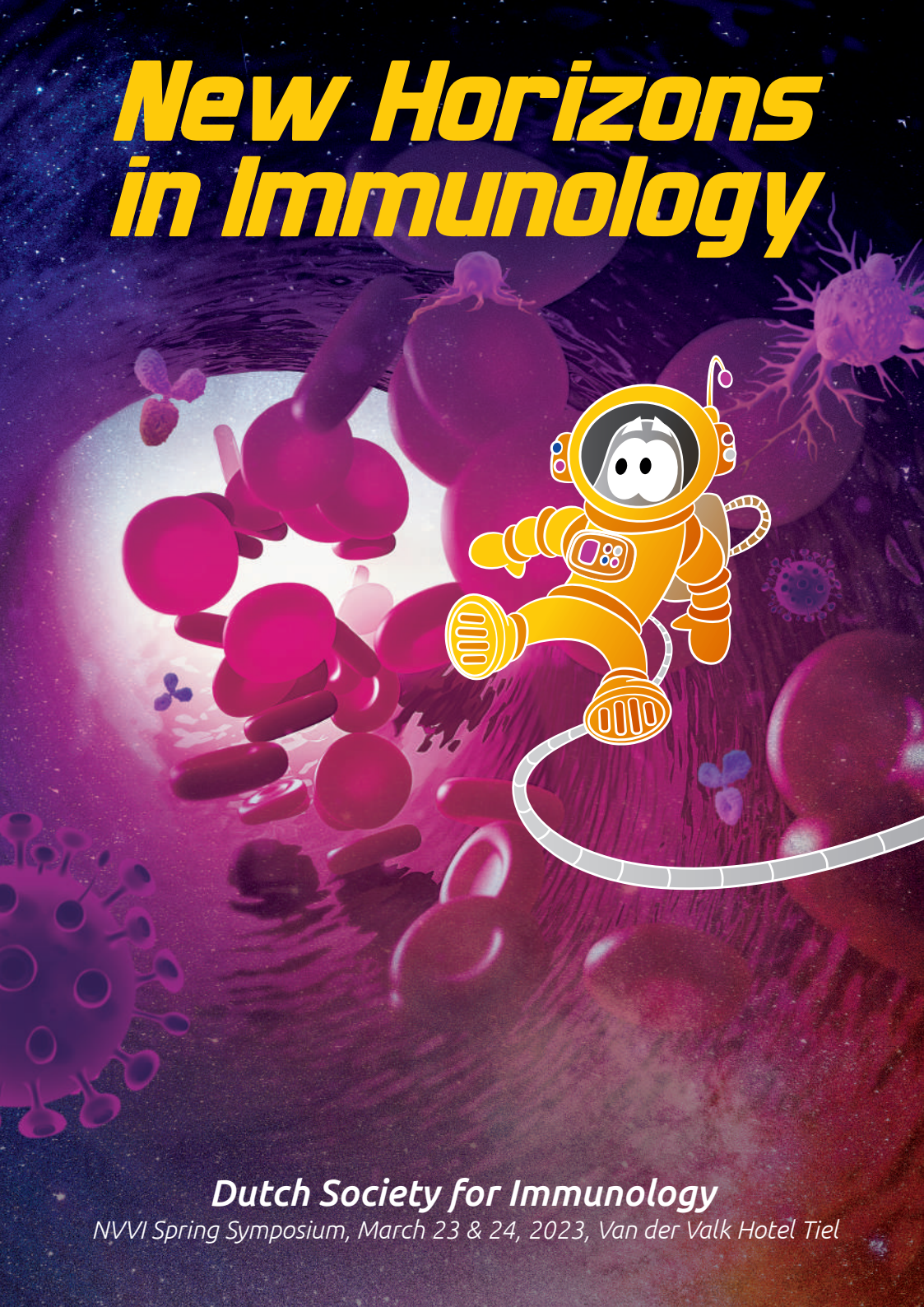
New Horizons in Immunology



Dutch Society for Immunology

NVVI Spring Symposium, March 23 & 24, 2023, Van der Valk Hotel Tiel

Sponsors

The following companies have made the 2023 symposium possible through their financial support:

MAIN SPONSORS

SPONSORS

Cover and design: **designGenerator**

Committee

Organized by the Spring Symposium Committee
of the Dutch Society for Immunology (NVVI)

