthralgia patients.

We included 174 ACPA and/or RF positive patients with arthralgia, but without clinical arthritis. US was performed at baseline in bilateral MCP2-3, PIP2-3 and wrist. Images were scored semiquantitatively for synovitis and Power Doppler (PD) on a scale of 0-3. Grades 2 to 3 for synovitis and grades 1 to 3 for PD were regarded as abnormal.

In total, 51 (29%) patients developed arthritis after a median follow-up time of 12 (IQR 6-23) months. Patients that did not develop arthritis had a median follow-up of 23 (IQR 12-48) months. Synovitis and PD signal in at least one joint was observed in 14 (8%) and 7 (4%) patients, respectively. The presence of synovitis was associated with arthritis development (OR 7.3, CI 2.1-24.4, p<0.01), whereas the presence of PD signal was not (OR 1.0, CI 0.1-5.2, p=1.0). Corresponding positive predictive values were 71% and 29%, respectively. Patients with synovitis or PD in at least one joint developed arthritis earlier than patients without US abnormalities (synovitis: median time to arthritis 9 versus 13 months, p<0.01; PD: median time to arthritis 5 versus 12 months, p<0.05) with corresponding HRs of 2.6 (CI 1.2-5.7, p=0.01) and 4.6 (CI 1.1-20.6, p=0.04) respectively.

Synovitis on US predicted arthritis development in seropositive arthralgia patients. This association was not found for PD, however PD frequency was low and therefore the power to predict arthritis was low. However, when taking into account the time to arthritis development, both synovitis and PD were associated with an increased hazard on developing arthritis.

Notes:
Background
Pneumonia is the world’s leading infectious killer, responsible for an estimated 3.1 million deaths annually. The incidence of community-acquired pneumonia (CAP) is U-shaped in terms of age; it is common in children younger than 5 years and adults older than 65 years. Monocytes and neutrophils are important cellular components of the innate immune response, and evidence indicates that the function of monocytes and neutrophils is disturbed in elderly subjects. Ageing is associated with progressive changes in DNA methylation. DNA methylation involves the addition of a methyl group to the fifth position of a cytosine base by DNA methyltransferases (DNMTs). Demethylation of DNA is primarily regulated by the Ten-Eleven Translocation (TET) family of enzymes. DNA methylation of blood leukocytes might influence innate immune response and thereby the susceptibility for infections. Knowledge of DNA methylation and its impact on antibacterial defense is highly limited. CAP represents a major health care problem and mortality and morbidity associated with severe pneumonia remain considerable, despite state of the art care. Therefore there is an urgent need to expand our knowledge of the pathogenesis of pneumonia.

Overall objective
To obtain insight into the role of altered DNA methylation in blood leukocytes in innate immune responses and host defense in patients with CAP and the influence of ageing hereon.

Approach
Multicenter observational study among patients with CAP at the Emergency Department, Intensive Care unit and Internal Medicine Ward of the Academic Medical Center Amsterdam and BovenIJ hospital. Blood is drawn to analyze DNA methylation patterns of purified monocytes and neutrophils, which will be analyzed in connection with DNMTs and TET activity, RNA and DNA gene expression and a selection of standard innate immune functions. Moreover, isolated monocytes and neutrophils are stimulated in vitro with Streptococcus pneumoniae and Klebsiella pneumoniae. Patient material is obtained upon inclusion and on day 28, when patients will be seen in the outpatient clinic for follow up.

Current status
At present 61 patients and 40 healthy elderly controls have been enrolled in the study.

Relevance and expected results
Pneumonia represents a major health care problem and especially affects the elderly population. We aim to explore a completely novel research area linking the extent of DNA methylation in blood leukocyte on the influence of innate immune responses to and host defense against CAP. This study will provide novel knowledge of host defense against pneumonia and may pave the way for new preventive and/or therapeutic interventions for pneumonia.
Cross-talk of dendritic cells with neutrophils that have been activated with immunoglobulin A
Breedveld AC, R. Braster, M. Bögels, R. Mebius, M. van Egmond
Molecular Cell Biology and Immunology, VUmc

Immunoglobulin A (IgA) is the most prevalent antibody at mucosal sites and a potent stimulus of neutrophils (PMN) via the IgA Fc receptor (FcαRI). In patients with inflammatory bowel disease, mucosal infiltration of PMNs as well as interaction with dendritic cells (DCs) is seen. We hypothesize that mucosal pathology and immune responses are influenced by FcαRI induced PMN activation. The aim of this study is to investigate crosstalk of IgA activated PMN with DCs.

Tolerogenic DCs (RADCs) and monocyte-derived DCs (moDCs) were derived from monocytes after being cultured for 6 days in the presence of IL-4 and GM-CSF, with or without retinoic acid (RA) respectively. Fresh isolated PMNs phagocytosed IgA coated latex beads (IgA PMN) after which they were co-cultured with DCs. Subsequent, maturation marker expression on DCs was assessed. Cell-cell interactions were analyzed with Imagestream and live cell microscopy, and cytokine production in supernatants of co-cultures was measured.

RA-DCs differed in morphology, expressed more CD103 and CD89, and had higher aldehyde dehydrogenase activity compared to moDCs. RA-DCs expressed less CD80, CD83, CD86 and HLA-DR after co-culture with IgA PMN compared to moDCs. Cell interactions, bead transfer between IgA PMN and DCs and IgA PMN uptake by DCs were seen in live cell microscopy and Imagestream. IL-6 and IL-12 was produced by moDCs after co-culture with IgA PMN, but not by RA-DCs.

IgA PMN induces the maturation of moDCs, but not of RADCs. DCs interacted with IgA PMN, and bead transfer from PMNs to DCs was observed. However, after co-culture with IgA PMN, RADCs did not produce IL-6 and IL-12. These findings suggest that moDCs become pro-inflammatory and have higher potential for antigen presentation after stimulation with IgA PMN, while RADCs induce immune tolerance.

Notes:
A two-component self-assembling nanoparticle for the multivalent display of native-like HIV-1 Env trimers


Medical Microbiology, AMC

The development of near-native mimics of the HIV-1 Env spike using the SOSIP framework has enabled the ability to induce autologous neutralization against resistant (Tier-2) viruses in several animal models. However, SOSIP-induced antibody titers remain relatively weak, short-lived, and narrow in specificity. Displaying antigens in a multivalent fashion on nanoparticles or virus-like particles is a well-established strategy to increase their immunogenicity. Here, we present the design and characterization of two-component protein nanoparticle systems displaying twenty native-like Env trimers per particle (SOSIP-NPs). The nanoparticles self-assemble with high efficiency into stable, monodisperse and well-ordered icosahedral particles as observed by negative-stain electron microscopy and dynamic light scattering. The protruding SOSIP spikes maintain their antigenic structure and, in contrast to their soluble counterpart, induce strong and sustained activation of several bNAb-expressing B cells in vitro. Immunization studies with these SOSIP-NPs in rabbits, Kymice and germline Ab knock-in mice have provided valuable insights for the usage of these particles as immunogens. These two-component nanoparticles displaying multiple SOSIP trimers represent a versatile platform for vaccine strategies aimed at inducing bNAbs.

Notes:
On the mechanism of IVIG-associated hemolysis by antibodies against A and B blood group antigens in IVIG products
Bruggeman C.W., Christine W. Bruggeman, Wendy Lau, Sietse Q. Nagelkerke, Elisabeth Meulenbroek, Timo K. van den Berg, Cedric Malhiot, Brain W. McCrindle, Rae S. M. Yeung, Taco W. Kuijpers
Blood Cell Research, Sanguin

Intravenous immunoglobulin (IVIG) is used as immunomodulating agent in multiple autoimmune and inflammatory diseases, such as Kawasaki disease (KD) and immune thrombocytopenia (ITP). Although a rare side-effect of IVIG treatment, anemia may be induced and can sometimes be so severe that erythrocyte transfusions are required. We analyzed an IVIG brand that caused severe, IgG-mediated anemia in more than the expected number of cases in Canada and the USA when it was broadly used during a period in 2012-2013.

To investigate the cause of this, we analyzed the antibody content of this specific IVIG product and found that it contains higher antibody titers against A and B blood group antigens than other IVIG products. We found these blood-group-specific antibodies to be of the IgG2 subclass and to cause opsonization of erythrocytes of the A/B/AB blood group. These opsonized erythrocytes could be phagocytized by monocyte-derived macrophages to low extent.

As IVIG-associated hemolysis occurs more often in KD - a known inflammatory condition with evidence of marked systemic inflammation, we hypothesized a 'two-hit' model for antibody-mediated erythrophagocytosis, in which antibody-mediated phagocytosis was facilitated by the inflammatory condition. Stimulating macrophages with LPS or TNFα prior to phagocytosis, strongly increased phagocytosis by macrophages of the opsonized erythrocytes in a strictly FcγRIIa-dependent manner. These findings show that the increased frequency of severe anemia following IVIG was caused by blood-group-specific IgG2 antibodies leading to FcγRIIa-dependent clearance of opsonized A- or AB-positive erythrocytes.

Notes:
Improving BCG vaccination and bladder cancer treatment by heterologous secretion: using LipY as a carrier

Burggraaf MJ, L.S. Ates, C. Kuijl, W. Bitter

Medical Microbiology & Infection control, VUmc

Mycobacterium bovis Bacillus Calmette-Guérin (BCG) is well known for its use as vaccine for tuberculosis and for its use as intravesical therapy to prevent recurrence of bladder tumors. Although BCG has been used for years in both therapeutic strategies, there is still an urgent need for improvement because of limited efficacy rates. Both vaccination and bladder cancer treatment could benefit from heterologous secretion by BCG. Secretion of pathogen specific antigens, human cytokines or tumor targeting proteins are promising strategies to improve therapeutic outcome.

To enable heterologous secretion by mycobacteria we searched for an endogenous protein that could serve as a carrier protein to facilitate heterologous secretion. As a proof of concept we examined ESX-5 substrate LipY as a carrier for the secretion of egg white protein Ovalbumin (OVA) and investigated which domains of LipY are essential for secretion and which mutations can enhance secretion of the fusion protein.

Here we show that LipY can be used as a carrier for heterologous secretion in mycobacteria. M. marinum showed an expected ESX-5 dependent secretion and surface localization of LipY-OVA as measured by flow cytometry and cell fraction analysis. Since secretion of proteins can be hampered by protein structure, we hypothesized that small changes in the structure introduced by error-prone PCR might enhance secretion. And indeed, specific mutations in the OVA domain enhanced surface localization. Furthermore, deletion of the LipY linker domain linking the secretion signal and the OVA domain (originally the lipase domain) enhanced secretion of the fusion protein. Moreover, this linker domain appeared to be involved in surface localization of full length LipY. Together, this data provides new insights in heterologous secretion by mycobacteria, which can be useful for improving BCG therapies.

Notes:

________________________________________________________________________________________________________________________

________________________________________________________________________________________________________________________

________________________________________________________________________________________________________________________

________________________________________________________________________________________________________________________

________________________________________________________________________________________________________________________

________________________________________________________________________________________________________________________

________________________________________________________________________________________________________________________

________________________________________________________________________________________________________________________

________________________________________________________________________________________________________________________

________________________________________________________________________________________________________________________

________________________________________________________________________________________________________________________

27
**Immunophenotyping macrophages via Folate receptor beta and other macrophage markers in Rheumatoid Arthritis**

chandrupatla DMSH, gerrit jansen, conny van der laken, carla molthoff

*Rheumatology, VUmc*

Background Macrophages play a key role in the pathophysiology of rheumatoid arthritis (RA). The folate receptor β (FR-β) is expressed on these macrophages and [18F]fluoro-PEG-folate positron emission tomography ([18F]fluoro-PEG-folate PET) can be used to visualize arthritis *in vivo*. In addition, [18F]fluoro-PEG-folate PET could be a highly interesting tool for therapeutic monitoring of MTX therapy, the corner stone for RA therapy.

Methods Arthritic rats [3] (n = 3-6 per group) received interventions with either MTX [i.p., 1mg/kg; 2 times (group A) or 4 times (group B)] or PBS (control group)] with a time interval of 3-4 days. [18F]fluoro-PEG-folate PET-CT were acquired for one hour after tracer injection. Sixty minutes after the PET scan, ex-vivo tissue distribution (expressed as percentage of the injected dose/gram tissue (%ID/g)). For histopathology (Haematoxylin-Eosin (HE) and immunohistochemistry with macrophage specific antibodies ED1 (~CD68) and ED2 (~CD163) were applied.

Results PET scans clearly visualized significantly lower SUVs (1.5-fold, p<0.01) in arthritic knees of both MTX-treated groups, approximating the levels observed seen in healthy rats. Corroborating [18F]fluoro-PEG-folate PET, *ex vivo* tissue distribution [18F] fluoro-PEG-folate demonstrating a 2- and 4-fold decrease (group A and B, respectively) in tracer uptake in arthritic knees after MTX therapy (0.12 and 0.06 for groups A and B, and 0.22 %ID/g for controls, respectively) (Figure). This reduction in uptake of [18F] fluoro-PEG-folate in arthritic knees was also associated with a significant decrease in ED1 and ED2 positive synovial macrophages in arthritic knees (~4 fold) for both treated groups compared with control rats knees (p < 0.01).

Conclusion This study in arthritic rats underscores the potential and usefulness of [18F] fluoro-PEG-folate PET as tool to monitor of MTX therapy and potentially other anti-folate treatments of RA.

Notes:
**IL-17D is expressed by stromal cells during bone formation**

Chen S, D.L.P. Baeten / N.G. Yeremenko  
Department of Experimental Immunology (EXIM), Academic Medical Center/University of Amsterdam, The Netherlands, AMC

IL-17 cytokine family consists of six family members. IL-17A, IL-17F and IL-17E are proinflammatory cytokines produced by immune cells. The origin and function of IL-17D remain poorly understood. Spondyloarthritis (SpA) is known to be an IL-17A-driven chronic inflammatory disease, characterized by joint inflammation, bone destruction and new bone formation.

To our surprise, RNA sequencing of SpA synovitis revealed minuscule expression of all IL-17 family members except for IL-17D, which was relatively highly expressed. We therefore examined the cellular source of IL-17D and whether IL-17D plays a role in the pathogenesis of SpA.

Immunofluorescence analysis revealed that IL-17D-positive cells in SpA synovitis are CD45-negative. Additionally, IL-17D-positive cells stained positive for typical stromal markers CD90, vimentin and markers for mesenchymal progenitor CD44, CD73 and CD105, suggesting a role for IL-17D in mesenchymal progenitors which can differentiate to bone and cartilage. Correspondingly, IL-17D positive cells were positive for osteocalcin, a hormone expressed by osteoblasts and a subset of mesenchymal progenitor cells.

Furthermore, as we found the highest IL-17D gene expression in synovial fibroblasts, chondrocytes and bone, we assessed the level of IL-17D during osteogenic differentiation of synovial fibroblasts derived from patients with SpA. Analysis of IL-17D expression confirmed its increase during osteogenic differentiation, reaching a peak at day 14, whereas IL-17D expression was significantly decreased after addition of pro-inflammatory cytokines TNF, IL-17A and IL-1β. In these SpA synovial fibroblasts, during osteogenic differentiation and already at baseline, expression of IL-17D correlated with the expression of SOX9, a master transcription factor involved in bone and cartilage differentiation.

Collectively, our data demonstrate that in contrast to immune cell-derived IL-17A, the cellular source of IL-17D is of stromal origin. Furthermore, IL-17D is increasingly expressed during bone formation. We will investigate the molecular mechanism of IL-17D and its putative role in pathogenic bone formation in SpA.

Notes:
Tissue-resident memory (TRM) have been recently established as an important subset of memory cells that provide early and essential protection against reinfection in the absence of circulating memory cells. In addition, CD8, T cells with a TRM-like phenotype have been found to infiltrate tumor tissue. The presence of these TRM-like cells are correlated with patient survival, suggesting that TRM have a protective role in cancer. Immunotherapy using adoptive transfer of in vitro reactivated T cells from the tumor site has provided a breakthrough in the treatment of cancer patients. Despite promising results further improvement of therapy is required for the majority of patients. Currently, it is unknown whether TRM can be expanded in culture for use in adoptive transfer therapy. The aim of our research is to study how cultures of tissue-resident memory CD8, T lymphocytes can be achieved.

We have found that TRM from the small intestine can proliferate in vitro after stimulation with anti-CD3/28 antibodies. Further expansion of the cells can be achieved using culture on IL-2 and the homeostatic cytokines IL-7 and IL-15. The in vitro expanded cells retain their TRM phenotype during culture, including expression of the TRM signature molecules CD69 and CD103. Culture of TRM was optimal under low O2 tension, which indicates that these cells have the ability to resist the hypoxic conditions of the tumor microenvironment. TRM were able to efficiently take up glucose from the medium, which is required to compete with highly glycolytic tumor cells. For expansion in vitro, TRM also relied on fatty acids, which are considered as a readily available energy source in the tumor microenvironment.

These findings suggest that in vitro re-stimulation of existing TRM is possible under conditions reflecting the tumor microenvironment, which is promising in terms of future usage for tumor treatment via adoptive transfer.

Notes:
Autologous Vγ9Vδ2-T cells as effector T cells in CLL immunotherapy


EXIM, AMC

Introduction: Although T cell therapy has curative potential in chronic lymphocytic leukemia (CLL), the efficacy of autologous αβ-T cell-based therapy has been modest. Vγ9Vδ2-T cells are a conserved subset of cytotoxic T lymphocytes with potent antilymphoma activity. The aim of this study is to assess the potential of Vγ9Vδ2-T cells for autologous cellular immunotherapy in CLL.

Results: Vγ9Vδ2-T cells from healthy controls (HCs) had an activated phenotype after coculture with CLL cells. Moreover, Vγ9Vδ2-T cells from HCs effectively lysed primary CLL cells.

Subsequently, Vγ9Vδ2-T cells derived from CLL patients and HCs were compared. Vγ9Vδ2-T cells from CLL patients are skewed towards effector phenotype. Despite an increased frequency of the effector type subset, Vγ9Vδ2-T cells from CLL patients contained less granzyme B.

Moreover, Vγ9Vδ2-T cells from CLL patients produced less effector type cytokines than HC-Vγ9Vδ2-T cells. Similarly, Vγ9Vδ2-T cells from CLL patients degranulated less than HCs. Correspondingly, the cytotoxicity of CLL-derived Vγ9Vδ2-T cells against CLL cells was significantly reduced in comparison to HCs. Coculture of CLL cells with HC Vγ9Vδ2-T cells resulted in a similar impairment in cytokine production and degranulation, pointing towards leukemia-specific causative mechanisms.

Next, the reversibility of Vγ9Vδ2-T cell dysfunction was tested. After coculture with phosphoantigen-expressing dendritic cells, the cytokine production and cytotoxicity of Vγ9Vδ2-T cells from HCs and CLL patients was comparable.

Since the clinically used kinase inhibitor ibrutinib not only targets CLL cells, but potentially also influences T cells, its effect on Vγ9Vδ2-T cells was studied. Ibrutinib pretreatment led to TH1-skewing in Vγ9Vδ2-T cells. Degranulation of Vγ9Vδ2-T cells was not impaired by ibrutinib.

Conclusion: CLL-mediated dysfunction of autologous Vγ9Vδ2-T cells is fully reversible upon ex vivo expansion, resulting in potent cytotoxic capacity towards CLL cells. Combined approaches with autologous Vγ9Vδ2-T cells and novel targeted therapies should be further explored, and ibrutinib may be specifically suitable as it promotes an anti-tumor TH1 profile.

Notes:
**Initiation of anti-tumor immune responses via targeting of human skin DC using tumor specific glyco-conjugates**


MCBI, VUmc

Skin-resident dendritic cells (DCs) are actively explored for the use in anti-cancer vaccination strategies. Human skin DC subsets comprise epidermal Langerhans cells (LCs) and dermal CD1a+, CD14+ and CD141+ DCs, which express different C-type lectin receptors (CLRs). To elicit an adaptive anti-tumor immune response, DCs need to take up, process and (cross-) present tumor-associated antigens (TAA) to CD4+ and CD8+ T cells while giving co-stimulatory signals. Using a human skin explant model, we set out to investigate LC and dermal DC cross-presentation of novel tumor specific glyco-conjugates that specifically target the CLRs Langerin and DC-SIGN. We showed that glycan-conjugation of melanoma specific peptide epitopes enhanced binding and uptake via Langerin or DC-SIGN expressed by LCs and dermal DCs, respectively. However, although Langerin and DC-SIGN have a shared specificity for the glycan epitope Lewis Y (LeY), they differed in their requirements regarding the molecular size of the glyco-conjugates. While Langerin effectively mediated cross-presentation in response to glycopeptides, DC-SIGN was most efficient when targeting was performed via glycoliposomes indicating that size matters. (Fehres et al. JCR 2015) By design of multivalent glyco-peptide nanomers with intermediate sizes we aim to target both Langerin and DC-SIGN positive skin DCs for optimal stimulation of CD4+ and CD8+ tumor specific T cells.