MEMBERS OF THE COMMITTEE

Martijn Nawijn (UMCG) - *Chair*
Gerty Schreibelt (Radboudumc) - *Treasurer*
Sandra van Vliet (AmsterdamUMC, locatie VUmc) - *Secretary General*
Jeroen den Dunnen (AmsterdamUMC, Locatie AMC)
Wendy Unger (Erasmus MC)
Uli Scherer (LUMC)
Lotte Wieten (MUMC)
Klaas van Gisbergen (Sanquin)
Michiel van der Vlist (UMC Utrecht)

NVVI SECRETARIAT

nvvi@amsterdamumc.nl
www.nvvi-dsi.nl

CONGRESS SECRETARIAT

Connect MEETings
k.vonk@connectmeetings.nl
www.connectmeetings.nl

Table of contents

Programme

Thursday March 23, 2023

11.00 **Welcome and Introduction**
Martijn Nawijn

Session I Immunology and individuality
Chair: Jeroen den Dunnen

11.15 **Memories are made of this**
José Borghans, Utrecht

12.00 **Testosterone-induced immune system adaptation during female-to-male sex-reassignment**
Petter Brodin, Stockholm, Sweden

12.45 **Lunch**

Session II Single cell technologies
Chair:Martijn Nawijn

13.45 **Immune circuit engineering to delineate cellular communication**
Jurjen Tel, Eindhoven

14.30 **A spatially resolved atlas of the human lung characterizes a gland-associated immune niche**
Kerstin Meyer, Cambridgeshire, UK

15.15 **Tea break**

>>>

Programme

Session III Neuro-immunology

Chair: Michiel van der Vlist

16.00 **Neuroimmune Ecosystems**
Henrique Veiga-Fernandes, Lisbon, Portugal

16.45 **Immunoception: Neuronal representation and control of immunity**
Asya Rolls, Haifa, Israel

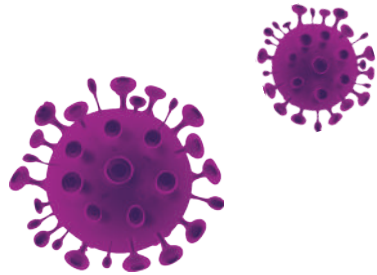
17.30 **Drinks and Dinner**

Keynote evening lecture

Chair: Lotte Wieten

20.00 **TREM2 in neurodegeneration and cancer**
Marco Colonna, St. Louis, USA

21.00 **Party**



>>>

Programme

Friday March 24, 2023

8.15 **'Meet-the-speaker' breakfast sessions**

Session IV Modelling immune responses

Chair: Klaas van Gisbergen

9.00 **Computational models of immune dynamics of T lymphocytes**
Johannes Textor, Nijmegen

9.45 **Towards a tissue-level understanding of homeostasis and inflammation**
Stephan Uderhardt, Erlangen, Germany

10.30 **Coffee break**

Session V Tumor immunology

Chair: Sandra van Vliet

11.00 **Tissue-resident gamma delta T cell function during tumor initiation, metastasis and immunotherapy**
Seth Coffelt, Glasgow, Ireland

11.45 **TCR gene therapy for patients with cancer. Optimization and prevention of tumor escape**
Mirjam Heemskerk, Leiden

12.30 **Lunch**

>>>

Programme

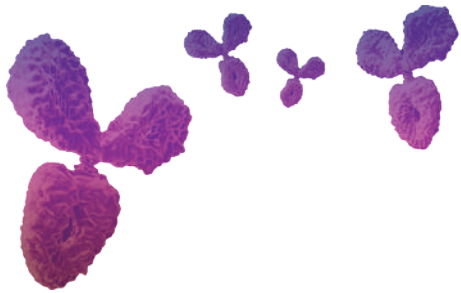
Session VI Infection & immunity

Chair: Martijn Nawijn

13.30 **How host-commensal interactions can shape mucosal immune responses**
Hermelijn Smits, Leiden

14.15 **Cytokines: Orchestrating immune responses in lung disease**
Anne O'Garra, London, UK

15.00 **Closure by Martijn Nawijn**

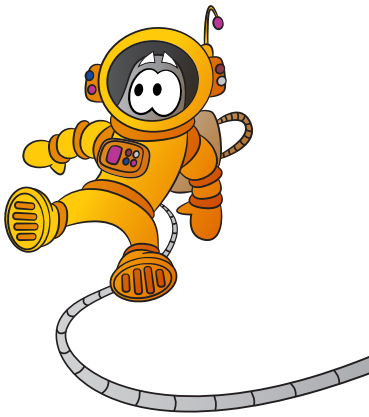


Session I

Immunology and individuality

Thursday March 23, 2023

Chair: Jeroen den Dunnen



Memories are made of this

José Borghans
UMC Utrecht

The adaptive immune system can store immunological memories against encountered antigens for up to decades. This allows the immune system to respond more efficiently upon reinfection through a combination of T and B cell memory. While the advantage of immunological memory is clearly illustrated by the success of vaccination, chronic autoimmune diseases illustrate that T and B memory responses can also lead to severe pathology. In order to interfere with such undesired memory responses, we need to know how memory T and B cell responses are maintained. Are these cells long-lived, do they maintain themselves through cell division, or are they dependent on a continuous influx of cells from another compartment?

For practical reasons, most research has been based on cells isolated from the circulation, i.e. from the blood of humans or the lymph nodes and spleen of mice. This research has revealed the, perhaps counterintuitive, insight that memory T and B cells have considerably shorter lifespans than naive T and B cells. Immunological memory is, however, in large part due to cells that do not continuously recirculate through the blood and lymph. These non-recirculating immune cells include tissue-resident T cells in non-lymphoid tissues, and plasma cells and memory T cells residing in the bone marrow. Much less is known about their dynamics.

We investigated the in vivo dynamics of memory T and B cells in different lymphoid and non-lymphoid tissues of mice and humans. Using deuterated water labelling, we followed the incorporation of deuterium in sorted memory T and B cell subsets over time. Because commonly used specific-pathogen free (SPF) mice do not mirror humans in the amount of memory T cells in tissues, we used wildling mice, i.e. C57BL/6 mice born to wild mice. These wildling

>>>

mice have a wild microbiome and hence a more natural immune phenotype. To study the in vivo dynamics of memory cells in humans, we isolated T and B cells from blood, bone marrow, skin and adipose tissue of otherwise healthy humans undergoing elective surgeries, who drank deuterated water in the weeks preceding their operation.

We found that, even in tissues, immunological memory is maintained in a dynamic way, meaning that cells have relatively short lifespans. We did find clear differences between tissues, however. In mice, memory T cells in bone marrow were longer-lived than memory T cells in the spleen, while memory T cells in non-lymphoid tissues, such as lung and liver, lived even shorter. Such tissue specialization was also observed in humans. Memory T cells from human skin lived at least as short as those from peripheral blood, while memory T cells from human bone marrow tended to live longer. In both mice and humans, we did not find any evidence for long-lived memory T cells in non-lymphoid tissues, which challenges the widely held view that tissue-resident memory T cells are static and maintained by longevity. Even for plasma cells in the bone marrow, we observed more uptake of deuterium than would be expected from the consensus view that plasma cells are extremely long-lived.

Taken together, our results suggest that immunological memory is maintained in a dynamic way, with clear differences between tissues, stressing the need for future studies into the in vivo dynamics of immune cells outside the blood.

Testosterone-induced immune system adaptation during female-to-male sex-reassignment

Petter Brodin

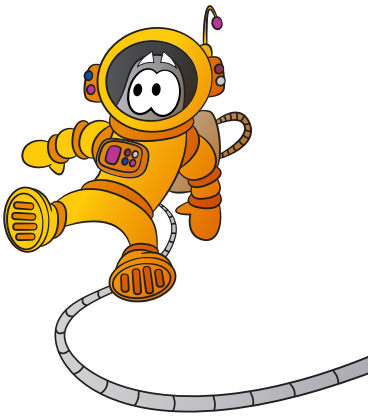
Karolinska Institutet, Sweden

Session II

Single cell technologies

Thursday March 23, 2023

Chair: Martijn Nawijn



Immune circuit engineering to delineate cellular communication

Jurjen Tel
TU Eindhoven

Much of the observed cellular heterogeneity is thought to originate or at least be influenced by signals from the microenvironment. Measuring the influence of environmental factors on cellular heterogeneity and investigating regulatory strategies of cell populations, however, is difficult with traditional methods as they do not give much opportunity for the control or design of the microenvironment. We exploit and develop an innovative single cell technology toolbox to fill the technology gap by enabling the compartmentalization of single cells or small groups of cells in chambers or droplets. This allows for the design of minimal environments under the omission of most external factors that could influence cellular behavior.