Notes:
Glioblastoma (GBM) is the most aggressive form of brain cancer in which tumor associated macrophages and microglia account for up to 30% of the tumor mass. Macrophage galactose lectin (MGL) is a carbohydrate-specific receptor that is expressed by myeloid cells. MGL contributes to immune suppression by mediating IL-10 secretion and inducing apoptosis of effector T cells through direct interaction with CD45. MGL-ligands include truncated O-linked glycans, which have long been associated with metastasis formation and poor survival in breast- and colorectal cancer. Here we aim to elucidate the role of the MGL–MGL-ligand interaction in GBM immune escape. To this end, GBM, low-grade glioma (LGG) and epilepsy surgical samples were collected after approved informed consent. Expression of MGL and its ligands was measured by flow cytometry, immunofluorescence microscopy, ELISA, and western blot. In a GBM mouse model (GI261), expression of MGL, and the effects of MGL ligands on GBM infiltrating immune cells were studied by RNA sequencing and mass cytometry. We detected significantly higher levels of MGL-ligand expression in patients derived GBM samples as compared to LGG and epilepsy samples. These MGL-ligands are expressed mainly in the perivascular space where we also find increased numbers of MGL+ perivascular macrophages. In the GI261 GBM mouse model we found increased expression of mouse MGL in the presence of a tumor compared to a mock injected mouse. Further, we created a stable mouse GBM model overexpressing MGL-ligands and studied the effects of MGL-ligands on the infiltrating and peripheral immune system (data to be analyzed). These results suggest that GBM overexpress truncated O-linked glycans, associated with metastasis and poor survival. MGL-MGL-ligand interactions form a possible new immune checkpoint contributing to GBM immune escape and poor prognosis.

Notes:
Integrating host genetic variants in clinical prediction rule for hearing loss after childhood bacterial meningitis: a model renewing study

El Tahir Q, A.M. van Furth, S.A. Morrè, S. Ouburg, M.W. Heymans

Lab van Immunogenetica/Kindergeneeskunde, VUMc

Background. Sensorineural hearing loss is the most common severe sequela in survivors of childhood bacterial meningitis. In the past we developed a validated prediction model to identify children at risk for postmeningitis hearing loss based on clinical factors. As genetic variation in host immune response genes is also associated with susceptibility to and severity of bacterial meningitis this study aims to determine whether host genetic risk factors to infection improve the performance of the prediction model.

Methods. The generated data of four hundred and seventy Dutch Caucasian survivors of childhood bacterial meningitis genotyped for five single nucleotide polymorphisms (SNPs) in four different genes involved in pathogen recognition and inflammation were used to improve the prediction model. Genetic data were included during model construction and performance of the model was compared to the original model by likelihood ratio tests and the area under the curve (AUC) of the receiver operating characteristic curves.

Results. Addition of genetic predictors improved the performance of the new model compared to the original clinical prediction rule (increase of AUC from 0.85(95% CI 0.78-0.91) to 0.91 (95% CI 0.84-0.97)). Independent predictors for hearing loss were S. pneumoniae, presence of ataxia during illness, CSF glucose level ≤ 0.6 mmol/L, duration of symptoms before admission > 2 days, TLR4+896 A>G and TLR9-1237 T>C.

Conclusions. Including host genetic factors during model construction results in a significantly improved prediction model for post-meningitis hearing loss in children. Future studies should focus on additional value of other SNPS and investigate SNP combinations (SNP traits) in larger cohorts but also assess applicability of the model.

Notes:
Loss of RALDH1 expression in the gut is compensated by expression of alternative ALDH enzymes

MCBI, VUmc

The vitamin A metabolite retinoic acid (RA) plays an important role in keeping the immune system in the gut in check. Here-to, ALDH enzymes, of which RALDH1, RALDH2 and RALDH3 are described most extensively, metabolize vitamin A into RA. In the gut, dendritic cells (DCs) can produce RA due to their expression of RALDH2. RA derived from DCs not only induces gut homing in T and B cells but also the expression of Foxp3 in T cells and IgA class switching in B cells. For RALDH2 expression in DCs, we have shown that DCs require an exogenous source of RA. We and others have shown that intestinal epithelial cells (IECs), which constitutively express RALDH1, are a potential source for RA mediated imprinting of DCs. To study this in more detail, we analyzed RALDH1 KO mice. Surprisingly, in these mice, no defect in the ability to convert vitamin A into RA by IECs and DCs could be observed, suggesting that compensatory mechanisms were activated due to the loss of RALDH1. Indeed, we found that several other ALDH enzymes showed an increased expression in IEC and in the draining lymph nodes. Additionally, we developed an activity based probe that can bind covalently to all active ALDH enzymes. Using click chemistry these enzymes could be pulled down, after which the characteristics of the active ALDH enzymes were determined by mass spectrometry. In conclusion, we show that other ALDH enzymes can compensate for the loss of RALDH1. Whether this is needed to maintain the epithelial integrity will need further study. Overall, these compensatory mechanisms could safeguard sufficient levels of RA to guarantee immune homeostasis.

Notes:
**Dissecting the Rheumatoid Factor response**

Falkenburg WJJ, P. Ooijevaar-de Heer, J. Koers, G. Wolbink, D. van Schaardenburg, T. Rispens

*Immunopathology, Sanquin*

Rheumatoid factors (RFs) are autoantibodies that bind to epitopes in the constant region (Fc) of IgG. They are assumed to play a role in the pathogenesis of rheumatoid arthritis (RA) and have value in predicting development of disease in patients at-risk for RA and disease course in RA patients. RF status and level are important criteria in the classification of RA and are used for diagnosing the disease. However, since RFs can also be present in other rheumatic diseases, chronic infections and even in healthy individuals the specificity of RF assays for diagnosing RA and their predictive value are currently not optimal.

The rheumatoid factor response is a polyclonal response that consists of various RFs binding to different epitopes on IgG-Fc. We characterized the binding profiles of RF responses from healthy donors and RA-patients in the clinical and pre-clinical phase using recombinant IgG targets with mutated RF epitopes. This will potentially lead to a better classification of pathological and physiological RF responses and improve the diagnostic and predictive value of RF testing.

Notes:
Neutrophils express Fc receptors, which recognize the antibodies IgA (FcαRI) or IgG (Fcγ receptors). We previously demonstrated that neutrophil migration is only induced after IgA activation, which may play a detrimental role in IgA-autoantibody mediated autoimmune diseases. The aim of this study is to investigate in depth the differences between IgA- versus IgG-induced neutrophil effector functions and to elucidate the specific signaling pathways of FcαRI, responsible for IgA-induced neutrophil migration and activation. Although no differences in phagocytosis, NETosis and ROS production was found, IgA stimulation led to enhanced release of cytokines, chemokines and metabolites. Additionally, IgA induced a stronger calcium release and a stronger and more sustained phosphorylation of different signaling molecules. Interestingly, these effects could also be observed after IgA triggering of monocytes. These results are against the current dogma which dictates that both FcαRI and Fcγ receptors use similar signaling pathways via immunoreceptor tyrosine-based associated motifs (ITAMs). The delineation of the exact signaling pathways of FcαRI and Fcγ receptors is the subject of further studies, as specifically targeting IgA signaling pathways may represent a novel therapeutic strategy to prevent tissue damage in IgA-mediated autoimmune diseases.

Notes:
THE NETHERLANDS CHLAMYDIA COHORT STUDY (NECCST): RISKS OF LONG-TERM COMPLICATIONS FOLLOWING CHLAMYDIA TRACHOMATIS INFECTIONS IN WOMEN.
Hoenderboom B.M., Morré S.A., Van den Broek I.V.F.
Immunogenetics, VUmc

Background: The Netherlands Chlamydia Cohort Study (NECCST) follows a cohort of women of reproductive age for ≥10 years to investigate Chlamydia trachomatis (CT) related risk (factors) for late complications, including the role of host genetic biomarkers. The NECCST-cohort builds on a prior large-scale Chlamydia Screening Implementation (CSI, 2008-2011). Here, outcomes from the first NECCST collection round are described.

Methods: In 2015-16 CSI women were invited to participate in NECCST. Data on CT-infections, pregnancies and the late complications Pelvic Inflammatory Disease (PID), ectopic pregnancy (EP) and tubal infertility (TI) were collected by questionnaires. CT Immunoglobulin G (IgG) was measured in self-collected blood samples. A positive CT history was defined as ≥1 positive outcome, either a positive CSI CT Polymerase Chain Reaction (PCR) result, a self-reported CT-infection or CT IgG presence. Current NECCST data were combined with background CSI-data.

Results: Among the 5,704 women enrolled, CT IgG prevalence was 14.5%. Of women with self-reported CT-infection or who had been CSI-PCR positive, 38.1% was CT-IgG positive. Of women without a self-reported CT-infection and who had been CSI-PCR negative, 7.0% was CT IgG positive. Overall, 29.2% (n=1,665) had a positive CT-history. Women with a positive CT-history reported less often planned pregnancies compared to a negative CT-history (19.5% versus 27.4%, P<0.001). In contrast, unplanned pregnancies were more common among women with a positive CT-history (24.7% versus 12.4% p<0.001). Women with positive CT-history had significantly higher risk of PID and TI compared to women with negative CT-history: 5.0% versus 2.0% (p<0.001) and 1.1% versus 0.3% (p<0.001), respectively.

Conclusion: Intermediate outcomes of NECCST after 4-7 years follow-up from initial CT screening suggest a higher risk for PID and TI in women with a positive CT-history. NECCST is expected to yield valuable results for identification of risk factors for CT-complications, which might enable targeted secondary preventive measures.

Notes:
TLR-FcγR cross-talk induces inflammation by human myeloid antigen-presenting cells through IRF5 dependent gene transcription


EXIM, KIR, AMC

Myeloid antigen presenting cells (APCs) are crucial for initiation of both physiological and pathological inflammatory responses, which critically depends on the cooperation of different families of receptors. An important recently identified route of induction of inflammation by human APCs involves cross-talk between Toll-like receptors (TLRs), recognizing microbial structures or dead/damaged host cells, and low-affinity Fc gamma receptors (FcγRs), recognizing IgG immune complexes. The physiological function of this TLR-FcγR cross-talk is to provide protective immune responses against invading pathogens. However, erroneous activation of TLR-FcγR cross-talk, e.g. by auto-antibodies, also plays a major role in the development of chronic inflammatory disorders such as rheumatoid arthritis (RA). Since interfering with TLR-FcγR cross-talk may have great therapeutic potential, we here set out to identify the molecular mechanisms responsible for TLR-FcγR cross-talk-induced inflammation. Strikingly, we identified that production of pro-inflammatory cytokines by TLR-FcγR cross-talk critically depends on the induction of two independent signaling pathways that ultimately converge on activation of the transcription factor IRF5. First, TLR stimulation results in phosphorylation of TBK1/IKKe, which is required for IRF5 phosphorylation and subsequent transcriptional activation. Second, we identified that FcγR stimulation signals via Syk and TRAF6 to induce IRF5 nuclear translocation. Combined, these two receptors amplify pro-inflammatory cytokine production by human APCs through IRF5-dependent gene transcription. Taken together, these data provide new potential targets for treatment to suppress inflammation in auto-antibody associated diseases such as RA, systemic sclerosis, and systemic lupus erythematosus (SLE).

Notes:
Phenotype and function of Natural Killer cells in CLL
Experimental Immunology, AMC

Chronic lymphocytic leukemia (CLL) is the most common leukemia in the western world. CLL patients experience viral reactivations and other opportunistic infections, which have been linked to an acquired immunodeficiency. Until now, the main focus of research has been on T-cell dysfunction. Natural Killer (NK) cells are important directly cytolytic cells which recognize and kill both virus infected as well as tumor cells. Despite their important role in tumor surveillance and in therapeutic strategies like monoclonal antibodies, little is known about NK cell function in CLL. We studied NK cell phenotype in CLL patients and age-matched healthy donors (HC) by flow cytometry. NK cell function was analyzed by co-culturing with a K562 cell line and measuring cytokine production and target cell death.

There was no difference in subset (CD56brightCD16-; CD56dimCD16+; CD56negCD16+) composition of NK cells in CLL patients compared to HC. Activating and inhibitory receptors on NK cells are mostly similarly expressed between CLL and HC, except NKG2D, which is slightly downregulated. Despite the similarity of these expression levels, CLL-derived NK cells displayed reduced cytotoxicity towards K562 in the presence of CLL cells. In contrast, pure fractions of NK cells, isolated from CLL patients by flow-assisted cell sorting, had full cytotoxic abilities compared to HC-derived NK cells. To analyze whether CLL cells directly influenced NK cell function, we co-cultured HC-derived NK cells with allogeneic CLL or HC-derived B-cells for 48 hours. We found that HC-derived NK cells were significantly impaired in the killing towards K562 cells after co-culture with CLL cells, but not HC-derived B-cells. Interestingly, cytokine production and degranulation by CLL-derived NK cells seems not to be affected. We are currently studying the relation between NK cell activation, cytokine production and cytotoxicity, to further determine which NK cell function are critically impaired in CLL, and whether aberrant activation of the NK cell plays a role in reduced function.

Notes:
**Palmitoylated antigens efficiently target skin-resident dendritic cells for treatment of cancer.**


MCBI, VUmc

To counteract tumor-derived immune suppression, effective immunotherapy should boost existing or elicit de novo, tumor-specific immune responses. Vaccines for the induction of these responses are not yet realized, because of knowledge gaps concerning the choice of suitable antigens and mode of targeting these antigens to dendritic cells (DCs), vital cells for the instruction of tumor-specific T cells. To address the problems concerning the inadequate uptake of antigen by DCs we modified tumor-associated antigens (TAAs) with a palmitic acid, known for its ability to target cellular membranes. *In vitro* monocyte-derived dendritic cells displayed enhanced activation of tumor-specific CD4+ and CD8+ T cells, due to superior uptake of the palmitoylated antigen. Strikingly, the routing of the palmitoylated antigen differed from that of the unmodified peptide, resulting in a more efficient entry in the MHC class I pathway. Enhanced uptake of the TAAs conjugated to a palmitic acid was also observed in our novel ex-vivo human skin model in dermal dendritic cell (dDC) present in the dermal and Langerhans cells (LCs) present in the epidermal layer of the skin. By mimicking the migration of dDCs and LCs to the lymph nodes we confirmed that the migratory APC subsets were eliciting the enhanced CD8+ T cell activation. Thus, targeting of dermal dendritic cells with palmitoylated antigens represents a novel and attractive vaccine strategy for the activation of skin antigen-presenting cells for the treatment of cancer.

Notes:
Semi-quantitative bacterial culture is the standard method to diagnose urinary tract infections (UTI), but bacterial growth rate limits diagnostic speed and it is unreliable when patients have been pre-treated with antibiotics. Metagenomics could increase diagnostic speed and accuracy by sequencing the microbiome and resistome directly from urine samples, bypassing culture. However, a semi-quantitative approach – as needed for diagnosing UTIs – has not been established.

Metagenomics was deployed to identify and semi-quantify bacterial presence indicative of UTI, predict antimicrobial susceptibility (AMR), and results were compared to semi-quantitative culture. Whole genome sequencing of the corresponding uropathogens was done for comparison. Analysis time and cost were tracked.

Forty-one consecutive urine samples underwent metagenomic analysis. All culture positive samples contained >200ng of DNA, suggestive of a threshold below which UTI could be ruled out solely based on DNA quantity. A semi-quantitative Diagnostic Index (DI) was created by multiplying the total DNA quantity by the relative abundance of uropathogens per urine sample. The DI allowed discrimination of UTI from non-UTI samples in all but 1 case. Metagenomic detection of AMR determinants correctly predicted the phenotype of uropathogens in 20 of 32 cases. The metagenomic workflow took 31h and cost €121 per sample.

The genomic determinants of AMR and their distribution across uropathogens need to be better understood for prediction of AMR phenotypes by metagenomics. The introduction of the DI demonstrates the potential of semi-quantitative metagenomics to replace culture as rapid diagnostic method for UTI.

Notes:
Targeting of noncanonical NF-κB signaling in endothelial cells significantly enhances the blocking effects of bevacizumab on angiogenesis in an in vitro 3D minitumour model of colorectal cancer

Jeucken K.C.M, C.X. Maracle, B. Helder, A. Steins, H.W.M van Laarhoven, S.W. Tas
Experimental Immunology - Div. of Clinical Immunology and Rheumatology, AMC

Angiogenesis is involved in tumour growth and metastasis of colorectal cancer. Current anti-angiogenic therapies focus mainly on targeting vascular endothelial growth factor (VEGF). Unfortunately, most tumours eventually develop resistance against anti-VEGF therapies. Recently, we identified NF-κB inducing kinase (NIK), a key component of noncanonical NF-κB signaling, to be expressed in blood vessels in colorectal cancer tissue. Since noncanonical NF-κB signaling is an important regulator of angiogenesis, NIK could be a potential anti-angiogenic target in colorectal cancer.

We used a in vitro 3D model of tumor angiogenesis modified to study colorectal cancer. This model utilizes spheroids consisting of colorectal cancer cells (colo320-HRS), human umbilical vein endothelial cells (HUVEC) and normal human dermal fibroblasts (NHDF). We compared the effects of siRNA-mediated silencing of NIK in endothelial cells (EC) and VEGF-blocking monoclonal antibody bevacizumab in this model. HUVEC sprouting was detected by confocal microscopy, quantified and used as marker for angiogenesis.

Noncanonical NF-κB signaling was activated by the lymphotoxin β receptor (LTβR) ligands LTβ and LIGHT, which was validated by production of pro-angiogenic factors IL-6, IL-8, CXCL1 and CXCL5. HUVEC sprouting was induced upon stimulation with LTβ, LIGHT or growth factor (GF, combination of VEGF and basic fibroblast growth factor (bFGF)). Targeting of NIK in HUVEC using siNIK attenuated angiogenesis after LTβ- and LIGHT-stimulation, but not after GF stimulation. Bevacizumab treatment inhibited angiogenesis after LIGHT- and GF-stimulation. Combining siNIK and bevacizumab led to greater reduction of angiogenesis after LT, LIGHT or GF stimulation compared to bevacizumab treatment alone.

These findings show that targeting of noncanonical NF-κB signaling leads to an attenuation of angiogenesis in a 3D minitumour model. Importantly, combined targeting of NIK and bevacizumab has additional value in reducing tumour angiogenesis. This may identify NIK as a potential new target for anti-angiogenic therapy, which may improve current treatment strategies in colorectal cancer.

Notes:
The contribution of non-canonical NF-κB signalling in endothelial cells to pathological bone formation in spondyloarthritis

Kaaij M.H., Sander Tas
EXIM, KIR, AMC

Background: Spondyloarthritis (SpA) is characterized by inflammation, as well as extensive angiogenesis and pathological bone formation. Of note, angiogenesis is required for osteogenesis. Transmembrane (tm)TNF transgenic (tg) mice that overexpress tmTNF and exhibit features of SpA, develop chronic inflammatory features and pathological osteogenesis. tmTNF ligation to TNF receptor 2 (TNFR2), which is restricted to specific cell types including endothelial cells (ECs), can induce noncanonical NF-κB signaling which is involved in pathological angiogenesis. A capillary subtype, termed type H, has recently been demonstrated to couple angiogenesis and bone formation.

Objective: To investigate the link between pathological angiogenesis and osteogenesis in tmTNF tg mice and the potential contribution of noncanonical NF-κB signaling in EC to this process and infiltration of immune cells into the bone marrow (BM).

Methods: Ankles and vertebrae from 12 week old tmTNF tg mice or littermate wild type (WT) mice were collected. Bone tissues were prepared by cutting 60 µm thick cryosections for immunofluorescence staining and 3D confocal imaging.

Results: Compared to WT mice, BM of tmTNF tg mice contained extensive lymphoid aggregates that predominantly consisted of B cells. Vertebrae of tmTNF tg mice also exhibited increased osteogenesis and ectopic osteogenesis, which was not observed in WT mice. Preliminary data suggests that ectopic osteogenesis may be associated with type H vessels at the enthesis.