Type I Interferon (IFN-I)-mediated antiviral responses are central to host defense against viral infections. Crucial is the tight and well-orchestrated control of cellular decision-making leading to the production of IFN-Is. Over the past decades, multilayered stochasticity driving cellular heterogeneity and subsequent cellular decision-making during IFN-I responses has become increasingly apparent. Innovative single-cell approaches revealed that the initiation of IFN-I production is only limited to a fraction of 1-3% of the total population, both found in vitro and in vivo, which were thought to be stochastically regulated. In short, IFN-I responses are elicited by fractions of so-called first responding cells which start the initial IFN-I production upon viral detection. Their production is further enhanced via autocrine signaling, inducing a feedforward loop. Besides, first responders elicit additional IFN-I production in so-called second responders, which are activated upon IFN-mediated paracrine signaling in combination with viral detection. These two major events have also been described as the early phase and later phase of

>>>

IFN-I responses. Especially the regulation of the early phase is of increasing interest because this phase is currently thought to orchestrate population-wide IFN-I signaling. Up till today it remains unclear whether cellular decision-making to become an IFN-I-producer during the early phase is as a stochastic process (e.g., dictated by host intrinsic factors), or a deterministic process (e.g., dictated by epigenetics). The later phase seems mainly driven by stochastic processes, as the outcome is mainly dictated by host-intrinsic factors (e.g., intrinsic and extrinsic gene expression noise).

Using epigenetic drugs and the classical Luria-Delbrück fluctuation test, we challenged the dogma on stochasticity dictating early IFN-I responses, thereby proving heritability in responsiveness instead. Finally, we assessed the effects of quorum sensing driving population-wide responsiveness, which we substantiated with an ordinary differential equation (ODE) model. Together, this systems immunology approach highlights the ability to challenge the fundamentals of cellular decision-making during early IFN-I responses, and potentially other immune signaling systems. Ultimately, these new insights challenge how we believe systemic cytokine responses are generated. Moreover, with our unique technology platform we can deconstruct the generation of these responses one cell at a time by immune circuit engineering.

A spatially resolved atlas of the human lung characterizes a gland-associated immune niche

Kerstin Meyer
Sanger Institute, UK

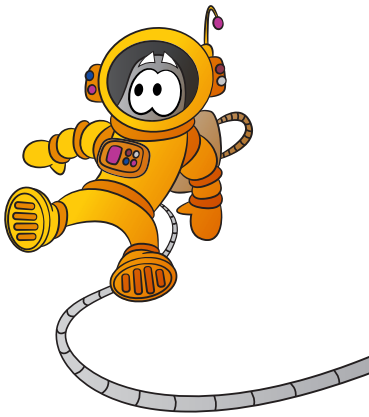
Single cell transcriptomics has allowed unprecedented resolution of cell types/states in the human lung, but their spatial context is less well defined. To (re)define tissue architecture of lung and airways, we profiled five proximal-to-distal locations of healthy human lungs in depth using multiomic single cell/nuclei and spatial transcriptomics (queryable at lungcellatlas.org). Using computational data integration and analysis, we extend beyond the suspension cell paradigm and discover macro and micro-anatomical tissue compartments including previously unannotated cell types in the epithelial, vascular, stromal and nerve bundle microenvironments. Importantly, we discover and validate a survival niche for IgA plasma cells in the airway submucosal glands (SMG). We show that gland epithelial cells recruit B cells and IgA plasma cells, and promote longevity and antibody secretion locally through expression of CCL28, APRIL and IL6. We find increased expression of MHC class II genes in selected regions of the SMG and find evidence of direct interaction with CD4 memory T cells, suggesting antigen presentation directly by SMG epithelial cells. We further examine the ciliated cell innate immune response to viral challenge in the nasopharynx in a time-resolved manner, observing rapid MHC class II upregulation. Our novel "gland associated immune niche" has implications for respiratory health and we report higher levels of IgA plasma cells in the airways of COVID-19 patients.