Conclusions: Overexpression of tmTNF in mice leads to extensive lymphoid aggregates in BM, as well as ectopic type H vessels associated with ectopic osteogenesis. The increase in ectopic bone formation may be the result of enhanced noncanonical NF-κB activation in ECs, which is the subject of current studies.

Notes:
Introduction: The host response to infection is characterized by a complex interplay of cellular transcriptional responses. Both hyper-activation as well as severely depressed host response mechanisms are detrimental to cells and tissue systems. This argues for a better understanding of the quantitative nature of the host response.

Objective: To identify host innate immune mechanisms that underlie the quantitative genomic response to lipopolysaccharide (LPS) in humans.

Methods: The study included whole blood gene expression microarray data from the human endotoxemia model (n=42; 4 hours post-LPS). Publicly available data of Illumina microarrays were retrieved for 1ng/kg and 4ng/kg LPS doses. We recruited an additional 8 healthy volunteers who received 2ng/kg LPS intravenously with blood for microarrays (Affymetrix human genome U219 chips) collected at 4 hours post-LPS. After background correction, the different microarray studies were combined for meta-analysis by firstly re-mapping probes to the current Genome Reference Consortium Human genome build 38 (GRCh38) available via GenCode. Gene expression data were subsequently normalized and adjusted for non-experimental chip effects. Data were analyzed by moderated t statistics and enrichment of canonical signaling pathways performed by Ingenuity Pathway Analysis.

Results: Differential gene expression analysis of 1 ng/kg, 2ng/kg and 4ng/kg LPS doses relative to pre-LPS (baseline) uncovered 6044, 4796 and 5893 significantly altered genes, respectively. Both unique and common transcriptional signatures were identified, with 638 commonly altered and 2267 uniquely altered genes dependent on LPS dose. Pathway analysis of elevated genes as a function of LPS dose revealed 27 canonical signaling pathways that included death receptor signaling, protein ubiquitination, PI3K/AKT and IL-6 signaling. Genes with decreased expression as a function of LPS dose significantly over-represented 12 pathways including EIF2 signaling, oxidative phosphorylation and mitochondrial dysfunction.

Conclusion: Shared and distinct cellular biological pathways were identified that responded quantitatively to different LPS doses. Genes involved in translation (EIF2 signaling), metabolism (oxidative phosphorylation, mitochondrial dysfunction) and cell death (death receptormsignaling) represented major cellular biological pathways influenced by LPS dose.

Notes:
NLRX1 deficiency prevents diet-induced hepatic steatosis by promoting mitochondrial oxidative phosphorylation in hepatocytes

Pathology, AMC

Background and aim: Non-alcoholic fatty liver disease (NAFLD), in which mitochondria play a central role, is a precursor for the metabolic syndrome and related diseases. NOD-like receptor (NLR)X1 (NLRX1) is an inflammasome-independent innate immune receptor that is uniquely localized in mitochondria, with as yet unknown effects on metabolic diseases. The aim of this study was to investigate the crosstalk between metabolism and NLRX1 during metabolic overload by studying hepatocyte energy metabolism, NAFLD, metabolic syndrome and associated kidney injury in the absence and presence of NLRX1.

Methods: In vivo male wild-type (WT) and NLRX1 knock-out (KO) mice (n=9-12) were fed a control diet (CD, 11% kcal derived from fat) or western-type diet (WD, 43% kcal derived from fat) for 16 weeks. In vitro we generated by CRISPR/Cas-9 genome editing NLRX1-deficient AML12 hepatocytes and analyzed energy metabolism during lipid overload.

Results: Hepatic NLRX1 expression in WT mice was upregulated during WD-induced NAFLD. In contrast to WT mice, NLRX1 KO mice were protected from WD-induced NAFLD, hepatic fibrosis, metabolic syndrome, and kidney dysfunction independent from inflammation. While mitochondrial content was equal, NLRX1-deficient hepatocytes showed increased mitochondrial fatty acid-dependent oxidative phosphorylation (OXPHOS) metabolism and decreased steatosis compared to controls. Moreover, glycolysis was decreased in NLRX1-deficient cells versus controls.

Conclusions: We found that NLRX1 plays an important role in promoting diet-induced NAFLD, hepatic fibrosis, obesity, insulin resistance, and related kidney dysfunction by directly regulating the energetic metabolic activity of hepatocytes via the restriction of OXPHOS and the enhancement of glycolysis. As such NLRX1 may be an attractive novel therapeutic target for hepatic steatosis and metabolic syndrome.

Notes:
A novel ILC/NK-like cell may be involved in Inflammatory Bowel Disease
Krabbendam L, J.H. Bernink, H. Spits
Experimental Immunology, AMC

Inflammatory Bowel Disease (IBD) comprise a group of severe inflammatory disorders, including Crohn’s disease and Ulcerative Colitis, which arise as a consequence of dysregulated immune responses.

Innate Lymphoid Cells (ILCs), including helper ILCs and NK cells are important in maintaining the intestinal homeostasis and providing protective immune responses, but also contribute to inflammatory diseases when not properly regulated. Three major subsets of helper ILCs can be distinguished on the basis of their cytokine secretion profile, but each subset has the capacity to adapt its phenotype and cytokine secretion profile in response to changing environment cues. However, this plasticity has mainly been observed between the family of helper ILCs, but whether these ILCs can convert into cytotoxic ILCs (NK cells) is unclear.

We identified a human ILC/NK-like cell that expresses both the ILC marker CD127 (IL-7R) and the NK cell marker CD94. This cell type was found more prominent in adult inflamed intestine when compared to healthy samples, and nearly absent in fetal intestine, suggesting that an inflammatory environment favors this cell type. Indeed, we found that bona fide helper ILCs can convert into this ILC/NK-like cell type when exposed to in inflammatory cytokine milieu in vitro. These ILC/NK-like cells share characteristics with both ILCs and NK cells, based on phenotypic markers, transcription profile and cytokine secretion suggesting that helper ILCs can acquire features of NK cells and possibly vice versa. These newly identified cells that seem to be at the interface of NK cells and ILCs may contribute to IBD pathology.

Notes:
**HIV-1 infection in macrophages reduces expression of pro-inflammatory genes.**

*Kruize Z., Cobos Jiménez, V., Boorman, T., Martinez, F., Gordon, S., & Kootstra, N.A.*

*Experimental Immunology, AMC*

Background: Macrophages are effectors of the innate immune response, responsible for sensing and clearing pathogens. HIV-1 is able to infect macrophages without inducing a strong innate immune response, establishing a viral reservoir in tissues and spreading infection to CD4+ T cells. We studied genome-wide transcriptome profiles in macrophages infected with HIV-1 to get new insights in antiviral mechanisms and evolution of HIV-1 to circumvent these mechanisms.

Methods: Monocytes were cultured in presence or absence of cytokines, to generate monocyte-derived macrophages, M1 (IFN-γ/TNF-α), M2a (IL-4) and M2c (IL-10) polarized macrophages. Cells were inoculated with CCR5-using HIV-1 (NL4-3.Ba-L) and cultured for 24h to allow completion of the viral replication cycle. Total RNA was used for genome-wide gene expression arrays. Functional analysis was performed using Ingenuity Pathway Analysis software.

Results: The expression levels of 1078 genes were regulated either upon infection with HIV-1 or cytokine polarization (>2-fold change). Of these genes, 690 genes changed in IL-4 treated cells, whereas IFNγ+TNFα and IL-10 stimulation induced changes in 141 and 152 genes, respectively. HIV-1 infection of macrophages induced changes in 257 genes. When cells were stimulated with IL-4 and subsequently infected with HIV-1, 745 genes changed in their expression levels. Infection of IFNγ+TNFα and IL-10 stimulated cells exhibited changes in 258 and 279 genes respectively.

Changes in gene expression associated with HIV-1 infection, corresponded to a decrease in the following functions: “Inflammatory Response”, “Cellular movement”, “Immune cell trafficking” and “Haematological System Development”. Remarkably, we observed an overlap between the HIV-1 and IL-4 profile, but not with the other activation profiles.

Conclusion: HIV-1 infection in macrophages induces changes in the transcriptional profile similar to IL-4 stimulation, consistent with a more anti-inflammatory phenotype. This indicates that HIV-1 dampens the ability of macrophages to mount inflammatory responses, probably to support replication and evade recognition by the immune system.

Notes:
SF3B1 mutations in cancer lead to an altered DNA damage response, and splice products that are substrates for nonsense-mediated decay

Leekstra AC, Alexander C. Leekstra1,2, Ingrid A. Derks1, Doreen te Raa1,2, Arjan A. van de Loosdrecht3, Joop H. Jansen4, Annelies de Klein5, Jeroen E. Guikema5, *, Arnon P. Kater2, *, and Eric Eldering1 *
Experimental Immunology, AMC

Next generation sequencing of cancer genomes has uncovered mutations in splicing factors in a variety of malignancies. Notably, heterozygous mutations of the U2 snRNP subunit SF3B1 were found in myelodysplastic syndrome (MDS), chronic lymphocytic leukemia (CLL), and uveal melanoma (UM), with both positive or negative effects on prognosis. Especially in CLL SF3B1 mutations are associated with worse prognosis and chemorefractoriness. We found that SF3B1 mutations in CLL have an impact on the DNA damage response (DDR) similar to an ATM defect (te Raa et al Leukemia 2014), although the mechanism remains unknown. Here, we report on the effect of SF3B1 mutations in cycling and non-cycling cells on the DDR, apoptosis and alternative splicing in various cancer types.

We detected an impaired apoptotic response after DNA damage in SF3B1mut CLL cells and UM cell lines. Secondly, we established that SF3B1 mutations are associated with increased alternative splicing products in CLL, UM and MDS that account for only 0.01-10% of the normal (WT) mRNA expression, and can therefore hardly explain the pathological effect. Using SF3B1mut and WT cell-lines, we found that those alternative transcripts are subject to nonsense-mediated decay (NMD), the process that degrades aberrant transcripts containing premature stop codons. We are currently testing isogenic SF3B1mut and SF3B1wt cancer cells to find out whether SF3B1 mutations can affect expression of normal transcripts involved in the DDR.

Notes:

________________________________________________________________________

________________________________________________________________________

________________________________________________________________________

________________________________________________________________________

________________________________________________________________________

________________________________________________________________________

________________________________________________________________________

________________________________________________________________________

________________________________________________________________________

________________________________________________________________________

________________________________________________________________________

________________________________________________________________________

________________________________________________________________________

________________________________________________________________________

49
Dendritic cells (DCs) are key inducers of the adaptive immune response. They possess a multitude of different pattern recognition receptors (PPRs), including Toll-like receptors (TLRs), NOD-like receptors and C-type Lectin Receptors (CLRs) in order to elicit tailor-made immune response against invading pathogens. Over the last few decades, CLRs have gained much attention not only as endocytic PRRs, but also for their immune modulatory functions. Strikingly, several carbohydrate ligands are shared among different CLRs, yet each seems to propagate a unique signalling cascade. Additionally, CLR triggering can modulate the signalling pathways of other PRRs, such as the TLRs, to modify or prolong the TLR-induced response.

We recently showed that carbohydrates can have a huge impact on how DC polarize or suppress T cell responses. Carbohydrates such as High Mannose-, LewisX-, or LewisY-containing ligands displayed differential IL-10 and IL-12 expression profiles by DC after concomitant LPS stimulation. On the other hand α2-3- or α2-6-sialic acid-containing ligands skew DCs towards the induction of T regs. These carbohydrates target differential CLRs such as DC-SIGN and Siglec, respectively. To gain further insight the immunogenic signalling pathways of the DC-expressed CLR DC-SIGN and its interference with TLR signalling, as well as the tolerogenic signalling pathways through Siglec, we coupled different carbohydrate ligands to a rigid dendrimeric structure, thereby offering multivalent presentation of these ligands. For insight in the underlying signalling pathways, we applied phosphoproteomics to investigate differences in DC protein phosphorylation upon specific CLR-ligand engagement, as well as next generation sequencing on a transcriptional level. Detailed analysis of these signalling pathways will reveal how carbohydrates contribute to creating an immunogenic or tolerogenic fingerprint by modifying DC through CLRs.

Notes:
For formation of plasma cells, B cells need be activated by specific antigen and subsequently present the ingested antigen via MHC class II molecules to antigen-specific follicular Thelper (Tfh) cells to receive additional signals from the Tfh cells. Costimulation of CD40 on the B cells by CD40L on Tfh cells and various cytokines secreted by Tfh cells, induce class switching and generation of IgG B cells. In addition, these factors drive formation of long-lived IgG-secreting Plasmablasts (PBs)/Plasma cells (PCs). It remains to be uncovered which combination of Tfh factors optimally tune B cells for PB/PC differentiation. Progress in this area is complicated by the poor success in priming of naïve B cells for PB/PC differentiation in vitro. Our novel data show that repeated costimulation in the right Tfh cytokine environment induces prominent PB and PC formation upon naïve B cell differentiation. In these repeated interactions, the strength of repeated CD40 costimulation and the interplay with Tfh cytokines signaling determine 1) the efficacy of PC formation, 2) the type of differentiation route leading to PB/PC formation and 3) the phenotypes of PB/PCs induced. Strikingly, the specific conditions also seem to regulate the chemokine receptor profile of the differentiating B cells and newly formed PCs. These chemokine receptors enable germinal center reactions and bone marrow homing for long-term PC survival. This suggests that the factors that drive formation of specific PB/PCs may also imprint PC longevity.

Notes:
Psoriasis is a uniquely human autoimmune disease mediated by IL-17 producing T cells. We studied non-lesional, lesional and previously lesional skin in patients who had cleared on etanercept therapy. We found expanded oligoclonal T cell populations in healed psoriatic lesions which produced IL-17 and/or IL-22 in active lesions from the same patients, suggesting they represent disease initiating T cells. Further alpha/delta TCR sequencing demonstrated that these putative disease initiating T cell clones were universally alphabeta T cells. In contrast to studies in mouse models, gammadelta T cells were rare in human psoriasis, previously lesional psoriatic skin and in healthy human skin, making up only 1.6%, 0.45% and 1.8% of the total T cell population respectively. By matching TCR alpha and beta sequences of initiating clones based on T cell frequency, we have obtained for the first time the complete TCR sequence of autoreactive T cells of origin in psoriasis. In short, we have identified and characterized the T cells of origin in psoriasis and find that they are universally alphabeta T cells producing IL-17 and/or IL-22. In dramatic contrast to experiments in mice. These results show that human psoriasis is a disease mediated by alphabeta T cells. Lastly, we have identified for the first time the full TCR sequences of initiating T cells in psoriasis, the first step in future studies designed to identify the autoantigen in psoriasis.

Notes:

________________________________________________________________________________________

________________________________________________________________________________________

________________________________________________________________________________________

________________________________________________________________________________________

________________________________________________________________________________________
How do glycans affect immune cells in RA?
MCBI, VUmc

Background and objectives
The fragment antigen-binding domain of Anti-Citrullinated Protein Antibodies (ACPAs) was recently shown to be extensively glycosylated. It is known that glycans play a key role in controlling innate and adaptive immunity and therefore we hypothesize that the glycans on ACPA may interact with glycan binding receptors and thus modulate immune responses in RA. Therefore, our aim is to elucidate the effect of glycan and ACPA binding to immune cells of RA patients to increase our understanding of RA pathogenesis.

Materials and methods
A whole blood flow assay is used to study glycan interactions with leukocytes. Leukocytes were isolated from blood and cells were incubated for 4 hours with 4 µg/ml of glycoconjugates at 4°C. Glycan binding and identification of immune cell subsets was assessed with flow cytometry.

Results
A whole blood flow assay of four healthy donors showed consistent high binding of mannose specifically to B cells and monocytes. Increased sialic acid binding to B cells, monocytes and neutrophils was observed after neuraminidase treatment to free sialic acid receptors on these cells. A more in-depth analysis of the interacting leukocyte cell subsets will be used to further investigate the binding of glycans to leukocytes and the expression of the corresponding receptors both in healthy donors and established RA patients.

Conclusion
This study examines the glycan-binding capacity of leukocytes in healthy donors and RA patients via the whole blood flow assay. Our preliminary data indicates specific binding of mannose and sialic acid to B cells. This is an important finding because B cells play a key role in the pathogenesis of RA, as they produce ACPA and are very efficient in antigen presentation. Further studies on glycan binding to other key immune cells in RA may aid in elucidating their role in the pathogenesis of RA.

Notes:
A two-hit hypothesis exists for Transfusion-Related Acute Lung Injury (TRALI), which suggests neutrophils and pulmonary endothelium have been previously activated as a “first hit”, before the “second hit”: transfusion of blood products which further aggravate the immune response, and result in endothelial damage and pulmonary edema. This hypothesis is supported by the observation that mice housed in a pathogen free environment, and mice selectively lacking toll-like receptor 4 (TLR4) on hematopoietic cells or pulmonary endothelium, are protected from the development of TRALI.

We have investigated the effect on endothelial activation and permeability, and transendothelial migration (TEM) by neutrophils at 0-1h, 4-6h and 20-24h stimulation of human umbilical venous endothelial cells (HUVECs) with LPS (a TLR4 ligand) or TNF (acting via the TNF receptor).

Western blotting was used to evaluate differences in vascular endothelial-cadherin (VE-cadherin; CDH5; CD144), intracellular adhesion molecule 1 (ICAM-1; CD54), vascular cell adhesion molecule 1 (VCAM-1; CD106) and endothelial selectin (E-selectin; CD62E). Expression patterns of these proteins was also evaluated with immunofluorescence (IF) staining. TEM was evaluated in static conditions and under flow using time-lapse confocal or wide field microscopy.

At 0h stimulation, no TEM was observed with pre-activated neutrophils isolated from whole blood of healthy volunteers. Maximum TEM was observed at the 4-6h time point in static and flow conditions for both LPS and TNF activated HUVECs, whereas TEM was ablated at the 20h time point for LPS, but not for TNF. Maximal TEM was not found to correlate with maximal ICAM-1 expression. No differences in VE-cadherin, E-selectin or VCAM-1 could be detected between the time points using Western blotting. IF staining showed E-selectin and ICAM-1 cluster at sites of TEM by neutrophils and has increased expression at the 4-6h and 20-24h time points. This study supports endothelial activation as the key moderator of TEM by neutrophils.

Notes:

________________________________________________________________________

________________________________________________________________________

________________________________________________________________________

________________________________________________________________________

________________________________________________________________________
IgG opsonization of viruses functions as an endogenous suppressor of type I and III IFN-related anti-viral immunity by human myeloid cells through FcγRIIa
Experimental Immunology/ Clinical Immunology and Rheumatology, AMC

While type I and type III interferons (IFNs) are fundamental for antiviral immunity, prolonged expression of these IFNs is also detrimental to the host. Therefore, type I and III IFN expression is tightly controlled upon viral infection, with high levels in the first few days followed by a strong and rapid decline. However, the endogenous mechanisms responsible for this suppression are still largely unknown. Here, we hypothesized that virus-specific IgG antibodies, which only emerge during late-phase of infection, function as an environmental cue for suppression of type I/III IFN responses. Strikingly, we indeed observed that IgG opsonization of model viruses influenza and respiratory syncytial virus (RSV) strongly suppressed type I/III IFN production by various human myeloid cells, including dendritic cells, macrophages, and Langerhans cells. Consequently, IgG opsonization also suppressed the expression of numerous IFN-stimulated genes (ISGs), while other anti-viral genes such as IL-27 and CD70 were unaffected. We identified Fc gamma receptor IIa (FcγRIIa) as the main receptor responsible, which strongly and selectively suppressed type I/III IFN gene transcription via cross-talk with TLR3,9, RIG-I/MDA5 and cytosolic DNA sensors, such as cGAS. Taken together, we have identified IgG opsonization of viruses as a novel endogenous suppression mechanism of type I and III IFN responses by the myeloid cell compartment. Considered the specificity for type I and III IFNs, this suppression mechanism may be a valuable potential tool for future treatment of type I IFN-associated diseases such as SLE and Sjögren’s Syndrome.

Notes:
T cells mediate adaptive immune responses. While circulating T cells protect against systemic infections, non-circulating resident memory T cells (TRM) are critical for protection in barrier tissues such as the lungs, skin and intestine. While the mechanisms by which TRM are recruited have become increasingly appreciated, major question remains regarding the heterogeneity and spatial organisation of these cells. While CD8+ TRM are extensively investigated, the role of the more abundant CD4+ TRM remains elusive. Single-cell analysis based on flow cytometry revealed three subsets of lung CD4+ TRM: CD69+CD103+, CD69+CD103- and CD69-CD103- cells. Using bulk-cell transcriptome analysis, we identified the transcriptional profile of human lung CD4+CD103+ TRM and compared this to blood CD4+ T cell subsets. We demonstrated that lung CD4+CD103+ TRM represent an unique subset of CD4+ T cells that is very different from circulating CD4+ T cells, but closely related to CD8+ TRM. CD4+ TRM expressed unique sets of chemokine receptors and adhesion molecules necessary for tissue homing. We revealed that resting CD4+CD103+TRM constitutively express high mRNA levels of cytotoxic mediators, but no protein. Functionally this was reflected by a fast recall response, magnitude of cytokine production, and a high degree of polyfunctionality. Phenotypic validation of target genes revealed a previously unrecognized complexity of the CD4+ TRM compartment in the lungs, as the expression of VLA-1, CXCR6 and the inhibitory 2B4 did not strictly associate to any of the conventional TRM phenotypes. Future single-cell approaches may deal with this caveat of population-averaged measurements in conventional transcriptome analysis experiments. Better understanding of the molecular and functional characteristics of heterogeneous CD4+ TRM populations may improve rational vaccine design and the efficacy of adoptive T cell therapy.

Notes:
Objectives. Recently it was reported that the cellular metabolism of immune cells is tightly linked to the functional phenotype of these cells. Pro-inflammatory cells metabolize pyruvate predominantly to lactate through a process called "aerobic glycolysis", whereas cells with an immunotolerant phenotype preferentially use oxidative phosphorylation to metabolize glucose. The regulation of this process is not yet fully understood. One of the key regulators of glycolysis is Hypoxia Inducible Factor (HIF)-1alpha. We are investigating the role of HIF1alpha during the inflammatory response against bacterial components (LPS) and during pneumonia.

Methods. We use the HIF1a inhibitor PX-478 and the HIF1a-stabilizing compound IOX2 to, respectively, inhibit and overexpress HIF1a in PBMCs and monocytes. IOX2 has also been used in in vivo pneumonia-models with WT mice. Furthermore, we are generating LysM-cre;HIF1a-flox and MRP8-cre;HIF1a-flox conditional knock-out mice to knock-out HIF1a in the myeloid lineage and granulocytes respectively.

Results. Inhibition of HIF1a by PX-478 in monocytes has proven difficult to show. However, we do see a trend towards reduced cytokine expression of TNF, IL-1b and IL-10 whilst IL-6 is unaffected. Stabilization of HIF1a is clearly shown by Western Blot. Ex vivo treatment of PBMCs and monocytes with this compound in combination with LPS in low dosage (0.1 ng/ml), increases IL-1b and TNF production. In vivo stabilization of HIF1a does not affect clearance of Pseudomonas aeruginosa in the lung. However, IOX2 treated mice do show higher expression of activation markers CD11b and CD62L on the alveolar macrophages. Cytokine production did not differ. The experiments with Klebsiella pneumoniae and the conditional knock-out mice remain to be performed.

Conclusion. HIF1a seems to be important for the activation of monocytes and macrophages and the production of IL1b and TNF. However, repeated experiments and additional in vivo experiments will have to be performed.

Notes:
The introduction of biologicals constituted a major step forward in the treatment of autoimmune disease. However, immunogenicity of these drugs has been associated with loss of effectivity and other unwanted side effects. Until recently research into antidrug responses has mainly focussed on the serological responses themselves, mainly due to technological limitations. Consequently, little insight has been gained in the cellular responses that precede the formation of antidrug antibodies.

To better investigate timing and characteristics of cellular responses we developed a novel technology to identify, fingerprint and quantify clonal T- and B-cell receptor responses with very high resolution using next-generation sequencing (NGS)–based repertoire analysis. NGS based analysis allows us to monitor clonal B- and T-cell receptor responses over time, and correlate responses across different time points in different tissues.

To link these fingerprints to phenotype and antigen specificity, we tested different formats of in vitro T-cell assays. While most assays showed high variability at the clonal level, the peptide based proliferation assays in the group of Bernard Maillère at CEA in France proved to be extremely robust. A novel methodological and bioinformatics approaches we then developed to create a statistical basis for identifying responding clones. The final results of this combine effort is a standardized, robust, highly sensitive, high-resolution tool to identify and phenotype (in vitro), and monitor and track (in vivo) specific fingerprinted T-cell responses at the clonal level.

The application of this methodology to cross-correlate in vitro and in vivo clonal responses in pre- and post-treatment samples of patients starting e therapy with a biological will help to elucidate the mechanisms and kinetics of cellular antidrug responses and develop a predicting (early) markers for immunogenicity.
**mTOR inhibition enhances the immune response of bronchial epithelial cells in response to Gram-negative pathogens but not LPS**

*Ramirez Moral I, Yu, X; de Vos, AF; de Jong, MD, van der Poll, T.*

**CEMM, AMC**

**Introduction**

Epithelial cells play a crucial role during bacterial infections of the airways by sensing of pathogens and orchestrating immune responses. Interfering the metabolism of immune cells is a novel approach to modulate their function and the mTOR pathway has been identified as a key regulator of physiological processes and immune effector responses. Inhibition of mTOR activity by metformin has been shown to diminish cytokine secretion by myeloid cells stimulated with bacterial ligands.

**Objective**

To determine the effect of mTOR pathway inhibition on immune mediators by human bronchial epithelial cells in response to pneumonia evoking bacteria.

**Methods**

Human primary bronchial epithelial cells (HBE) were generated from healthy resection tissue of cancer patients and cultured in a polarized manner on an air-liquid interface. Cells were pretreated for 1 hour with the mTOR inhibitor metformin (0-5 mM) and subsequently stimulated for 24 hours with lipopolysaccharide (LPS) (0.1-1 μg/ml), flagellin (1-10 μg/ml), UV-irradiated Klebsiella (K.) pneumoniae or Pseudomonas (P.) aeruginosa.

**Results**

In response to apical exposure with K. pneumoniae, P. aeruginosa or flagellin, but not LPS, HBE cells upregulated the expression of innate immune mediators such as IL-8, β-defensin 2 and CXCL20, and secretion of IL-8 protein at the basolateral side. Inhibition of the mTOR pathway by metformin notably enhanced the transcription of these immune mediators in HBE cells after exposure to the bacterial ligand flagellin. This effect was accompanied by a selective up-regulation of the mitochondrial enzyme superoxide dismutase 2, pointing towards a mitochondrial-related mechanism.

**Conclusion**

Our findings demonstrate that inhibition of the mTOR pathway with Metformin enhances the immune response of bronchial epithelial cells upon bacterial challenge, and suggest that cell-specific differences in mTOR-regulated processes occur.

**Notes:**
Personalisation of Intravenous Immunoglobulin Therapy for Childhood Immune Thrombocytopenia

Schmidt DS, de Haas M, Vidarsson G, van der Schoot CE
Experimental Immunohematology, Sanquin

My PhD project aims to personalize the treatment of pediatric immune thrombocytopenia (ITP) with intravenous immunoglobulins (IVIG). I work with data and material from two randomized controlled trials: the TIKI trial in which 200 children with newly diagnosed ITP were randomized to IVIG or observation only, and the HOVON64 study in which 138 adults with treatment-refractory ITP were allocated to different treatment schemes of Rituximab. Furthermore, we utilize material and data from a Dutch multicenter cohort study of 40 children with chronic ITP.

ITP is a rare disease, affecting annually about 120 children in the Netherlands, but it has profound impact on the quality of life of children and their families. In childhood ITP, children show variable bleeding symptoms and 30% do not respond to treatment with IVIG. Furthermore, 90% of children recover from ITP within a year, irrespective of routine treatment, but 10% develop chronic disease. It is currently unknown what determines this variation.

Using the abovementioned cohorts, we try to (1) explain bleeding symptoms in children with ITP, to indicate who would benefit from treatment, (2) predict which children respond to treatment with IVIG, and (3) identify which patients will develop chronic ITP and possibly require other immune-modulatory therapy. With this information, treatment decisions could be better suited to a particular ITP patient.

Notes:
Inefficient mucosal CD8+ effector T cells set the stage for increased susceptibility to viral infections during infancy
Schreurs RRCE, F. Steinert, A.A. Drewniak, R. Bakx, S. The, A. Sagebiel, D. Perez, K. Reinshagen, T.B.H. Geijtenbeek, J.B. van Goudoever (promotor), M.J. Bunders (co-promotor/supervisor)
Experimental Immunology, AMC

Newborns and infants are more susceptible to gastrointestinal viral infections. Cord blood studies suggest that this is due to a largely naïve and immature CD8+ T cell compartment. However, we have recently identified CD8+ T cells with memory characteristics in the newborn intestinal mucosa. To further understand ontogeny of CD8+ T cells in the intestinal mucosa, we analyzed fetal, infant and adult intestinal mucosal tissue using flow cytometry.

In the fetal mucosa, 38% (interquartile range 32-45%) of CD8+ T cells demonstrated an effector phenotype (CD45RA+/-CCR7-) which increased to 59% (54-74%) in the infant and to 97% (96-99%) in adults. Likewise, the frequency of tissue-resident CD8+ effector (CD69+103+) T cells increased from 40% (24-62%) in the fetus, to 46% (23-51%) in the infant and to 83% (65-88%) in the adult. The production of TNF-a, IFN-y, and IL-2 by both fetal and infant CD8+ T cells was 2-to-3-fold less compared to the 47% (37-50%) of TNF-a, 56% (40-61%) IFN-y, and 33% (22-47%) IL-2-producing mucosal CD8+ T cells in adults. Fetal and infant CD8+ T cells had a reduced capacity to produce more than two cytokines at once. Furthermore, fetal and infant CD45RA+/−CCR7− CD8+ T effector cells were predominantly CD27+28+ with corresponding little production of granzyme B and perforin (0-4% in fetus and 0-21% in the infant) compared to adult cells which were primarily CD27−28− and readily (50-84%) produced both these cytolytic molecules.

These data suggest that though effector CD8+ T cells can be found in the early-life intestinal mucosa, their functional capacity is inferior to adult cells. The reduced capacity to induce high-quality CD8+ T effector responses in the intestine early in life may set the stage for infants’ increased susceptibility to gastrointestinal viral infections.

Notes:
Pregnancy outcome in women with systemic lupus erythematosus, a multicenter cohort-study
Schutte-Blomjous BS, BS Blomjous, CNH Abheiden, SJ Kroese, JM van Laar, RHWM Derksen, IEM Bultink, AE Voskuyl, AT Lely, MA de Boer, JIP de Vries, RDE Fritsch-Stork
Rheumatology, VUmc

Background: Systemic lupus erythematosus (SLE) predominantly affects women during their fertile period. SLE patients are prone to pregnancy complications and may experience increased disease activity.

Objectives: To investigate disease activity pregnancy and pregnancy complications according to antiphospholipid antibody (aPL) status. Additionally, first and consecutive pregnancies were compared.

Methods: All ongoing pregnancies of >16 weeks gestation of SLE patients (according to the ACR revised criteria) receiving joint care from rheumatologists and gynecologists in two tertiary centers in the Netherlands between 2000-2015 were included. Disease activity (SLE(P)DAI), flare rate (SELENA-SLEDAI) and pregnancy complications were assessed.

Results: From 96 women (84% Caucasian) 144 pregnancies were included. Before (<6 months), during and after pregnancy (<6 months) the median SLE(P)DAI score was 2 and mild/moderate flare rates were 6.3%, 18.8% and 13.9% respectively. Three patients developed a severe flare during pregnancy, 2 patients postpartum. Severe maternal complications (preeclampsia, eclampsia or HELLP-syndrome) occurred in 16.2% of aPL negative, 21.4% of aPL positive SLE patients, and in 30.8% of SLE patients with antiphospholipid syndrome (APS). HELLP-syndrome occurred in 23.1% of SLE patients with APS and in 3.1% of SLE patients without APS. The perinatal complications intrauterine fetal death, preterm birth, small-for-gestational age and neonatal lupus occurred in 4.1%, 32.7%, 14.8%, 1.4%, respectively. Maternal and perinatal complication rates were similar in first (18.6% and 41.4%) and consecutive (17.6% and 35.1%) pregnancies. Of all patients, 42.7% developed a complication during all of their pregnancies (obstetrical history included).

Conclusions: This is the first study in patients with SLE demonstrating that incidence rates of pregnancy complications do not decrease in consecutive pregnancies compared to first pregnancies, in contrast to findings in the general population. Despite overall low disease activity and the absence of aPL in the majority of patients, almost half of the patients developed a complication during their pregnancies.

Notes:
Introduction: It is thought that an unbalanced vaginal microbiome composition can lead to pre-term births, miscarriage, and fertility problems. However, some studies suggest a beneficial effect of dysbiotic vaginal microbiota on conception or live birth. This meta-analysis focuses on the effect of specific vaginal microbiome compositions regarding the outcome of IVF treatments and efficacy of ways to modulate vaginal microbiome compositions that are in dysbiosis.

Methods: A systematic review was performed in the Medline, EMBASE, and Cochrane databases, using terms for healthy vaginal microbiota, dysbiotic vaginal microbiota, IVF, fertility, and pregnancy, and attenuation or modulation of the vaginal microbiota. Articles written in English matching the search terms were included for screening in this study until March 2017.

Results: A meta-analysis of 6 studies showed that a microbiome that is not in dysbiosis provides a significant advantage regarding successful conception and live birth after IVF treatment (OR = 0.680, P = 0.034, CI95% = 0.476 - 0.971). Notable is that, even though there was non-significant heterogeneity between included studies, one included study did find an opposite effect. A meta-analysis of 6 other studies showed that treatment of bacterial vaginosis using both the standard metronidazole as well as a lactobacillus based probiotic had more success than treatment with only metronidazole (OR = 2.585, P<0.001, CI95% = 1.664 - 4.016).

Conclusion: These meta-analyses show firstly that dysbiosis of the vaginal microbiota has a significant effect on conception and live birth after IVF treatment. Secondly, the meta-analyses show a significant improvement in the treatment of bacterial vaginosis by adding a lactobacillus based probiotic to the normal therapy.
Lipo-based vaccines against melanoma: an in situ approach to target skin resident immune cells and invariant natural killer T cells
Stolk DA, Jana Vree, Martino Ambrosini, Hans J. van der Vliet, Tanja D. de Gruijl, Yvette van Kooyk
Department of Molecular Cell Biology and Immunology, VUmc

Introduction
Effective vaccination strategies for the treatment of cancer require strong activation of the innate and adaptive immune system which is driven by antigen presenting cells (APC). Therefore, skin resident APCs such as dermal dendritic cells (dDCs) and Langerhans cells (LCs) are an attractive target for in situ anti-tumor vaccination. In an ex vivo skin explant model we have shown that glycan modification of melanoma tumor antigens enhances endosomal routing and cross-presentation by targeting C-type lectin receptors. dDCs can also activate invariant natural killer T (iNKT) cells, which operate at the boundary of innate and adaptive immunity, through presentation of α-galactosylceramide (αGC) in a CD1d dependent manner. Indeed, we have shown before that skin APCs take up injected αGalCer which results in activation of iNKT while simultaneously enhancing the efficacy of a tumor vaccine.

Aim
This study aims to develop a skin-delivered vaccine containing synthetic long peptides (SLP), incorporated in an αGC-containing liposome, to specifically target both innate and adaptive players of the immune system.

Results
So far, our in vitro model systems have shown that the combination of SLP and αGC in one lipo formulation induces strong CD8+ T-cell responses and potent activation of iNKT. Also, addition of a C-type lecting targeting ligand could improve uptake by moDCs and enhance CD8+ T-cell and iNKT activation in an ex vivo skin model. All together these data indicate that our delivery platform could be a potential new vaccination strategy against melanoma.

Future
Future plans for our APC targeting vaccine are focused on 1) their potency to target skin draining lymph node APCs 2) the in vivo validation in hDC-SIGN Tg mice and their ability to eliminate B16 melanoma in a therapeutic setting 3) their efficacy in combination with local checkpoint inhibition.

Notes:
RNA helicase DDX3 is a novel sensor for HIV-1
Stunnenberg MS, Theo Geijtenbeek, Sonja Gringhuis
Experimental immunology, AMC

Background
Antiviral immune responses are paramount in limiting HIV-1 replication. However, the virus is able to evade innate sensing in DCs and this underlies immune dysfunction leading to severe co-morbidities in patients. HIV-1 is able to infect dendritic cells (DCs) by exploiting DC-SIGN and TLR-8 signaling, leading to induction of transcription from the integrated provirus. Upon transcription, RNA polymerase is unable to transcribe beyond the first 58 nucleotides, hereby forming so-called ‘abortive RNAs’. A novel player, RNA helicase DDX3, is involved in recognizing abortive RNA transcripts which can lead to induction of mitochondrial-associated viral proteins (MAVS)-induced type I antiviral IFN responses. By triggering DC-SIGN, HIV-1 leads to the formation of functional full-length HIV-1 transcripts and subsequent PLK-1 activation actively suppresses the novel DDX3-dependent innate sensing mechanism. By targeting DDX3 with synthetic abortive RNA constructs we can elucidate the role of DDX3 in antiviral immunity in DCs.

Methods
We investigated immune responses of monocyte-derived DCs upon stimulation with a synthetic 58 nucleotide abortive RNA construct that triggers DDX3. We studied whether this construct can induce antiviral immunity, induce DC maturation and limit viral replication capacity.

Results
Here we show that a synthetic abortive RNA construct can trigger DDX3 and allows for the induction of antiviral type I IFN in DCs. Upon stimulation with abortive RNA, DCs show IFN-dependent maturation by increased CD86 expression levels. Furthermore, stimulation with abortive RNA strongly inhibits replication capacity of HIV-1 infected DCs. Interestingly, these findings show that viral transcripts can be used to induce antiviral immunity.

Conclusion
We identify abortive RNAs as key players in the induction of antiviral immunity by (i) inducing type I IFN responses, (ii) induction of DC maturation and (iii) decreasing viral replication capacity. These findings are important to take along in vaccine design.

Notes:
Immune-mediated mechanisms of maladaptive renal repair
Tammaro A, Leemans J.C., Florquin S. and Dessing M.C.
Pathology, AMC

Infiltrating inflammatory cells play a role in the development of and recovery from acute kidney injury (AKI), common during renal transplant procedures. TREM-1 is expressed on granulocyte and macrophages and enhances the inflammatory response. In order to prevent excessive inflammation and recovery from AKI, fine tuning of the immune response is critical. Here we investigated whether TREM-1 plays a role in the pathology of renal ischemia/reperfusion injury (IRI). WT and TREM-1/3 KO mice were subjected to renal IRI and sacrificed 1, 5 and 10 days later. Renal function, inflammation and repair processes were assessed in vivo whereas in vitro we used several tools to unravel the mechanism. Unexpectedly, TREM1/3 KO mice displayed no major differences in renal injury and inflammation during the acute phase. However, TREM1/3 KO mice showed significantly increased mortality in bilateral IRI during the repair phase. In unilateral AKI, we observed a maladaptive repair, namely increased renal damage, reduced tubular epithelial cells (TECs) proliferation, increased macrophages infiltration, fibrosis and senescence. In vitro, we found that TREM-1 is up-regulated following IRI in TECs. Moreover, TREM-1 deficient TECs displayed an altered mitochondrial homeostasis and cellular senescence following in vitro IRI, which resulted in delayed wound healing. Altogether these data suggest that TREM-1 is a key player in limiting the development of maladaptive repair following AKI, possibly by promoting TEC proliferation through regulation of mitochondrial integrity, hence favoring the restoration of tubular homeostasis following IRI

Notes:
Harnessing intracellular mycobacteria for therapy against cancer and infectious disease
Troost R., C. Kuijl / W. Bitter / Y. van Kooyk
Medical Microbiology and Infection Control, VUmc

Introduction:
Mycobacterium tuberculosis is a successful human pathogen. The specific molecular pathways that this pathogen uses to manipulate host biology remain to a large extend a mystery. Mycobacteria are attractive as a vaccine because of the safety record of BCG in humans. However, BCG is a poor vaccine against tuberculosis itself. By decorating BCG with specific antigens we aim to elicit a strong immune response that will confer protection to Mycobacterium tuberculosis.

Methods:
We made mutants through bacterial transformation of BCG. These mutants contain a plasmid encoding a mycobacterial surface protein under the control of a ribozyme switch which enables tight control over protein expression. In the sequence of the protein a Spytag was incorporated which allows for conjugations of Spycatcher fusion proteins. By making use of the protein-peptide bond between the Spytag peptide and the Spycatcher protein we show that we are able to decorate the surface of mycobacteria with a nanobody recognizing GFP.