Session III

Neuro-immunology

Thursday March 23, 2023

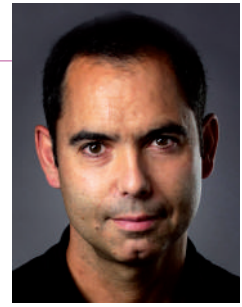
Chair: Michiel van der Vlist



Neuroimmune Ecosystems

Henrique Veiga-Fernandes

Champalimaud Foundation, Portugal



Neuroimmune interactions have been revealed to be at the centre-stage of tissue defence and organ homeostasis. Neuronal and immune cell subsets can colocalise in discrete tissue environments, forming neuroimmune cell units that constitute the basis for bidirectional interactions and which drive coordinated neuroimmune responses to local and systemic challenges. Nevertheless, whether neuronal and immune cells cooperate in inter-organ communication axes to orchestrate organism homeostasis and defence remains elusive. Here, we will discuss how neuroimmune circuits integrate and respond to their environment to regulate organismal physiology, in health and disease.

Immunoception: Neuronal representation and control of immunity

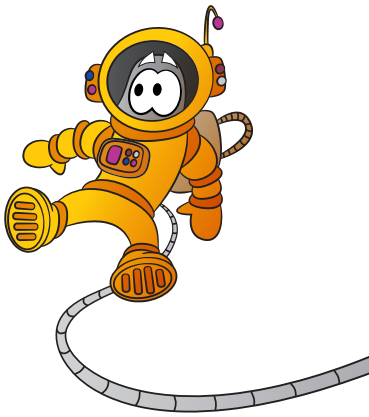
Asya Rolls
Israel Institute of Technology, ISR

Thoughts and emotions can impact physiology. This connection is evident in the emergence of disease following stress, psychosomatic disorders, or recovery in response to placebo treatment. Nevertheless, this fundamental aspect of physiology remains largely unexplored. In this talk, I will focus on the bidirectional communication between the brain and the immune system, a mechanism that we recently coined as immunoception. The brain represents that state of the immune response and forms "immunengrams" that can regulate different immune reactions. In this talk, I will discuss how the brain stores and represents immune information and how the brain's predictive capacity can be implemented in central control of the immune system. A mechanistic understanding of the neuro-immune dialog has potential implications for understanding and treating psychosomatic and autoimmune disorders and may offer new avenues for immune-modulation.

Keynote evening lecture

Thursday March 23, 2023

Chair: Lotte Wieten



Keynote: TREM2 in neurodegeneration and cancer

Marco Colonna
Washington University School of Medicine, USA

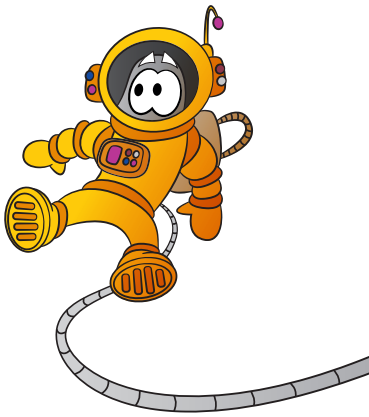
TREM2 is an activating receptor of the Ig-superfamily expressed in tissue resident macrophages that binds lipids and transmits intracellular signals through the adaptor DAP12. DAP12 recruits the protein tyrosine kinase Syk, which initiates a cascade of tyrosine phosphorylation events that activate downstream mediators ultimately leading to cell activation. We have studied TREM2 in microglia for its capacity to sustain microglial responses to Alzheimer's disease (AD). Rare hypomorphic variants of TREM2 are associated with increased risk of developing AD in human. We found that TREM2 deficiency in a mouse model of AD results in reduced microglia activation and increased amyloid pathology. I will illustrate the mechanisms by which TREM2 sustains microglia at the transcriptional and biochemical level and explore the potential of TREM2 agonists for AD immunotherapy. In addition to microglia, TREM2 is also expressed in several peripheral macrophage populations involved in host defense and metabolism. In the adipose tissue, TREM2 sustains the presence of a population of lipid-associated macrophages that prevent the dysmetabolism engendered by a high-fat diet. In atherosclerosis, TREM2+ macrophages are enriched in atherosclerotic lesions and specialize in lipid catabolism. In the liver, a TREM2+ subset of macrophages expands during liver cirrhosis and contributes to fibrosis. We found that TREM2+ macrophages are abundant in different types of human tumors. Moreover, TREM2-deficient mice were more resistant to tumor growth than wild-type mice in different mouse models and more responsive to anti-PD1 treatment. I will highlight how TREM2 deficiency is associated with alterations in macrophage subsets that promote an increase in intratumoral CD8+ T cells. Moreover, I will show that TREM2 blockade with a monoclonal antibody can enhance antitumor responses mediated by checkpoint immunotherapy. Thus, reshaping of tumor-associated macrophages by anti-TREM2 mAb is a promising avenue for complementing checkpoint immunotherapy.