Results:
We generated several mutants of the mycobacterial surface protein containing a HA-tag at various positions. Induction of expression with theophylline showed that the expression at two out of four different positions was well tolerated. The HA-tag was exchanged with a Spytag to allow for conjugation of a nanobody recognizing GFP. With flow cytometry we assessed conjugation efficiency and determined that of the remaining two sites one was conjugated with higher efficiency than the other.

Discussion/Conclusions:
We showed that it is possible to conjugate proteins to the surface of BCG by using the novel Spytag-Spycatcher system. This allows us to conjugate any protein to the surface of mycobacteria. These proteins will include mycobacterial and tumor antigens. With this tool we aim to develop BCG into a more immunogenic vaccine.

Notes:
NFkB1 haploinsufficient patients show impaired proliferation, plasmablast formation and immunoglobulin production ex vivo

Background:
Common Variable Immunodeficiency (CVID) is a heterogeneous disorder characterized by recurrent infections, low immunoglobulins and poor vaccination response, admixed with variable autoimmune and inflammatory features. In a cohort of 846 primary immunodeficiency cases with genomes sequenced as part of the NIHR BioResource – Rare Diseases project, we identified NFkB1 as the gene most enriched for likely disease-causing variants.

Methods:
Whole-genome sequence data were analyzed to identify rare loss-of-function variants. PBMCs were isolated from both clinically affected and unaffected NFkB1 variant carriers (NFkB1+/-), including 4 sporadic cases and 5 pedigrees (n=17, age: 11–77 years). Apart from extensive immunophenotyping, PBMCs were CFSE-labeled and cultured with T-cell-dependent and independent stimuli to examine B cell proliferation, plasmablast formation and immunoglobulin production ex vivo.

Results:
Of the 390 cases with a CVID diagnosis, 16 (4%) had a novel heterozygous frameshift, nonsense, splice-site, gene deletion or conserved missense NFkB1 variant. The analyzed sporadic and familial NFkB1+/- individuals showed symptoms ranging from none to recurrent respiratory tract infections, autoimmunity, and lymphomas. Partial penetrance is noted, disease correlating with older age. Serum IgG and IgA levels were low in all adult NFkB1+/- individuals. The low-to-absent switched memory B cells in all NFkB1+/- individuals was accompanied by low surface IgG and IgA. All clinically affected NFkB1+/- patients had an increase in CD21low B cells. Proliferation, plasmablast formation and immunoglobulin production (apart from IgM) was impaired upon stimulation with CpG/IL-2 and anti-IgM/anti-CD40/IL-21 in all NFkB1+/- individuals.

Conclusion:
NFkB1 haploinsufficiency is the commonest monogenic cause of CVID and results in a defect in the formation of immunoglobulin-producing B cells.

Notes:
Immunity against measles before and after allogeneic hematopoietic stem cell transplantation
van Aalst M, R. Verhoeven, J. Schinkel, A. Goorhuis, M.P. Grobusch, S.S. Zeerleder, G.J. de Bree
Center of Tropical Disease and Travel Medicine, AMC

Introduction:
Recently, measles outbreaks have been reported in many European countries. Allogeneic hematopoietic stem cell transplantation (allo-HSCT) recipients are at particular risk of contracting the potentially serious, and fatal infection, since they often lose measles immunity post-HSCT. The development of adequate vaccination strategies in these vulnerable patients is hampered by a lack of insight in the number of patients losing protective immunity against measles and lack of insight in risk factors for losing immunity during transplantation. Insight in these factors is essential to improve vaccination recommendations. Particularly, because the few studies elaborated on measles immunity in allo-HSCT patients date from the eighties or nineties, and since then many has changed in HSCT procedures and conditioning regimens.
In the present study we aim to characterize levels of, and factors associated with humoral immunity against measles before and post-HSCT. Furthermore, we aim to evaluate the contribution of donor derived immunity.

Methods:
We will analyze measles-specific humoral immunity of adult allo-HSCT recipients with >1 year disease-free survival between 2012-2017. Antibody measurements will be performed at three time points on stored sera/plasma: before HSCT, three months and one year post-HSCT. We collected information on medical history, vaccination status, relation to donor, and conditioning regimen retrospectively.

Results
Currently, we are assessing measles-specific antibody titers in 91 allo-HSCT patients. In 20 patients we are only able to assess immunity pre-HSCT and at one time point post-HSCT. We expect to complete assessments by the end of August, and to finalize analyses by the end of September.
Of included patients, 55 (60%) patients were male. Median age was 54 years. Fourteen patients (15%) received a myelo-ablative (MA) conditioning regimen and the other 77 (85%) patients received a reduced intensity conditioning regimen (RIST).

Conclusion
We expect data to be available in September which will be discussed by then.

Notes:
Chronic lymphocytic leukemia impairs metabolic fitness in CD8 T cells
van Bruggen J.A.C., S. Endstra, M.D. Levin, P.J. Siska, J.C. Rathmell, A.P. Kater, and G.J.W. van der Windt
Experimental Immunology, AMC

T cell exhaustion is acquired in chronic lymphocytic leukemia (CLL), impeding development of effective immunotherapeutic strategies. While the mechanisms behind impaired T cell function in context of CLL are not fully understood, it is known that aerobic glycolysis is essential for effector function. We therefore hypothesized that interaction of T cells with CLL cells leads to an altered metabolic programming resulting in impaired T cell function.

We have shown previously that mitochondrial metabolism contributes to the ability of T cells to rapidly induce glycolysis upon stimulation. In the current study we found that resting CLL-derived CD8 T cells showed increased basal respiration, mitochondrial membrane potential and ROS compared to healthy donors (HD) CD8 T cells. However, the spare respiratory capacity was reduced in CLL-derived CD8 T cells, indicating a lower capacity to deal with an enhanced energy demand.

Recently we have also shown that T cells derived from CLL patients exhibited impaired activation compared HD correlating with reduced glucose uptake. In the present study we found that impaired glucose uptake was independent of T cell-CLL cell contact, and this could be restored by depleting CLL cells. Furthermore we demonstrated that impairment of glucose uptake was not due to competition between T cells and CLL cells. In addition, extracellular flux analysis of CLL derived CD8 T cells showed impaired induction of glycolysis immediately after stimulation. CLL derived CD8 T cells also showed impaired expression of the surface glucose transporter GLUT1 and the degranulation marker CD107a, of which the latter correlated with glucose uptake.

Taken together, these data indicate that the metabolic fitness of CD8 T cells is impaired in CLL. Boosting T cell metabolism in CLL might therefore improve emerging immunotherapies such as CAR-T cell therapy.

Notes:
Performance of the multi-target Mikrogen Chlamydia trachomatis IgG ELISA in the prediction of tubal factor infertility (TFI) in subfertile women: comparison with the Medac MOMP IgG ELISA plus

van Ess E.F., Ouburg S., Morré S.A.
Immunogenetics, VUmc

Background: There is a need for more accurate Chlamydia trachomatis (CT) IgG antibody tests for tubal factor infertility (TFI) diagnostics. We evaluated the predictive value for TFI of Medac ELISA plus (MOMP) and multi-target Mikrogen ELISA (MOMP-CPAF-TARP).

Method: Based on Medac ELISA plus results 183 subfertile women underwent either hysterosalpingography (HSG) or laparoscopy to diagnose TFI. TFI was defined as extensive adhesions and/or distal occlusion of at least one tube. Women not fulfilling the definition of TFI served as controls. Serum was subsequently tested with Mikrogen ELISA and results were compared.

Results: 48 patients had TFI, 135 were controls. Mikrogen ELISA tested 125 patients positive/borderline of which 32% had TFI. Medac ELISA plus tested 77 patients positive/borderline of which 29.9% had TFI. Mikrogen tested 40 out of 48 TFI patients positive/borderline, Medac 23 out of 48. Kappa value was 0.34. PPV of Mikrogen ELISA and Medac ELISA plus were respectively 32% (95%CI 26-39%) and 30% (95%CI 24-37%), and NPV 86% (95%CI 81-91%) and 76% (95%CI 70-82%).

Conclusion: Both tests were comparable in the prediction of TFI. However, Mikrogen ELISA had a higher NPV and might be more reliable in identifying patients without TFI. Kappa-value showed limited concordance between both tests.

Notes:
isolation and characterization of monoclonal antibodies from HIV envelope immunized rabbits
laboratory of experimental virology, AMC

Since years researchers have attempted to develop a fully protective HIV-1 vaccine. Although no working vaccine is yet available, the field has made huge steps towards reaching such a vaccine. Stable, soluble envelope (Env) proteins that adopt a native-like conformation are now widely tested in immunization studies in various animals. High neutralization titers against the autologous virus are reached during these immunization studies, however very little cross-neutralization is seen. Because of high sequence diversity within the HIV family this cross-neutralization is essential for full protection. Determining antibody specificities in immunized animals will help to establish important factors contributing to high autologous neutralization titers as well as give indications on how to improve the breadth of the responses. In order to do this we isolated monoclonal antibodies from HIV Env protein immunized rabbits. These rabbits developed autologous neutralizing serum responses with sporadic cross-neutralizing ability. Five autologous neutralizing monoclonals were isolated from two different rabbits, and three were analyzed in more detail. These antibodies were shown to be clonal family members and target an epitope on the bottom of the Env trimer near a, strain specific, hole in the glycosylation shield. In addition, antibodies were able to cross-bind several subtype B Env proteins, and showed competition in binding with known broadly neutralizing antibodies targeting the gp41-gp120 interface on the Env protein. Lastly, cross-neutralization of SHIVp3 was shown for these antibodies which correlated with the serum cross-neutralization ability of the rabbit. Thus, we determined that this clonal antibody family target an immunodominant epitope at the bottom of the Env trimer, which is likely not present on most other HIV subtypes. It will therefore be important to shield this region of the Env trimer in future immunization experiment to elicit proper responses that can develop into broadly neutralizing responses.

Notes:
Treatment options for patients with intestinal graft-versus-host disease (GvHD) are limited when conventional treatment with steroids fails. Recently, it has become clear that gut microbiota have a major influence on intestinal health and disease. Studies have shown that allogeneic HSCT recipients with a less diverse fecal microbiome have a greater chance of developing intestinal GvHD and are more prone to succumb to transplant-related complications. This led us to the hypothesis that reversal of intestinal dysbiosis could improve intestinal GvHD. Here, we report data of a single-arm pilot study that assessed the safety and effectiveness of fecal microbiota transplantation (FMT) as treatment for steroid-dependent or steroid-refractory, intestinal GvHD.

Seven allogeneic HSCT recipients with biopsy-proven, steroid-dependent or steroid-refractory intestinal GvHD received fecal suspension via nasoduodenal infusion from an unrelated, healthy, CMV negative donor. Primary endpoints were reduction of GvHD and changes in fecal microbiota composition at 1, 4, 12 and 24 weeks after fecal transplantation. In addition, blood samples were collected to perform lymphocyte subset analysis. Importantly, there were no serious adverse events observed that could be attributed to the FMT. Follow-up, ranging from 3 to 6 months, identified 3 complete responders. In these patients defecation frequency and consistency normalized and immunosuppressants were tapered successfully, without relapse of diarrhea. Microbial analysis of fecal samples and lymphocyte analysis of blood samples will be performed in the near future, when all patients have completed follow-up, to get a better understanding of the specific microbiota and immune cells involved. We demonstrate that a single FMT is safe and effective in allogeneic HSCT patients with steroid-refractory or steroid-dependent GvHD of the intestine. Further prospective studies are needed to confirm these findings and to identify those patients that may benefit from this treatment.

Notes:
Expanded B-cell receptor clones in blood samples of patients with Chronic Inflammatory Demyelinating Polyneuropathy


Neurology, AMC

Following reports that pathogenic antibodies are present in a minority of patients with chronic demyelinating inflammatory neuropathy (CIDP), we here study whether oligoclonal expansions of B-cell clones are present in patients with this disease. B-cell receptor (BCR) repertoire was analysed using next generation sequencing on RNA extracted from blood samples in 30 subjects: 10 CIDP patients with active disease before starting treatment; 10 CIDP patients in remission (i.e. no treatment in the last 12 months) and 10 healthy controls. Healthy controls did not have highly expanded BCR clones, whereas most CIDP patients did, regardless of disease activity and response to treatment. Based on these preliminary data expanded BCR clones are observed in the peripheral blood of most CIDP patients, regardless of disease activity (active or remission/cure). Functional characterization of these expanded clones remains to be performed.

Notes:
Neutrophils kill antibody-opsonized cancer cells by a unique cytotoxic process of ‘trogoptosis’ that we have recently identified. Trogoptosis is essentially a lytic mechanism of cytotoxicity that involves physical destruction of the target plasma membrane, and this occurs when neutrophils rip fragments from the tumor cells by trogocytosis (trogos in Greek means ‘gnaw’). Both trogocytosis and the actual killing process occur as a consequence of neutrophil Fc-receptor signaling. However, whereas neutrophils are quite efficient in the trogoptosis of solid cancer cells, their capacity to kill hematopoietic cancer cells, including B lymphoma cells in the presence of the anti-CD20 monoclonal antibody Rituximab (Rmab), appears surprisingly limited. Clearly, this may also restrict the clinical efficacy of Rmab and in order to be able to improve this, it is important to understand the basis of this limitation.

Our initial studies to explore this suggest that the inability of neutrophils to kill anti-CD20 opsonized B lymphoma cells is due to intrinsic feature(s) of the CD20 target antigen. In particular, we demonstrate efficient trogocytosis in the apparent absence of killing, suggesting that the latter is not caused by a general absence of neutrophil Fc-receptor signaling. Furthermore, we show that antibodies against other target antigens on the B lymphoma cells, such as e.g. HLA-DR, are able to cause efficient neutrophil-mediated killing, thereby implying that these tumor cells do not have an inherent resistance to neutrophil-mediated killing, but rather one that is specifically related to the nature of the CD20 target antigen.
Role of the mycosin proteases in Type VII secretion systems of pathogenic mycobacteria
van Winden VJC, Wilbert Bitter and Edith N.G. Houben

Medical Microbiology & Infection Control, VUmc

The pathogen Mycobacterium tuberculosis, the causative agent of the human disease tuberculosis (TB), is responsible for over 1 million deaths annually. One of the research focus points of mycobacteria, are the type VII secretion (T7S) systems, as pathogenic mycobacteria strictly rely on these systems to infect host cells. T7S systems act as a conduit for the transfer of various proteins across the highly impermeable mycobacterial cell envelope. Pathogenic mycobacteria can have up to five T7S systems, ESX-1 to ESX-5, of which at least three are crucial for the virulence and/or viability of M. tuberculosis.

One of the conserved T7S components is the membrane-embedded serine protease mycosin (MycP). Strikingly, whereas MycP is essential for secretion, protease activity of MycP1 in M. tuberculosis has been shown to be dispensable for secretion (1). The essential role of MycP therefore remains unclear. Here, we show that both MycP1 and MycP5 of M. marinum have a similar phenotype as MycP1 in M. tuberculosis, confirming that MycP has a second unknown, proteolytic-independent, function that is essential for protein transport via its respective T7S system.

To investigate whether this essential role is related to proper functioning of the T7S membrane complex, we first analyzed the composition of the ESX-1 membrane complex and showed that this complex consists of EccBCDE1, similarly as was previously shown for ESX-5. Surprisingly, while mycosins are not an integral part of these purified core complexes, we noticed that the stability of both the ESX-1 and ESX-5 complex is compromised in the absence of their MycP subunit. Additional crosslinking and pulldown experiments showed that mycosins do associate with the membrane complex, but that this interaction is lost during complex purification. We hypothesize that this loose association of MycP with the core membrane complex is crucial for the integrity and functioning of the T7S machinery.

Notes:
**Human B cells internalize pathogens through BCR-Nck-PI3K signaling without CD19 co-receptor involvement**

Verstegen NJMC, Unger PA, Walker JZ, Nicolet BP, Jorritsma T, Bar-Ephraïm YE, Marsman G, de Wit J, Spaapen R, ten Brinke A, van Buul JD and van Ham SM

*Immunopathology, Sanquin*

B cells internalize foreign particles during infection or self-particles during autoimmunity. These internalized particles are subsequently processed and presented as antigenic-derived peptides to enable a cognate interaction with helper T cells necessary to promote humoral immunity. Although it has been appreciated that B cells internalize small antigens (<0.75m), their phagocytic ability has long been denied. Recent findings clearly demonstrate phagocytosis when large particulate antigens are recognized via the B cell antigen receptor (BCR). However, the molecular pathways that control BCR-mediated phagocytosis remain unknown and are therefore investigated in the current study. We developed a high-throughput quantitative image analysis approach using Salmonella typhimurium as an antigenic model particle to quantify BCR-mediated phagocytosis. We demonstrate that PI3K, but not one of its downstream targets AKT, is required for BCR-mediated phagocytosis, thereby potentiating antigen-derived peptide presentation to ensure CD4 T cell help. PI3K can be recruited to the BCR via CD19, as part of the co-receptor, and the adaptor protein Nck. It is currently not clear whether activation of PI3K through CD19 and Nck occur at the same time and potentiate each other, or whether both are used in different situations. Conditional knockout, using CRISPR/Cas9, demonstrated that phagocytosis in human B cells is dependent on Nck-PI3K module without CD19 co-receptor involvement. Subsequent its activation, PI3K promotes phagocytosis through activation of cytoskeleton remodeling molecules. Our findings might aid in discovering new therapeutic targets in auto-immune diseases in which phagocytosis of large particles containing self-antigen (e.g. apoptotic/necrotic particles) by B cells play a central role.

Notes:
Asthma is a chronic disease of the airways that is characterized by inflammation and airway hyperresponsiveness (AHR). In the airways of asthma patients contact system activation occurs following allergen challenge. High molecular weight kininogen (HMWK) is a key substrate in the contact system. Upon action HMWK is cleaved and liberates bradykinin which interacts with its receptors on numerous cells to exert a pro-inflammatory response. Several animal models demonstrated that bradykinin causes bronchoconstriction when administered into the airways. Additionally, blocking the interaction between bradykinin and its receptors alleviates AHR in these models. However, there is limited data on the role of the contact system on lung inflammation and how this is related to the observed AHR.

In this study we determined the effect of HMWK depletion in our clinically relevant asthma mouse model on AHR and allergic lung inflammation. Our three weeks asthma mouse model consists of a sensitization phase with house dust mite extract in the first week and a challenge phase in the second and third week. Using HMWK KO mice or a HMWK specific anti-sense oligonucleotide the mice were depleted of HMWK before or after sensitization respectively. We observed attenuation of AHR following HMWK depletion regardless of the timing. Interestingly, the pulmonary and airway inflammation was not significantly altered by HMWK depletio.

In conclusion, we demonstrate a mechanistic pathway to attenuates AHR which is independent of the extent of inflammation. This is potentially an interesting therapeutic target for its additional effect next to the majority of the existing asthma medication that strives to control symptoms by inhibiting inflammation.

Notes:
The biggest cause of death in children under 5 years is infection. The most common symptom presenting the infection is fever. Most of the febrile illnesses are caused by viral infections, but a small number are life-threatening bacterial infections, such as meningitis, pneumonia or osteomyelitis. In the clinic it is nowadays difficult to distinguish between a bacterial or viral infection based on clinical grounds. This results in treatment with antibiotics when children are suffering from a viral infection for the fear of missing a bacterial infection, leading to unnecessary use of antibiotics. There is an urgent need for the development of improved methods to distinguish between bacterial and viral infections. This project will focus on the identification of new personalized discriminators of bacterial and viral infection. Candidate biomarkers, including neutrophil proteins, granzymes and cytokines, will be validated in cohorts of patients using Luminex, multiplex assays and ELISA. A reliable diagnostic tool will lead to more accurate diagnoses and thereby reduce hospital admissions and help the reduction of antibiotic resistance.
Abstracts
Posters
IDENTIFYING THE AS PATIENT AT RISK: IS AORTIC ROOT DILATATION ASSOCIATED WITH HLA-B27?
Baniaamam MB, promotor: Nurmohamed M.T.N. , co-promotor: Paulus W.J.S.P.
Rheumatology, VUmc/Reade

Background: Cardiac involvement is more common in Ankylosing Spondylitis (AS) patients with HLA-B27 genotype, especially aortic valvular regurgitation (AVR). AVR in AS is caused by aortic root dilatation and fibrotic thickening of the aortic cusps, both linked to inflammation. Inflammation of the aortic root might lead to a weakening in aortic wall strength and dilatation with AVR. Severe AVR can result in heart failure and is an indication for valve replacement or repair. The prevalence of AVR in AS is estimated at 14-18%, which is significantly higher compared to the general population. Therefore, some advocate regular echocardiographic screening of AS patients[1]. Hence, we should aim to identify a specific ‘at risk’ AS population that might benefit from routine echocardiographic monitoring.

Objectives: Primary: To assess the association between the aortic root diameter in HLA-B27 positive versus HLA-B27 negative patients.
Secondary: To assess the association between the aortic root diameter with disease duration and inflammation biomarkers.

Methods: We performed a cross-sectional study in AS patients between 50-75 years. Patients underwent echocardiography, with 2D, spectral and colour flow Doppler. The aortic root diameter was corrected for body surface area (BSA). Correlation between aortic root diameter/BSA and disease duration and inflammation biomarkers were assessed.

Results: 132 Consecutive AS patients were included with a mean age of 60.5 years, of whom 110 (83%) were HLA-B27 positive. The median aortic root diameter/BSA ratio was significantly higher in HLA-B27 positive patients compared to HLA-B27 negative patients: 1.75 ± 0.22 mm vs. 1.61 ± 0.14 mm (p=0.001). Eight AS patients (6%) had aortic root dilatation (>2.1mm corrected for BSA), who were all HLA-B27 positive. Patients with and without aortic root dilatation did not significantly differ in age or disease duration. The median aortic root diameter/BSA ratio was correlated with disease duration (r=0.229, p=0.012), but not with inflammatory biomarkers.

Notes:
The composition of the vaginal microbiome and pro-inflammatory status as predictors in idiopathic subfertility: the research protocol of a pilot study

Borg MB, dr. J.P.W. Vermeiden / prof. dr. S. A. Morré

Department of Medical Microbiology and Infection Control, VUmc

Background: There is strong evidence that an unfavorable composition of the vaginal microbiome can prevent conception. The innate immune system is suggested to influence the composition of the microbiome, and vice versa. It remains unknown whether the innate immune system and the vaginal microbiome predict one another. However, it has not been elucidated whether this relationship can affect the probability of pregnancy in women undergoing fertility treatment.

Methods: In this open prospective study, a cohort of women of reproductive age undergoing intrauterine insemination at the Nij Barrahûs fertility center in Wolvega, with and without idiopathic subfertility (n =100), is followed during their artificial insemination treatment cycle. Additional to the standard insemination treatment, women experience one extra venipuncture as well as a vaginal swab shortly before insemination. Women without history of subfertility inseminated with donor semen represent controls whereas women with idiopathic subfertility are referred to as cases. In order to examine the pro-inflammatory status of the women we assess the concentration of five cytokines. Of these, two cytokines (sTNFαR1, IL-6) will be determined directly in plasma, three (TNFα, IL-10, IL-12) will be assessed after 24 hours cultivation of whole blood after LPS stimulation. Determination of the composition of the vaginal microbiome will be carried out by means of the IS-pro technique. The two primary study parameters will be statically analyzed for significant differences between the two groups. With the aid of logistic regression analyses we will examine which particular agents contribute to the differentiation of the two groups.

Discussion: The proposed study will investigate the relationship of the innate immune system with the vaginal microbiome in the setting of artificial fertility treatment. This may contribute to the development of a prognostic model to estimate the probability of conception in subfertile women before starting fertility treatment. Subsequently, this knowledge may enable additional treatment to modulate the vaginal microbiome compositions that are in dysbiosis increasing the probability of pregnancy.

Notes:
The role of retinoic acid on IgA regulation in inflammatory bowel disease
Bos A.V., Egmond M. van, Mebius R.
MCBI, VUmc

Background
Inflammatory bowel disease is characterised by a disrupted epithelial barrier that allows infiltration of bacteria into the tissue leading to immune activation and recruitment. Chronic inflamed intestine contain a disrupted immune homeostasis, which in healthy gut is regulated by immunoglobulin A (IgA). Mechanisms by which IgA regulates homeostasis include pathogen neutralization and opsonisation. Additionally, IgA is a potent neutrophil activator which leads to pathogen phagocytosis and initiates neutrophil recruitment. The production of IgA is directly regulated by the release of retinoic acid by ‘tolerogenic’ dendritic cells residing in the lamina propria. Although the role of retinoic acid on IgA production and intestinal homeostasis in healthy humans is becoming clear, its potential role in inflammatory bowel disease development is yet unknown.

Hypothesis
We hypothesize that retinoic acid is differently regulated in IBD and therefore affects IgA production, causing an abnormal and continues neutrophil response that leads chronic disease.

Research plan
1) Assess the phenotype of leukocytes residing in inflamed gut vs. controls, to (among others) determine whether indeed RALDH enzymes (responsible for retinoic acid) and IgA production is enhanced in human IBD samples.
2) Assess whether elevated levels of RALDH enzymes are responsible for enhanced IgA production in human IBD.
3) Confirm findings in IBD animal models

Anticipated results
Retinoic acid expression is enhanced in IBD patients and affects IgA production, leading to a constant trigger for neutrophils to be activated. Hopefully, interference in this pathway leads to suppressed inflammation and improvement of disease.

Notes:
High Prevalence of Circulating Enterovirus C in Malawi
Virology, AMC

Over the past decades, intense vaccination programs and surveillance have significantly reduced the global incidence of poliovirus infections, with polio now being endemic in only three countries (Afghanistan, Pakistan and Nigeria). However, outbreaks of circulating vaccine derived poliovirus (cVDPV) have been reported, and outbreaks of other enteroviruses with severe symptomatology, such as EV71 and EV68, have occurred in various countries in North-America, Europe, Asia and in Australia. As a result, the enterovirus prevalence in Europe and the United States has been extensively studied. However, the status of enterovirus prevalence in African countries remains largely unknown.

We conducted a prevalence study in fecal samples obtained from Malawian children between 2002 and 2004. We found high levels of Enterovirus C, which is in accordance with findings in Cameroon and Madagascar, but in contrast to findings in other parts of the world, where Enterovirus C is scarcely found.

The findings of this study have implications for polio eradication. Poliovirus, a member of species Enterovirus C, is known to be able to recombine with other serotypes from the same group. Therefore, countries such as Malawi, where the oral polio vaccine (OPV) is used and Enterovirus C is widely circulating, are thought to have an increased risk at outbreaks of cVDPV.

Notes:
Neutrophil-extracellular traps and complement activation in the pathogenesis of thrombosis in autoimmune hemolytic anemia

Delvasto L.V., S.S Zeerleder
Immunopathology, Sanquin

Autoimmune hemolytic anemia (AIHA) is a rare disease characterized by autoantibody-mediated hemolysis of red blood cells. Thromboembolic complications occur in up to 27% of AIHA patients and form a major clinical challenge in the treatment. AIHA patients with detectable complement activation and intravascular hemolysis appear particularly prone to -mainly- venous thrombosis, which contributes to the high morbidity and mortality of this disease. From studies on paroxysmal nocturnal hemoglobinuria (PNH), which is characterized by intravascular hemolysis and marked thrombophilia, it is known that complement activation plays a crucial role in the pathogenesis of thrombosis. Moreover, inhibition of complement activation effectively prevents thromboembolic complications in PNH. Complement activation is a strong activator of polymorphonuclear neutrophils (PMN). And, as shown recently by our group and others, PMN activation and formation of Neutrophil Extracellular Traps (NETs) contribute to venous thrombus development. We hypothesized that complement-mediated PMN activation and subsequent NET formation may result in the induction of a procoagulant state in AIHA patients and may hence account for the thromboembolic events. Indeed, we obtained preliminary results suggesting that neutrophil activation is present in AIHA patients. We also found that treatment of AIHA patients with an inhibitor of the classical pathway of complement efficiently blocks intravascular hemolysis. The aim of this project is to determine the occurrence of complement-mediated neutrophil activation and NET formation in patients with AIHA, its relation with the development of thrombosis and to evaluate whether treatment of these patients can be improved by complement inhibition to prevent the thromboembolic complications.

Notes:
In vivo evaluation of AMP-based formulations (single and multi-component) on printed femur implants
Guarch Pérez CMGP, Riool M. and Zaat S. A. J.
Medical Microbiology, AMC

The insertion or implantation of medical devices such as prosthetic joints, prothetic heart valves, catheters and many other applications has become essential during the treatment of patients with diverse conditions. However, these medical devices are highly threatened by multi-drug resistant infections often found as bacteria in biofilms embedded on their surfaces. Biofilm formation on protheses is a serious global healthcare issue, which endangers the patient life and often results in the implant removal to avoid infection propagation.

A novel way of manufacturing implants is by additive manufacturing, more often referred to as “3D-printing”. This will allow production of highly personalized implants, but they will still have the same risk of infection as conventional implants. In order to avoid biofilm formation on these new type of medical devices, the European Training Network PRINT-AID has been established as a partnership to develop 3D-printing systems to produce personalized implants with antimicrobials incorporated to their structure.

As part of PRINT-AID, this study will focus on development, characterization and in vivo evaluation of novel 3D-printed femur implants by incorporating newly developed highly potent Synthetic Antimicrobial and Antibiofilm Peptides (SAAPs) to prevent Staphylococcus aureus and Staphylococcus epidermidis infection in a murine model. Furthermore, anti-biofilm activity and possible influence on the host immune response and tissue repair using invasive and non-invasive techniques will also be evaluated.

Overall, this study aims to develop alternative strategies to prevent infection of additively manufactured implants, in order to avoid the difficulties related to the treatment of persistent infections promoted by the implantation of medical devices.

Notes:
Inflammatory cell aggregates in protocol renal allograft biopsies show a trend of association with renal function decrease, but are not associated with allograft survival

Hoelbeek J.J.H., J. Kers, F.J. Bemelman, J.J. Roelofs, S. Florquin
Pathology, AMC

Immune cell aggregates, ranging from cell clusters to tertiary lymphoid organs (TLO) with germinal center reaction, are observed in settings of chronic immune activation. In kidney transplantation, all data on TLO are based on chronically rejected allografts, therefore it remains unclear whether these TLO affect inflammation in the renal allograft. To get more insight in the role of TLO, we evaluated TLO presence (aggregates of ≥30 inflammatory cells) in protocol renal biopsies taken 6 and 12 months after transplantation (T6 and T12), during a clinical trial comparing cyclosporine and tacrolimus. Seventy-four biopsies from the AMC were reviewed, T6= 36 of which 6 (17%) with aggregates, T12= 38 including 5 (13%) with aggregates, 1 patient had aggregates in T6 and T12. The eGFR at T6 from patients with aggregates was 58mL/min (IQR 50-88), compared to 49mL/min (IQR 39-65) for patients without aggregates (p=0.1). The ΔGFR (T12-T6) for patients with aggregates in T6 shows a numerical difference of -12mL/min (IQR -19-3) compared to 0mL/min (IQR -8-7) for patients without aggregates (p=0.06). Aggregate incidence was similar in both treatment groups (p=0.3).

Allograft survival of patients with aggregates in T6 was 83% compared to 86% for patients without aggregates (median follow up, p=0.9). The same analysis for T12 biopsies showed 75% survival with aggregates compared to 80% in the group without aggregates (median follow up, p=1.0).

We conclude that immune cell aggregates in protocol renal biopsies show a trend of association with renal function decrease, however in our cohort this did not associate with graft survival. Since we reviewed protocol biopsies, aggregates do not likely represent an inflammatory epiphenomenon. Further investigation in a larger, preferably independent cohort, is needed to evaluate the significance of our findings and characterize subtypes of aggregates and their effects on kidney allografts.

Notes:
Detection of anti-Borrelia miyamotoi antibodies in retrospective and prospective cohorts

CEMM, infectious diseases, internal medicine, AMC

Introduction:
Borrelia miyamotoi is found in 0.5-4% of Ixodes ricinus ticks in Europe and is the causative agent of hard tick-borne relapsing fever (HTBRF). The first human cases were found in Russia in 2011 and subsequently in Europe, the US and Asia by experimental molecular and serological tests. These patients presented with an acute febrile illness, and three similar immunocompromised cases were described with a meningoencephalitis due to B. miyamotoi. However, the extent of human exposure to B. miyamotoi after a tick-bite in Western-Europe remains unknown. During infection with B. miyamotoi, antibodies against several antigens, including the variable major proteins (Vmps) and glycerophosphodiester phosphodiesterase (GlpQ), develop in humans. We here aim to estimate the exposure to B. miyamotoi after a tick-bite in Western-Europe by detecting Vmp- and GlpQ-specific antibodies.

Methods:
Several cohorts containing acute and convalescent sera of individuals from Western-Europe bitten by ticks are available for serological testing. Detailed clinical information, e.g. history of fever or previous Borrelia burgdorferi exposure, is also available. Detection of B. miyamotoi antibodies will be performed by ELISAs and Western blots using several recombinant Vmps and GlpQ as antigens. Healthy blood donors from the same geographical location represent the control groups.

Results:
Recombinant proteins for ELISA and Western blot have been generated, experimental ELISA and Western blot protocols have been optimized, and sera from a Dutch and Swedish tick-bite cohort have been obtained. At the meeting we will be able to share our preliminary data on IgM and IgG antibodies against Vmps and GlpQ in acute and convalescent sera from these patient cohorts. In the near future a prospective study will be initiated to follow-up patients who develop fever after a tick-bite.

Conclusion:
We strive to estimate exposure to the emerging hard tick-borne pathogen B. miyamotoi and the incidence of HTBRF in Western-Europe.

Notes:
The role of the gut microbiota in pneumonia and sepsis
Jacobs M.
Center for Experimental Molecular Medicine, AMC

Intro
Pneumonia accounts for more deaths than any other infectious disease worldwide and constitutes the primary cause of sepsis. Each year 3.5 million deaths are attributed to pneumonia which probably is an underestimation since deaths from sepsis and deaths attributed to other conditions (e.g., cancer and Alzheimer; in which pneumonia is the terminal event) are coded separately. The Gram-positive Streptococcus pneumoniae is the most frequent cause of community-acquired pneumonia. Klebsiella pneumoniae is a common Gram-negative cause that is increasingly difficult to treat due to emergence of antibiotic resistance. New impulses in this field are thus urgently needed. The gut microbiota can be seen as an exteriorized organ that exerts numerous beneficial functions in the host response against infections. The gut hosts 1,000-2,000 different bacterial species of which the Firmicutes and Bacteroides divisions dominate. The microbiota protects against epithelial cell injury and pathogen colonization. Recently, spectacular advances have been made in our understanding of the intestinal microbiota in health and disease. The trillions of bacteria that resides within our gut play a fundamental role in development of the immune system and local support of mucosal immunity. Most recent data suggest that gut bacteria also modulate innate immune responses at systemic sites such as the lung. Its role in pneumonia however remains ill defined. In the current project we aim to increase our understanding of the microbiota in systemic infection, with an emphasize on lung host-pathogen interaction, which is the area of expertise in our lab.

Methods
The current project is part of a translational umbrella project that focusses on both preclinical and clinical aspects.
Murine models: We work with a well-established murine model of (bacterial) pneumonia and sepsis. By giving mice a regimen of broad-spectrum antibiotics, which depletes their gut microbiome, we found that these mice had deteriorated outcome, in terms of bacterial outgrowth and dissemination, to a bacterial challenge. Within this model we can test many hypotheses, e.g. the role of diet on gut microbiome, and gut-derived compounds of interest like pathogen associated molecular patterns and short-chain fatty acids (SCFAs) on the host defense against infection. Besides this, we are writing a research proposal to the Animal Ethics Committee to expand this model in order test novel research questions. These include the usage of germ-free animals, which are an ideal model to test the effect of gut microbiomes of interest. Furthermore, we have proposed surgical procedures by which we think we can learn more about the interaction between the gut and the lung (popularly coined the gut – lung axis), and also potentially find new gut-derived compounds of interest.
Genomics: 16s sequencing.
We explore microbiomes of interest, namely the gut but also respiratory tract, by illumina sequencing of faeces and lung samples. Hereby, we can attain detailed information on which species are present.

Results (expected)
We expect to get substantial insight in the role the gut microbiome plays in host defense against bacterial pneumonia. In particular, we aim to elucidate the differences between a ‘healthy’ and ‘dysbiotic’ microbiome and test microbiomes of interest in our murine infection model. In line with this we will perform experiments to gain insight in how the gut microbiome may be altered/manipulated, e.g. through diet and usage of probiotics (synbiotics). Furthermore, we will test gut derived compounds of interest like PAMPS and SCFA, while we also develop methods to find new gut-derived compounds of interest.
IgG Fab glycosilation: a novel mediator of humoral tolerance
Koers J, Theo Rispens
Immunopathology, Sanguin

Antibodies can acquire N-linked glycans in the variable (Fab) domains during antigen-specific immune responses. Previous findings suggest that these so-called Fab glycans have important immunomodulating features, altering antibody affinity, and possibly regulating B cell responses. It was also found that IgG4 antibodies - associated with humoral tolerance - contain elevated levels of Fab glycans. Furthermore, levels of Fab glycosylation vary widely between different specific antibody responses, suggesting antigen-associated selection. Interestingly, increased levels of Fab glycans against certain soluble antigens were observed, while the levels were very low in anti-RhD antibody responses.

IgG in complex with antigen can inhibit B cell activation by engaging the inhibitory Fc-Receptor (FcγR) FcγRIIb together with the B cell receptor. We therefore hypothesize that the emergence of Fab glycans during a specific antibody response represents an important, novel mechanism to limit B cell responses. IgG containing Fab glycans might additionally engage CD22, another inhibitory receptor, of the sialic acid-binding immunoglobulin-like lectin (Siglec) family, resulting in a much stronger inhibition and consequently in a higher threshold for activation. Alternatively, or in addition, once a B cell expresses Fab glycans on its B cell receptor, these might directly engage CD22 (‘in cis’) resulting in an altered threshold for B cell activation.

The strong immunogenicity of certain red blood cell antigens may be related to their inability to elicit Fab glycosylated antibodies: epitopes close to the negatively charged cell membrane may be poorly accessible to antibodies carrying (negatively charged) Fab glycans, resulting in strong negative selection of B cell clones with Fab glycans and enhanced chance of immunization - as was observed for RhD.

Notes:
CD19-CD3 DART induction of T cell mediated killing of malignant B cells
Martens AWJ, A.P. Kater & G.J.W. van der Windt
EXIM, AMC

Chronic lymphocytic leukemia (CLL) is the most common type of leukemia in the Western world. Although advances have been made in the treatment of CLL, it is still incurable. Allogeneic hematopoietic stem-cell transplantation can be highly effective in eliminating CLL cells, which is mediated by the T cells of the donor, indicating that T cell based immunotherapies hold promise. Unfortunately, this therapy cannot be used widespread, since patients often develop severe graft-vs-host disease. Other T cell based therapies, such as checkpoint inhibitors or CAR T cells have shown to be effective in other hematological malignancies, yet disappointing results have been obtained in CLL patients. The T cells of CLL patients have an exhausted phenotype, contributing to the ineffectiveness of these therapies.

Janssen has developed a new bispecific antibody, that targets both the T cell and CLL cell, that will lead to lysis of the CLL cell. In collaboration we are assessing whether this Dual Affinity Re-Targeting (DART) can be used in CLL despite the described exhausted phenotype of T cells of CLL patients.

Notes:
Towards personalized methotrexate therapy in Rheumatoid Arthritis: measuring Methotrexate Polyglutamates in PBMCs and defining the role of aberrant splicing of FPGS

Clinical Chemistry, VUmc

Low-dose methotrexate (MTX) is the anchor drug in the treatment of rheumatoid arthritis (RA), both as mono- and combination therapy with other chemical and biological disease-modifying anti rheumatic drugs (DMARDs). Moreover, its convenience, tolerability, safety and low costs contribute to the clinical and socio-economic benefits of MTX. An important parameter in the efficacy of MTX involves its intracellular retention by conversion to its active metabolites, i.e. MTX-polyglutamates (MTX-PGn), a process catalysed by the enzyme folylpolyglutamate synthetase (FPGS). An accurate LC-MS/MS-based method for measuring MTX-PGn has recently been described in erythrocytes, revealing that erythrocyte MTX-PGn accumulation is strongly correlated with suppression of disease activity and MTX response. Of note, however, RA patients exhibited a large inter-patient variability in erythrocyte MTX-PGn accumulation, for which the underlying mechanism remains elusive.