Session IV

Modelling immune responses

Friday March 24, 2023

Chair: Klaas van Gisbergen



Computational models of immune dynamics of T lymphocytes

Johannes Textor
RadboudUMC, Nijmegen

We could think of the adaptive immune system (AIS) as one of the two major complex adaptive systems in our bodies, next to the central nervous system (CNS). Interestingly, the basic mechanisms of learning in the AIS and CNS are entirely different from each other: while the CNS forms an intricate web of connections between cells, the IS instead relies on repertoires – large collectives of hyperdiverse, moving cells. Building computational models of such repertoires, we first investigate how – and under which conditions – a system like this can learn to distinguish between epitopes coming from "foreign" versus "self". This model will be used to interpret recent data that contradicts the decades-old theory that most self-reactive T cells are deleted during negative selection: in fact, many self-reactive cells survive, but collectively they may still be predisposed to recognizing foreign as long as certain conditions are met. Second, we will use machine learning to interrogate T cell receptor sequence data for signs of patterns that pre-disposes cells towards recognizing self versus foreign. We will show that fewer nucleotide insertions, usage of hydrophobic amino acids in the CDR3-beta sequence, and usage of certain V and J genes indeed can predict self-reactivity to some extent. Third, we will explore an inherent risk of such "anthropomorphic" theories of the immune system and show that intricate motility patterns found in T cells in various tissues, which have been interpreted as signs of an optimizing evolutionary process, may in fact just be consequences of physical constraints placed on T cell motility. Hence, by using computational models, we will explore three scenarios where the immune system learns – or perhaps does not learn after all.

Towards a tissue-level understanding of homeostasis and inflammation

Stephan Uderhardt
University Hospital of Erlangen, Germany

Immune responses occur in tissues and their course and outcome are strongly determined by them. To gain a comprehensive understanding of physiological and pathological inflammation, their analysis must take into account both the fine structure and composition of the tissue itself and the resulting contextual dynamics and functional phenotypes of the cellular effectors therein. In this talk, I will present recent and ongoing technical and analytical developments aimed at directly assessing cellular functions in vivo and understanding tissue organization through compartment-level spatial schematics. We will dicuss cellular behavior with a focus on macrophages, communication within the physical stroma network, and heterocellular synergies in the context of immunological homeostasis. To address such questions, our lab develops and employs AI-driven histocytometry workflows that allow us to translate multiplex 3D tissue images into single cell data with multidimensional local anatomical context. In combination with functional in vivo imaging, we use these data to generate spatially embedded models of individual cells to quantitatively analyze their immediate microenvironment in situ and correlate these with dynamic immune effector functions in healthy and diseased tissues.

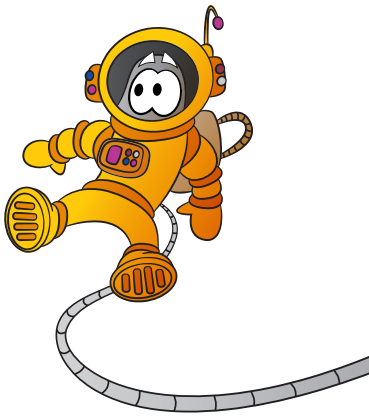
Specifically, we will take a detailed look at the biology of tissue-resident macrophages, their intricate interactions with the stroma of various tissues, and certain behavioral aspects and spatial functions that arise from their tissue connectivity and population dynamics. On this basis, we will discuss a theoretical framework of local inflammation thresholds that we hypothesize crucially determine the susceptibility or resistance of a tissue or a particular compartment to pathological inflammation and thus inflammatory disease.