We aim to extend and improve on this method by measuring MTX-PGn in peripheral blood mononuclear cells (PBMCs) representing the primary targets in disease pathogenesis. We validated the LC-MS/MS assay to measure MTX-PGn in FPGS-proficient and FPGS-deficient human T-cell lines and, in preliminary studies, showed the feasibility of detecting MTX-PGn in PBMCs of RA patients on MTX-therapy. In addition, screening for aberrant pre-mRNA splicing of FPGS in PBMCs of RA patients is in progress using a comprehensive PCR-based assay with 9 primer pairs covering the entire FPGS gene. Together, these analyses may contribute to improved personalized MTX-therapy in RA.

Notes:
Prevention and Restriction of Antimicrobial Resistance in Pneumococci by Multi-Level Modelling

Pereverzeva L., Van der Pol T.
Center of Experimental and Molecular Medicine (CEMM), AMC

Streptococcus pneumoniae is a major health threat in industrialized and developing countries. The pathogen affects both young and old people, immune-competent as well as immunocompromised individuals. By genetic recombination within diverse populations, individual strains are not only able to evade vaccination but also able to acquire antimicrobial resistance (AMR), which can then be transmitted onwards.

In 2017, a multinational consortium of researchers started a project to understand the mechanisms and distribution of this pneumococcal AMR repertoire at the genetic, bacterial, host and population levels to layout new strategies for risk assessment, prevention and reduction of AMR. In particular, the environmental, immunological and pharmacological drivers of resistance emergence and selection, the genetic population dynamics, as well as the fitness of the new traits in different host conditions will be analyzed and modelled.

In the Academic Medical Center Amsterdam, we will focus on the host-pathogen interaction in infections with highly virulent antimicrobial resistant strains of Pneumococci in in vitro/ex vivo models of human macrophages and epithelial cells and in mouse models of nasopharyngeal colonization, pneumonia and invasive disease. Hereby, a comprehensive characterization of the host-pathogen response on antimicrobial resistant strains of Pneumococci will be established. In a concerted effort, this consortium will develop countermeasures against antimicrobial resistance in a major health threat by multi-level modelling of its resistance emergence, selection, and transmission in diverse environments.

Notes:
Leukocyte DNA methylation and host defense mechanisms during pneumonia
Qin W, Qin, van der Poll T, Scicluna BP
CEMM, AMC

Innate immune cells are the first immune mechanisms recruited to defend against invading pathogens, these cells induce sophisticated transcriptional programs involving the regulation of thousands of genes once they sense an intruder. The regulation of this program is achieved through a series of epigenetic changes. DNA methylation is a kind of epigenetic changes and was closely related to gene silencing when this happened at promoter and enhancer regions. Furthermore, recent work has showed that DNA methylation was changed in many immune cells during bacterial or viral infection. Thus we speculate that DNA contributes to host immune responses against bacterial infection. To test this hypothesis, we first pretreated the THP1-MD2-CD14 cells, a monocyte-like cell line, with DNA Methyl Transferase Inhibitor N-Phtalyl-L-Tryptophan (RG108), and then stimulated with different Toll like receptor (TLR) ligands, pam3csk4 (TLR2), poly I:C (TLR3), LPS (TLR4), flagella (TLR5) and heat killed Klebsiella pneumoniae, the released cytokines were finally measured at different time points post stimulation. Our results showed that RG108 can significantly decrease interleukin (IL) 1β, IL-6 and tumor necrosis factor α (TNF-α) production. In addition, this inhibitory effect was independent of cell death and NF-κB activation. In conclusion, DNA methylation is involved in host innate immune response, and may serve as an immunomodulatory target in infectious diseases.

Notes:
Immunoglobulin A coated vaginal bacteria identify women at risk for preterm labor
Schuster H.J., A. Breedveld, D. Budding & R. Mebius
AMC: gynaecology, VUmc: medical microbiology, AMC and VUmc

One in thirteen children in the Netherlands is born prematurely. However, pathogenesis of preterm labor is still not well understood. The composition of vaginal microbiota has been associated with premature birth, especially L. iners abundant vaginal microbiota. Because the start of parturition is an immunological trigger, we hypothesized that certain Lactobacillus spp. can cause an immune response which leads to premature childbirth. With the understanding that labor is a localized inflammatory process, there is a potential role for vaginal mucosal immunoglobulin A (IgA). To test this hypothesis, we will use flowcytometry-based bacterial cell sorting and 16S rDNA sequencing to characterize taxa-specific coating of vaginal microbiota with IgA. We hypothesized that women who will deliver prematurely, have more IgA-coated L. iners within the L. iners dominant vaginal microbiota cluster compared to women with term delivery. This information can be used to develop a prediction model for preterm birth.

Notes:
The impact of host range and persistence of E. coli on the spread of antimicrobial resistance
van der Putten B.C.L., Matamoros S.P.F., Schultsz C.
AMC

The spread of antimicrobial resistance (AMR) ‘threatens the very core of modern medicine’, according to a 2015 WHO Global Action Plan. Important information on the transmission of AMR is still lacking, which is addressed in two projects I will be working on: HECTOR and COMBAT. HECTOR aims at determining the impact of host range of E. coli on the spread of AMR. Some E. coli clones, like ST131, seem to transmit readily between different hosts, while other clones are restricted to specific hosts. How this host restriction affects the transmission of AMR bacteria or plasmids is not currently known. The other project is COMBAT, which investigates the acquisition of extended-spectrum β-lactamase (ESBL) producing Enterobacteriaceae by international travelers. After their return, the travelers and their household members were followed up, to assess the persistence and spread of the ESBL-producing bacteria. COMBAT already revealed before that the probability of spread of ESBL-producing bacteria to household members of international travelers was 12%.

For both HECTOR and COMBAT, we will also be interested in which genetic elements are associated for the increased risk of AMR transmission. In the case of HECTOR, the association between colonization or virulence factors (important for host range) and AMR genes will be the center of attention. For COMBAT, we want to know which genetic elements are associated with strong bacterial persistence in the gut of travelers, and which elements are important for the onwards spread of AMR after return.

Notes:
T cells are essential for protective immunity to infection and cancer. However, their full therapeutic potential is often restrained by several inhibitory mechanisms, known as immune checkpoints. Novel therapies that block specific immune checkpoints have recently revolutionized the treatment landscape of advanced-stage cancer. Hence, there is now a large demand for novel therapeutic strategies to block T cell inhibition.

Toward this demand, our laboratory has recently discovered a new inhibitory mechanism of T cell responses that operates via the persistent downregulation of T cell antigen receptor (TCR) expression at the peak of clonal expansion, in proportion to the strength of the initial antigen recognition. This programmed TCR downregulation (PTD) is associated with degradation of the TCRzeta protein subunit and restricts T cell cytokine production, proliferation and pathogen control. However, the molecular pathways underlying PTD remain elusive, precluding manipulation of this potent inhibitory mechanism for therapeutic purposes.

In my PhD project, I aim to elucidate PTD using a multi-angled approach, by dissecting both mouse and human T cell responses during infection and cancer. My key objectives are (1) to block PTD by preventing TCRzeta protein degradation via site-directed mutagenesis, (2) to define the molecular mechanisms driving PTD by conducting functional genomics screens, and (3) to characterize PTD in humans by analysing tumour-infiltrating T cells from cancer patients. To meet these objectives, I will combine unique transgenic mouse models with a retroviral functional genomics toolkit, which enables me to manipulate gene expression in primary antigen-specific T cells in a high-throughput manner, creating vast opportunities to identify novel mechanistic players.

Overall, these studies are expected to greatly advance our understanding of T cell regulation via its primary surface receptor, the TCR, thereby providing new avenues to enhance protective immunity to infection and cancer.

Notes:
Towards new biomarkers in sepsis
van Engelen T.S.R., W.J. Wiersinga, T. van der Poll
CEMM, AMC

Background
Community-acquired pneumonia (CAP) is a major healthcare problem. In the emergency department it can be challenging to diagnose CAP correctly. Recent studies have found novel biomarkers that can discriminate patients with CAP from non-CAP patients in the intensive care setting. These and other new biomarkers may also aid in diagnosing CAP in the emergency department leading to adequate and timely antibiotic treatment, thereby contributing to antibiotic stewardship and better healthcare.

Objectives
To evaluate for patients with clinically suspected CAP, the value of new and previously described (molecular) biomarkers for the diagnosis and etiology of CAP. We will compare the expression / plasma levels of biomarkers in patients with confirmed CAP versus those in whom the diagnosis of CAP was refuted by a day 28 independent adjudication committee. We previously described a molecular biomarker (FAIM3/PLAC8 gene expression ratio) for the diagnosis of CAP upon admission to the Intensive Care Unit (ICU). We will compare this with the value of “established” plasma protein biomarkers in this context (e.g., C-reactive protein, procalcitonin, Interleukin (IL)-8, IL-6 and soluble TREM-1). In an unbiased approach, we will seek to discover new molecular biomarkers using whole genome RNA profiling (using micro-arrays or RNA sequencing) and DNA methylation profiling (using reduced representation bisulfite sequencing).

Approach
A single-center prospective observational study in adult patients presenting at the Emergency Department (ED) suspected of non-traumatic pulmonary disease, in two cohorts of consecutively enrolled patients (i.e. a discovery and validation cohort). Newly discovered biomarkers will be validated in the validation cohort.

Relevance and expected results
We aim to validate the previously discovered FAIM3/PLAC8 gene expression ratio in this cohort of less severely ill patients and to evaluate the value of other new and previously described biomarkers for the diagnosis and etiology of CAP. The long term benefits are to reduce the burden of pneumonia by timely diagnosis using novel biomarkers and providing targeted therapy.

Notes:
The pro-inflammatory role of immunoglobulin A
van Gool M.M.J., van Egmond, M., Mebius, R.E.
Molecular Cell Biology & Immunology, VUmc

Background
Immunoglobulin A (IgA) is the most prevalent antibody at mucosal sites and a potent stimulus of neutrophils (PMN) via the IgA Fc receptor (FcαRI). However, the function of monomeric serum IgA is still poorly understood. Serum IgA is considered an anti-inflammatory modulator by inhibiting inflammatory signals via activation of IgG receptors. This project aims to unravel the pro-inflammatory role of serum IgA by FcαRI induced PMN activation.

Methods
Fluorescent latex beads were coated with different ratios of human pooled-serum IgA and IgG and incubated with freshly isolated human PMNs. After incubation phagocytosis, migration and chemotaxis assays were performed. Subsequently, these assays were repeated with beads that had been coated with plasma samples of healthy individuals. Levels of total IgA and IgG in these plasma samples were determined by ELISA.

Results
Beads that had been opsonized with different ratios of IgA and IgG were equally phagocytosed by PMNs. However, migration of PMNs was only observed towards IgA coated beads and not towards IgG coated beads. Similarly, only beads with a high IgA:IgG ratio induced PMN migration, which was reduced when a lower IgA:IgG ratio was present on beads. This observation was confirmed in a chemotaxis assay, since migration of PMNs was only seen towards supernatants of neutrophils that had phagocytosed IgA coated beads. Plasma levels of IgA were determined at 1-3 mg/ml and IgG at 4-9 mg/ml depending on the donor.

Conclusions
Up till now it is thought that serum IgA induces inhibitory signals that reduce activation via IgG receptors. However, phagocytosis of IgG coated beads by PMNs was not inhibited by the presence of IgA. We hypothesize that the presence of IgA may enhance IgG induced inflammatory responses, as we demonstrated that IgG neither induces PMN migration nor release of chemoattractants in contrast to IgA.

Notes:
HIV continues to cause significant morbidity and mortality around the world, emphasizing the need for an effective preventive HIV vaccine. The induction of potent immunity by vaccines is the major focus of research efforts aimed to protect against HIV infection. However, vaccines against HIV have yet not been able to induce protective immune responses. Broadly neutralizing antibodies (bnAbs) isolated from HIV-1 infected individuals have revealed that the human immune system is capable of eliciting antibodies that can protect against infection, as confirmed in macaque challenge studies. As the Envelope glycoprotein (Env) trimer is the sole target of bnAbs, a recombinant trimer would represent an optimal starting point for immunogen design. Soluble antigenic mimics of the native HIV-1 Env can be made by introducing a disulfide bond between the gp120 and gp41, so called SOSIP trimers. These new SOSIP trimer vaccine immunogens show for the first time that an immunogen is capable of eliciting neutralizing antibodies against the homologous (relatively resistant) virus in vaccinated animals. How to broaden the response to target heterologous viruses is, however, still unclear. Understanding the hurdles that antibody maturation needs to overcome to protect against HIV infection will guide rational vaccine design.

In the proposed study, I will isolate antibodies from immunized animals and artificially simulate antibody maturation to study how the elicited antibodies develop into bnAbs. This will advance the design of sequential immunization regimens to guide the humoral immune response towards neutralization breadth and a protective HIV vaccine.

Notes:
Antacid use as facilitating factor for acquisition of extended-spectrum beta-lactamase and/or carbapenemase-producing Enterobacteriaceae.
Roel R.P.J., Prof. dr. C.M.J.E. Vandenbroucke-Grauls; dr. K. van Dijk; dr. S. de Greeff; dr. W. Altorf; dr. A.F. Schoffelen
Medical Microbiology & Infection Control, VU University Medical Center

Importance
Gastric juice is considered an important host defense mechanism against ingested bacterial pathogens. Low levels of gastric acid increase susceptibility to varied enteric infections. Acid-suppressive drugs might therefore facilitate the acquisition of Enterobacteriaceae that produce extended-spectrum beta-lactamases or carbapenemases (ESBL-CPE).

Objective
To examine the association between the use of acid-suppressive drugs and community-acquired urinary tract infection (UTI) with extended-spectrum beta-lactamase or carbapenemase-producing Enterobacteriaceae.

Design, Setting, and Participants
We will conduct a matched population–based case–control study using data from the Dutch Infectious Disease Surveillance Information System for Antibiotic Resistance (ISIS-AR) from October 2016 through October 2017. All individuals aged 18 years and older with urine isolates harbouring Enterobacteriaceae resistant to third generation cephalosporins or carbapenems, sent to the laboratory by general practitioners will be eligible for inclusion. We will randomly select 5 controls per case with matching age, sex, and residential area. Controls will be individuals harbouring sensitive Enterobacteriaceae isolates in their urine. Questionnaires on demographics, previous illnesses, travel history, medication use and animal contact will be collected.

Main outcome measures
Univariable and multivariable logistic regression analyses will be used for case-control comparisons in order to estimate the odds ratios (ORs) and their 99% confidence intervals (CIs).

Results
N.A.

Conclusions
N.A.