Session V

Tumor immunology

Friday March 24, 2023

Chair: Sandra van Vliet



Tissue-resident gamma delta T cell function during tumor initiation, metastasis and immunotherapy

Seth Coffelt

Institute of Cancer Sciences, University of Glasgow,
Cancer Research UK Beatson Institute, Ireland

Understanding how immune cells impact cancer progression and metastasis is the major focus of the Coffelt lab. Specifically, we are interested in the mechanisms by which gd T cells participate in tumour evolution: from early stages of initiation to late stages of cancer spread. gd T cells encompass several phenotypically and functionally different subsets, including those that traffic between organs and lymph nodes as well as those that remain fixed within specific organs. We and others have shown that these gd T cell subsets can be either tumour-promoting or tumour-opposing. Recently, we have focused our efforts on tissue-resident gd T cells of the lung and the bowel, asking questions about their regulation and behaviour in these tissues.

Our data show that IL-17A-producing gd T cells of the lung consist of two major subsets, Vg4+ and Vg6+ cells, that promote mammary cancer metastasis. Single cell RNA sequencing of these two subsets revealed that Vg4+ and Vg6+ cells are controlled by different co-inhibitory molecules in tumor-bearing mice. Lung Vg6+ cells expressed constitutive levels of PD-1, while Vg4+ cells upregulated TIM-3 in response to tumor-derived factors. Blockade of PD-1 or TIM-3 signaling in tumor-bearing mice increased IL-17A expression from Vg4+ and Vg6+ cells, respectively. We also found that IL-17A-producing Vg4+ and Vg6+ cells counteracted anti-PD-1 and anti-TIM-3 immunotherapy.

In the gut tissue, we found that tissue-resident gd T cells kill nascent cancer cells. However, these cells were largely excluded from established tumors. The biology behind this immune escape mechanism involved suppression of critical gd T cell survival signals in cancer cells by the b-catenin transcriptional complex. We believe that gd T cells are excluded from tumors due to the absence of these survival signals. Currently, we are exploring ways to reverse these processes.

TCR gene therapy for patients with cancer. Optimization and prevention of tumor escape

Mirjam Heemskerk
LUMC, Leiden

B-cell maturation antigen (BCMA) expressed on the cell surface of multiple myeloma (MM) cells is one of the most promising targets for CAR T-cell therapy of MM patients. Clinical studies with BCMA CAR T-cells demonstrated that patients with relapsed MM can achieve objective responses. However, due to heterogeneous expression of BCMA, antigen loss on targeted tumor cells caused relapses in treated patients demonstrating the importance of selection of antigens for the success of T-cell therapies. Unlike CARs, T-cell receptors (TCR) can recognize antigens from both extracellular and intracellular derived proteins presented by HLA molecules. This extends the range of antigenic options. Furthermore, T-cell therapies directed against genes essential for growth and survival will drastically reduce the chance of tumor escape, since shutting down of genes will result in growth retardation and death of tumor cells. The B-cell lineage specific B-cell Oct-binding protein 1 (BOB1) meets these important criteria. Several studies as well as our own data imply an important function for BOB1 in cell survival of B-cell neoplasia, especially in MM.

We have recently identified TCRs that recognize peptide sequences of BOB1. BOB1-TCR modified CD8 T-cells were highly reactive against primary MM samples, while ignoring any non-B-cell types. Moreover, BOB1-TCR T-cells, resulted in rapid and reproducible targeting of established bone-marrow located human MM tumors in a preclinical mouse model. The TCR expression was optimized by simultaneous deletion of endogenous TCR by CRISPR/Cas9 editing, and TCR engineering of primary NK cells was performed to therapeutically target tumors and tumor immune evasion. BOB1-TCR gene therapeutic strategy will be explored in a phase I clinical study in which we

>>>

will investigate the feasibility, safety, and first indication of clinical efficacy of BOB1-TCR gene therapy in MM.

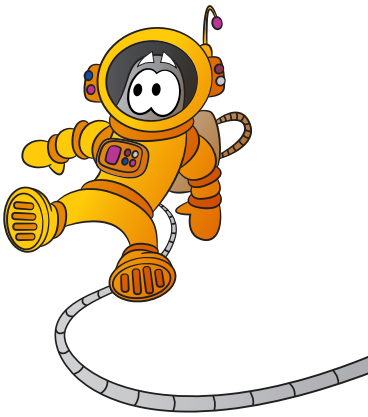
To prevent tumor escape, we anticipate that in the future combination therapies with CARs and TCR engineered T cells have to be developed as effective treatment. To investigate whether combination therapy would be beneficial we established MM xenograft models in which escape due to heterogeneous expression of tumor targets, using CRISPR/Cas9 editing systems, could be investigated. We have demonstrated that effective population control of these heterogeneous MM tumors demonstrated that simultaneous treatment with TCR as well as CAR T cells was required, while single treatment eventually led to outgrowth of tumor cells.

Session VI

Infection & immunity

Friday March 24, 2023

Chair: Martijn Nawijn



How host-commensal interactions can shape mucosal immune responses

Hermelijn Smits
LUMC, Leiden

Airway mucosa is constantly exposed to environmental triggers, including harmless, but foreign, allergens and potentially life-threatening pathogens. In healthy individuals, this does not lead to allergic responses or severe disease as our immune system forms a strong line of defence in conjunction with the epithelial lining. Crucial elements include combined antimicrobial activity with local immunomodulation to prevent excessive tissue damage.

Key is that our immune system should not respond to all foreign obstacles but recognizes what is potentially harmful or harmless. Excessive inflammatory responses must be avoided as this causes tissue damage and may form a gateway to other pathogens. Commensals at mucosal surfaces play an essential role in this process. The gut microbiota harbors the biggest community of commensals in the body, consisting of bacteria, archaea, fungi, viruses, and until recently also helminths. In addition to metabolic benefits, microbiota promote immune homeostasis, induce immune maturation in newborns, enhance intestinal epithelial barrier and prevent pathogen colonization. Gut microbiota strongly impact the development of both inflammatory and immunoregulatory responses, continuously driving education of host immunity. Not only in the gut, but also act systemically reaching out to other organs, such as the lungs. The composition of microbiota is strongly influenced by mode of birth, host genetic factors and immunity, diet, infections, antimicrobial agents (antibiotics, anti-helminth drugs) and environmental exposure.

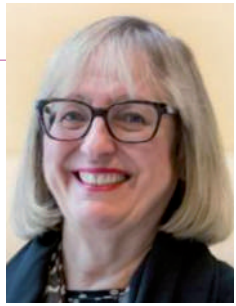
>>>

In westernized societies, non-communicable chronic diseases are rising and seem associated with changes and dysbiosis of gut microbiota. We only begin to understand how crucial the homeostatic relationship between commensals and host immune system is and the consequences of calibrating the activation threshold of cells and tissues to promote responses to infection, vaccines, and tumors, but remain silent to allergens or innocent microbes.

How can we translate these findings into medical or preventative practise in our modern society? Studies with pre- and probiotics, as well as controlled helminth infections, thus far showed some short-term beneficial effects in specific patient groups or people at risk, but with variable effects. This maybe related to differences in (long lasting) colonisation of those commensals, the effective dose, and the age group in which these approaches were applied. Therefore, our attention is shifting from the microorganisms itself to products derived from or related to commensals. Interestingly, commensals can produce bioactive molecules that act on the host immune system by inducing antimicrobial activity as well as tissue integrity-maintaining tolerance. Those commensal immunemodulators may therefore form an interesting new class of 'natural drugs' that can boost protective mucosal immunity in the host.

Cytokines: Orchestrating immune responses in lung disease

Anne O'Garra
The Francis Crick Institute, UK

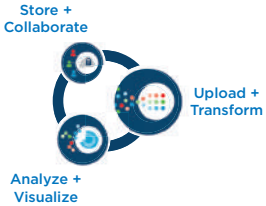


>>>

STEP INTO THE FUTURE

OF DATA ANALYSIS

Cytobank



Don’t let data analysis stop your research.

The field of flow cytometry has entered the era of machine learning-assisted data analysis.

Cytobank cloud enables comprehensive visualization and analysis of single-cell data with no need for coding knowledge:

- Integrated analysis algorithms
- Built-in statistical tools
- Automated gating option

BECKMAN COULTER *Life Sciences*

For Beckman Coulter’s worldwide office locations and phone numbers, please visit Contact Us at beckman.com

MAIN SPONSOR

BECKMAN COULTER
Life Sciences

SPONSORS

Agilent
Trusted Answers

BD

BioLegend®
Enabling Legendary Discovery™

EUSAPharma

Luminex®

MABTECH

MERCK

Miltenyi Biotec

SONY

STEMCELL™
TECHNOLOGIES