Notes:
<table>
<thead>
<tr>
<th>Surname</th>
<th>First name</th>
<th>Institute</th>
<th>Department</th>
<th>E-mail address</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aarts</td>
<td>Cathelijn</td>
<td>Sanquin</td>
<td>Blood Cell Research</td>
<td><a href="mailto:c.aarts@sanquin.nl">c.aarts@sanquin.nl</a></td>
</tr>
<tr>
<td>Baas</td>
<td>Inge</td>
<td>Sanquin</td>
<td>Immunopathology</td>
<td><a href="mailto:i.baas@sanquin.nl">i.baas@sanquin.nl</a></td>
</tr>
<tr>
<td>Baniaamam</td>
<td>Milad</td>
<td>VUmc/Reade</td>
<td>Rheumatology</td>
<td><a href="mailto:m.baniaamam@reade.nl">m.baniaamam@reade.nl</a></td>
</tr>
<tr>
<td>Barendregt</td>
<td>Anouk</td>
<td>AMC</td>
<td>Radiology/Pediatric rheumatology</td>
<td><a href="mailto:a.m.barendregt@amc.uva.nl">a.m.barendregt@amc.uva.nl</a></td>
</tr>
<tr>
<td>Berkhout</td>
<td>Lea</td>
<td>Sanquin</td>
<td>Immunopathology</td>
<td><a href="mailto:l.berkhout@sanquin.nl">l.berkhout@sanquin.nl</a></td>
</tr>
<tr>
<td>Blanken</td>
<td>Annelies</td>
<td>Reade/VUmc/AMC</td>
<td>Rheumatology</td>
<td><a href="mailto:a.blanken@reade.nl">a.blanken@reade.nl</a></td>
</tr>
<tr>
<td>Borg</td>
<td>Melisa</td>
<td>VUmc</td>
<td>Department of Medical Microbiology and Infection Control</td>
<td><a href="mailto:m.borg1@vumc.nl">m.borg1@vumc.nl</a></td>
</tr>
<tr>
<td>Bos</td>
<td>Amelie</td>
<td>VUmc</td>
<td>MCBI</td>
<td><a href="mailto:a.bos4@vumc.nl">a.bos4@vumc.nl</a></td>
</tr>
<tr>
<td>Brands</td>
<td>Xanthe</td>
<td>AMC</td>
<td>Center for Experimental and Molecular Medicine</td>
<td><a href="mailto:x.brands@amc.uva.nl">x.brands@amc.uva.nl</a></td>
</tr>
<tr>
<td>Breedveld</td>
<td>Annelot</td>
<td>VUmc</td>
<td>Molecular Cell Biology and Immunology</td>
<td><a href="mailto:a.breedveld@vumc.nl">a.breedveld@vumc.nl</a></td>
</tr>
<tr>
<td>Brouwer</td>
<td>Lieke</td>
<td>AMC</td>
<td>Virology</td>
<td><a href="mailto:lieke.brouwer@amc.uva.nl">lieke.brouwer@amc.uva.nl</a></td>
</tr>
<tr>
<td>Brouwer</td>
<td>Philip</td>
<td>AMC</td>
<td>Medical Microbiology</td>
<td><a href="mailto:p.j.brouwer@amc.uva.nl">p.j.brouwer@amc.uva.nl</a></td>
</tr>
<tr>
<td>Bruggeman</td>
<td>Christine</td>
<td>Sanquin</td>
<td>Blood Cell Research</td>
<td><a href="mailto:c.bruggeman@sanquin.nl">c.bruggeman@sanquin.nl</a></td>
</tr>
<tr>
<td>Burggraaf</td>
<td>Maroeska</td>
<td>VUmc</td>
<td>Medical Microbiology &amp; Infection control</td>
<td><a href="mailto:m.burggraaf@vumc.nl">m.burggraaf@vumc.nl</a></td>
</tr>
<tr>
<td>chandrupatla</td>
<td>durga</td>
<td>VUmc</td>
<td>Rheumatology</td>
<td><a href="mailto:d.chandrupatla@vumc.nl">d.chandrupatla@vumc.nl</a></td>
</tr>
<tr>
<td>Chen</td>
<td>Sijia</td>
<td>AMC</td>
<td>Department of Experimental Immunology (EXIM), Academic Medical Center/University of Amsterdam, The Netherlands</td>
<td><a href="mailto:S.Chen@amc.uva.nl">S.Chen@amc.uva.nl</a></td>
</tr>
<tr>
<td>Chuwonpad</td>
<td>Ammarina</td>
<td>Sanquin</td>
<td>Hematopoiesis</td>
<td><a href="mailto:a.chuwonpad@sanquin.nl">a.chuwonpad@sanquin.nl</a></td>
</tr>
<tr>
<td>de Weerdt</td>
<td>Iris</td>
<td>AMC</td>
<td>EXIM</td>
<td><a href="mailto:i.deweerdt@amc.nl">i.deweerdt@amc.nl</a></td>
</tr>
<tr>
<td>Delvasto</td>
<td>Laura</td>
<td>Sanquin</td>
<td>Immunopathology</td>
<td><a href="mailto:l.delvasto-nunez@sanquin.nl">l.delvasto-nunez@sanquin.nl</a></td>
</tr>
<tr>
<td>Duinkerken</td>
<td>Sanne</td>
<td>VUmc</td>
<td>MCBI</td>
<td><a href="mailto:s.duinkerken@vumc.nl">s.duinkerken@vumc.nl</a></td>
</tr>
<tr>
<td>Dusoswa</td>
<td>Sophie</td>
<td>VUmc</td>
<td>MCBI</td>
<td><a href="mailto:s.dusoswa@vumc.nl">s.dusoswa@vumc.nl</a></td>
</tr>
<tr>
<td>Name</td>
<td>First Name</td>
<td>Institution</td>
<td>Department/Function</td>
<td>Email</td>
</tr>
<tr>
<td>-------------------</td>
<td>------------</td>
<td>-------------</td>
<td>---------------------</td>
<td>--------------------------------</td>
</tr>
<tr>
<td>El Tahir</td>
<td>Omaima</td>
<td>VUmc</td>
<td>Lab van Immunogenetica/Kindergeneeskunde</td>
<td><a href="mailto:o.tahir@vumc.nl">o.tahir@vumc.nl</a></td>
</tr>
<tr>
<td>Erkelens</td>
<td>Martje</td>
<td>VUmc</td>
<td>MCBI</td>
<td><a href="mailto:m.erkelens@vumc.nl">m.erkelens@vumc.nl</a></td>
</tr>
<tr>
<td>Falkenburg</td>
<td>Willem</td>
<td>Sanquin</td>
<td>Immunopathology</td>
<td><a href="mailto:w.falkenburg@sanquin.nl">w.falkenburg@sanquin.nl</a></td>
</tr>
<tr>
<td>Gaurch Pérez</td>
<td>Clara Mar</td>
<td>AMC</td>
<td>Medical Microbiology</td>
<td><a href="mailto:claguape@gmail.com">claguape@gmail.com</a></td>
</tr>
<tr>
<td>Heineke</td>
<td>Marieke</td>
<td>VUmc</td>
<td>MCBI</td>
<td><a href="mailto:m.heineke@vumc.nl">m.heineke@vumc.nl</a></td>
</tr>
<tr>
<td>Hoelbeek</td>
<td>Joris</td>
<td>AMC</td>
<td>Pathology</td>
<td><a href="mailto:j.j.hoelbeek@amc.uva.nl">j.j.hoelbeek@amc.uva.nl</a></td>
</tr>
<tr>
<td>Hoenderboom</td>
<td>Bernice</td>
<td>VUmc</td>
<td>Immunogenetics</td>
<td><a href="mailto:bernice.hoenderboom@rivm.nl">bernice.hoenderboom@rivm.nl</a></td>
</tr>
<tr>
<td>Hoepel</td>
<td>Willianne</td>
<td>AMC</td>
<td>EXIM, KIR</td>
<td><a href="mailto:j.w.hoepel@amc.nl">j.w.hoepel@amc.nl</a></td>
</tr>
<tr>
<td>Hofland</td>
<td>Tom</td>
<td>AMC</td>
<td>Experimental Immunology</td>
<td><a href="mailto:t.hofland@amc.nl">t.hofland@amc.nl</a></td>
</tr>
<tr>
<td>Hoornstra</td>
<td>Dieuwertje</td>
<td>AMC</td>
<td>CEMM, infectious diseases, internal medicine</td>
<td><a href="mailto:d.hoornstra@amc.uva.nl">d.hoornstra@amc.uva.nl</a></td>
</tr>
<tr>
<td>Horrevorts</td>
<td>Sophie</td>
<td>VUmc</td>
<td>MCBI</td>
<td><a href="mailto:so.horrevorts@vumc.nl">so.horrevorts@vumc.nl</a></td>
</tr>
<tr>
<td>Jacobs</td>
<td>Max</td>
<td>AMC</td>
<td>Center for Experimental Molecular Medicine</td>
<td><a href="mailto:m.c.jacobs@amc.uva.nl">m.c.jacobs@amc.uva.nl</a></td>
</tr>
<tr>
<td>Janes</td>
<td>Victoria</td>
<td>AMC</td>
<td>Medical Microbiology</td>
<td><a href="mailto:v.a.janes@amc.nl">v.a.janes@amc.nl</a></td>
</tr>
<tr>
<td>Jeucken</td>
<td>Kim</td>
<td>AMC</td>
<td>Experimental Immunology - Div. of Clinical Immunology and Rheumatology</td>
<td><a href="mailto:k.c.jeucken@amc.uva.nl">k.c.jeucken@amc.uva.nl</a></td>
</tr>
<tr>
<td>Kaaij</td>
<td>Merlijn</td>
<td>AMC</td>
<td>EXIM, KIR</td>
<td><a href="mailto:m.h.kaaij@amc.uva.nl">m.h.kaaij@amc.uva.nl</a></td>
</tr>
<tr>
<td>Khan</td>
<td>Hina Naz</td>
<td>AMC</td>
<td>Center for experimental Molecular Medicine (CEMM)</td>
<td><a href="mailto:h.n.khan@amc.uva.nl">h.n.khan@amc.uva.nl</a></td>
</tr>
<tr>
<td>Koers</td>
<td>Jana</td>
<td>Sanquin</td>
<td>Immunopathology</td>
<td><a href="mailto:j.koers@sanquin.nl">j.koers@sanquin.nl</a></td>
</tr>
<tr>
<td>Kors</td>
<td>Lotte</td>
<td>AMC</td>
<td>Pathology</td>
<td><a href="mailto:l.kors@amc.uva.nl">l.kors@amc.uva.nl</a></td>
</tr>
<tr>
<td>Krabbendam</td>
<td>Lisette</td>
<td>AMC</td>
<td>Experimental Immunology</td>
<td><a href="mailto:l.krabbendam@amc.nl">l.krabbendam@amc.nl</a></td>
</tr>
<tr>
<td>Kruize</td>
<td>Zita</td>
<td>AMC</td>
<td>Experimental Immunology</td>
<td><a href="mailto:z.kruize@amc.uva.nl">z.kruize@amc.uva.nl</a></td>
</tr>
<tr>
<td>Leeksma</td>
<td>Alexander</td>
<td>AMC</td>
<td>Experimental Immunology</td>
<td><a href="mailto:a.c.leeksma@amc.nl">a.c.leeksma@amc.nl</a></td>
</tr>
<tr>
<td>Li</td>
<td>Eveline</td>
<td>VUmc</td>
<td>Molecular Cell Biology &amp; Immunology</td>
<td><a href="mailto:r.li@vumc.nl">r.li@vumc.nl</a></td>
</tr>
<tr>
<td>Marsman</td>
<td>Casper</td>
<td>Sanquin</td>
<td>Immunopathology</td>
<td><a href="mailto:c.marsman@sanquin.nl">c.marsman@sanquin.nl</a></td>
</tr>
<tr>
<td>Martens</td>
<td>Anne</td>
<td>AMC</td>
<td>EXIM</td>
<td><a href="mailto:a.w.martens@amc.uva.nl">a.w.martens@amc.uva.nl</a></td>
</tr>
<tr>
<td>Name</td>
<td>First Name</td>
<td>Department</td>
<td>Email</td>
<td></td>
</tr>
<tr>
<td>-------------------</td>
<td>------------</td>
<td>-----------------------------</td>
<td>------------------------------</td>
<td></td>
</tr>
<tr>
<td>Matos Tiago</td>
<td>Tiago</td>
<td>AMC Dermatology</td>
<td><a href="mailto:t.matos@amc.nl">t.matos@amc.nl</a></td>
<td></td>
</tr>
<tr>
<td>Molhoek Anoushka</td>
<td>Anoushka</td>
<td>VUmc MCBI</td>
<td><a href="mailto:a.molhoek@vumc.nl">a.molhoek@vumc.nl</a></td>
<td></td>
</tr>
<tr>
<td>Morsing Sofia</td>
<td>Sofia</td>
<td>Sanquin Plasma proteins</td>
<td><a href="mailto:s.morsing@sanquin.nl">s.morsing@sanquin.nl</a></td>
<td></td>
</tr>
<tr>
<td>Muller Ittai</td>
<td>Ittai</td>
<td>VUmc Clinical Chemistry</td>
<td><a href="mailto:i.muller@vumc.nl">i.muller@vumc.nl</a></td>
<td></td>
</tr>
<tr>
<td>Nadafi Reza</td>
<td>Reza</td>
<td>VU medical center MCBI</td>
<td><a href="mailto:nadafi.reza@vumc.nl">nadafi.reza@vumc.nl</a></td>
<td></td>
</tr>
<tr>
<td>Newling Melissa</td>
<td>Melissa</td>
<td>AMC Experimental Immunology/ Clinical Immunology and Rheumatology</td>
<td><a href="mailto:m.newling@amc.uva.nl">m.newling@amc.uva.nl</a></td>
<td></td>
</tr>
<tr>
<td>Oja Anna</td>
<td>Anna</td>
<td>Sanquin Hematopoiesis</td>
<td><a href="mailto:a.oja@sanquin.nl">a.oja@sanquin.nl</a></td>
<td></td>
</tr>
<tr>
<td>Otto Natasja</td>
<td>Natasja</td>
<td>AMC Center of Experimental and Molecular Medicine</td>
<td><a href="mailto:n.a.otto@amc.nl">n.a.otto@amc.nl</a></td>
<td></td>
</tr>
<tr>
<td>Pereverzeva Liza</td>
<td>Liza</td>
<td>AMC Center of Experimental and Molecular Medicine (CEMM)</td>
<td><a href="mailto:e.pereverzeva@amc.uva.nl">e.pereverzeva@amc.uva.nl</a></td>
<td></td>
</tr>
<tr>
<td>Pollastro Sabrina</td>
<td>Sabrina</td>
<td>AMC EXIM/KIR</td>
<td><a href="mailto:s.pollastro@amc.uva.nl">s.pollastro@amc.uva.nl</a></td>
<td></td>
</tr>
<tr>
<td>Qin Wanhai</td>
<td>Qin</td>
<td>AMC CEMM</td>
<td><a href="mailto:w.qin@amc.uva.nl">w.qin@amc.uva.nl</a></td>
<td></td>
</tr>
<tr>
<td>Ramirez Moral</td>
<td>Ivan</td>
<td>AMC CEMM</td>
<td><a href="mailto:i.ramirez@amc.uva.nl">i.ramirez@amc.uva.nl</a></td>
<td></td>
</tr>
<tr>
<td>Ravi Abilash</td>
<td>Abilash</td>
<td>Academic Medical Center</td>
<td><a href="mailto:a.ravi@amc.uva.nl">a.ravi@amc.uva.nl</a></td>
<td></td>
</tr>
<tr>
<td>Rodrigues Neves</td>
<td>Charlotte</td>
<td>VUMc Dermatology</td>
<td><a href="mailto:c.rodrigues@vumc.nl">c.rodrigues@vumc.nl</a></td>
<td></td>
</tr>
<tr>
<td>Schmidt David</td>
<td>David</td>
<td>Sanquin Experimental Immunohematology</td>
<td><a href="mailto:d.schmidt@sanquin.nl">d.schmidt@sanquin.nl</a></td>
<td></td>
</tr>
<tr>
<td>Schreurs Renee</td>
<td>Renee</td>
<td>AMC Experimental Immunology</td>
<td><a href="mailto:r.r.schreurs@amc.nl">r.r.schreurs@amc.nl</a></td>
<td></td>
</tr>
<tr>
<td>Schuster Heleen</td>
<td>Heleen</td>
<td>AMC and VUmc</td>
<td><a href="mailto:h.schuster@vumc.nl">h.schuster@vumc.nl</a></td>
<td></td>
</tr>
<tr>
<td>Schutte-Bloemjous</td>
<td>Birgit</td>
<td>VUmc Rheumatology</td>
<td><a href="mailto:b.blomjous@vumc.nl">b.blomjous@vumc.nl</a></td>
<td></td>
</tr>
<tr>
<td>Singer Martin</td>
<td>Martin</td>
<td>VUmc Medical Microbiology and Infection Control</td>
<td><a href="mailto:m.singer@vumc.nl">m.singer@vumc.nl</a></td>
<td></td>
</tr>
<tr>
<td>Stolk Dorian</td>
<td>Dorian</td>
<td>VUmc Department of Molecular Cell Biology and Immunology</td>
<td><a href="mailto:d.stolk@vumc.nl">d.stolk@vumc.nl</a></td>
<td></td>
</tr>
<tr>
<td>Stunnenberg Melissa</td>
<td>Melissa</td>
<td>AMC Experimental Immunology</td>
<td><a href="mailto:m.stunnenberg@amc.uva.nl">m.stunnenberg@amc.uva.nl</a></td>
<td></td>
</tr>
<tr>
<td>Name</td>
<td>First Name</td>
<td>Institution</td>
<td>Department</td>
<td>Email</td>
</tr>
<tr>
<td>---------------------</td>
<td>------------</td>
<td>---------------------</td>
<td>-------------------------------------------------</td>
<td>--------------------------------</td>
</tr>
<tr>
<td>Tammaro</td>
<td>Alessandra</td>
<td>AMC</td>
<td>Pathology</td>
<td><a href="mailto:a.tammaro@amc.uva.nl">a.tammaro@amc.uva.nl</a></td>
</tr>
<tr>
<td>Troost</td>
<td>Ran</td>
<td>VUmc</td>
<td>Medical Microbiology and Infection Control</td>
<td><a href="mailto:r.troost@vumc.nl">r.troost@vumc.nl</a></td>
</tr>
<tr>
<td>Tuijnenburg</td>
<td>Paul</td>
<td>AMC</td>
<td>Experimental Immunology</td>
<td><a href="mailto:p.tuijnenburg@amc.uva.nl">p.tuijnenburg@amc.uva.nl</a></td>
</tr>
<tr>
<td>van Aalst</td>
<td>Mariëlle</td>
<td>AMC</td>
<td>Center of Tropical Disease and Travel Medicine</td>
<td><a href="mailto:m.vanaalst@amc.uva.nl">m.vanaalst@amc.uva.nl</a></td>
</tr>
<tr>
<td>van Asten</td>
<td>Saskia</td>
<td>Sanquin</td>
<td>Immunopathology</td>
<td><a href="mailto:s.vanasten@sanquin.nl">s.vanasten@sanquin.nl</a></td>
</tr>
<tr>
<td>van Beek</td>
<td>Anna</td>
<td>Sanquin</td>
<td>Immunopathology</td>
<td><a href="mailto:a.vanbeek@sanquin.nl">a.vanbeek@sanquin.nl</a></td>
</tr>
<tr>
<td>van Bruggen</td>
<td>Armando</td>
<td>AMC</td>
<td>Experimental Immunology</td>
<td><a href="mailto:j.a.vanbruggen@amc.nl">j.a.vanbruggen@amc.nl</a></td>
</tr>
<tr>
<td>van der Putten</td>
<td>Boas</td>
<td>AMC</td>
<td>Amsterdam Institute for Global Health and Development</td>
<td><a href="mailto:b.vanderputten@aighd.org">b.vanderputten@aighd.org</a></td>
</tr>
<tr>
<td>van der Spek</td>
<td>Jet</td>
<td>AMC</td>
<td>Experimental Immunology</td>
<td><a href="mailto:jetvanderspek@gmail.com">jetvanderspek@gmail.com</a></td>
</tr>
<tr>
<td>van Engelen</td>
<td>Tjitske</td>
<td>AMC</td>
<td>CEMM</td>
<td><a href="mailto:t.s.vanengelen@amc.uva.nl">t.s.vanengelen@amc.uva.nl</a></td>
</tr>
<tr>
<td>van Ess</td>
<td>Eleanne</td>
<td>VUmc</td>
<td>Immunogenetics</td>
<td><a href="mailto:e.vaness@vumc.nl">e.vaness@vumc.nl</a></td>
</tr>
<tr>
<td>van Gool</td>
<td>Melissa</td>
<td>VUmc</td>
<td>Molecular Cell Biology &amp; Immunology</td>
<td><a href="mailto:m.vangool@vumc.nl">m.vangool@vumc.nl</a></td>
</tr>
<tr>
<td>van Haaren</td>
<td>Marlies</td>
<td>AMC</td>
<td>laboratory of experimental virology</td>
<td><a href="mailto:m.m.vanhaaren@amc.uva.nl">m.m.vanhaaren@amc.uva.nl</a></td>
</tr>
<tr>
<td>van Lier</td>
<td>Yannouck</td>
<td>AMC</td>
<td>Hematology</td>
<td><a href="mailto:y.f.vanlier@amc.nl">y.f.vanlier@amc.nl</a></td>
</tr>
<tr>
<td>van Lieverloo</td>
<td>Gwen</td>
<td>AMC</td>
<td>Neurology</td>
<td><a href="mailto:g.g.a.vanlieverloo@amc.uva.nl">g.g.a.vanlieverloo@amc.uva.nl</a></td>
</tr>
<tr>
<td>van Rees</td>
<td>Dieke</td>
<td>Sanquin</td>
<td>Dpt of Blood Cell Research</td>
<td><a href="mailto:d.vanrees@sanquin.nl">d.vanrees@sanquin.nl</a></td>
</tr>
<tr>
<td>van Schooten</td>
<td>Jelle</td>
<td>AMC</td>
<td>Medical Microbiology</td>
<td><a href="mailto:jellevanschooten@hotmail.com">jellevanschooten@hotmail.com</a></td>
</tr>
<tr>
<td>van Winden</td>
<td>Vincent</td>
<td>VUmc</td>
<td>Medical Microbiology &amp; Infection Control</td>
<td><a href="mailto:v.vanwinden@vumc.nl">v.vanwinden@vumc.nl</a></td>
</tr>
<tr>
<td>Verstegen</td>
<td>Niels</td>
<td>Sanquin</td>
<td>Immunopathology</td>
<td><a href="mailto:n.verstegen@sanquin.nl">n.verstegen@sanquin.nl</a></td>
</tr>
<tr>
<td>Verweij</td>
<td>Nicki</td>
<td>VUmc</td>
<td>Rheumatology</td>
<td><a href="mailto:n.verweij@vumc.nl">n.verweij@vumc.nl</a></td>
</tr>
<tr>
<td>Willems</td>
<td>Roel</td>
<td>VU大学</td>
<td>Medical Microbiology &amp; Infection Control</td>
<td><a href="mailto:r.willems@vumc.nl">r.willems@vumc.nl</a></td>
</tr>
<tr>
<td>Yang</td>
<td>Jack</td>
<td>AMC</td>
<td>CEMM</td>
<td><a href="mailto:j.yang@amc.nl">j.yang@amc.nl</a></td>
</tr>
<tr>
<td>Zandstra</td>
<td>Judith</td>
<td>Sanquin</td>
<td>Immunopathology</td>
<td><a href="mailto:j.zandstra@sanquin.nl">j.zandstra@sanquin.nl</a></td>
</tr>
<tr>
<td>Time</td>
<td>Thursday</td>
<td>Friday</td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>-------------------------------</td>
<td>------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9:30 - 9:15</td>
<td>registration</td>
<td>9:30 - 10:30 registration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9:15 - 9:20</td>
<td>opening</td>
<td>10:00 - 10:15 opening</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9:20 - 10:20</td>
<td>session 1</td>
<td>10:20 - 11:20 session 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9:30 - 10:30</td>
<td>session 5</td>
<td>10:30 - 11:30 session 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10:20 - 10:40</td>
<td>coffee break</td>
<td>11:40 - 12:00 coffee break</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10:40 - 11:40</td>
<td>session 2A</td>
<td>12:00 - 12:30 keynote speaker: Frank Miedema</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11:00 - 12:00</td>
<td>session 2B</td>
<td>12:30 - 13:00 keynote speaker: Maria Yazdanbakhsh</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12:00 - 13:00</td>
<td>lunch</td>
<td>13:00 - 14:30 lunch</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13:00 - 14:30</td>
<td>activity</td>
<td>14:00 - 15:00 activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15:00 - 15:30</td>
<td>coffee break</td>
<td>15:00 - 15:30 coffee break</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15:30 - 18:30</td>
<td>activity</td>
<td>15:30 - 16:30 activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15:30 - 18:30</td>
<td>activity</td>
<td>16:30 - 17:30 activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15:30 - 18:30</td>
<td>who's the rat? start in lecture hall</td>
<td>17:30 - 18:30 activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16:40 - 17:00</td>
<td>award ceremony</td>
<td>18:00 - 18:30 dinner</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17:00 - 18:00</td>
<td>dinner</td>
<td>18:30 - 19:30 dinner</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:30 - 19:30</td>
<td>dinner</td>
<td>19:30 - 20:30 Create a poster session</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19:30 - 20:30</td>
<td>Create a poster session</td>
<td>20:30 - 21:00 Poster session 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20:20 - 21:00</td>
<td>Poster session 1</td>
<td>21:00 - 21:30 Poster session 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21:00 - 21:30</td>
<td>Poster session 2</td>
<td>21:30 - 22:00 Poster session 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9:30 - 9:45</td>
<td>breakfast</td>
<td>9:30 - 9:45 registration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9:45 - 9:00</td>
<td>registration</td>
<td>9:30 - 9:45 registration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9:00 - 9:15</td>
<td>check out</td>
<td>9:15 - 9:30 opening</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9:30 - 9:45</td>
<td>registration</td>
<td>9:15 - 9:30 opening</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9:45 - 10:00</td>
<td>registration</td>
<td>9:15 - 9:30 opening</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10:00 - 10:15</td>
<td>registration</td>
<td>9:30 - 9:45 registration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10:15 - 10:30</td>
<td>registration</td>
<td>9:15 - 9:30 opening</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10:30 - 10:45</td>
<td>registration</td>
<td>9:30 - 9:45 registration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10:45 - 11:00</td>
<td>registration</td>
<td>9:15 - 9:30 opening</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>