‘Immunity and Science fiction: The next 50 years in Immunology’

NVVI Symposium Lunteren, 2015, March 26-27
Congrescentrum De Werelt, Lunteren, The Netherlands

Thursday March 26

11.00 Welcome and Introduction

Session 1: Perspective: In vitro vs In vivo
Chair: Annemiek van Spriel
11.15 Hans Clevers (Utrecht, The Netherlands)
12.00 Nienke Vrisekoop (Utrecht, The Netherlands)
12.45 *Lunch*

STEM CELLS: DR JEKYLL OR MR HYDE?

Hans Clevers

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Stem cells are the foundation of all mammalian life, including that of man. Two types of stem cells have been discovered.
1) Embryonic stem cells (ES cells) are briefly present in the early human or mouse embryo, a few days after fertilization. These ES cells can be grown indefinitely in the lab and have the potential to build each and every tissue in our body. Because of this ‘pluripotency’, ES cells hold great promise for therapeutic application in the field of regenerative medicine. However, derivation of ES cells leads to the destruction of the (mouse/human) embryo. This has caused intense debate from ethical, religious and logistical perspectives.
A recent development circumvents the destruction of embryos. It is now possible to take skin cells (or other cells) from adults and convert these in the lab into cells with ES properties, so called iPS cells. Many of the hurdles that ES cell technology have faced, do not exist for iPS cells.
Neither ES cells, nor iPS cells play a significant role yet in the clinic.
2) Adult stem cells. Every organ in our body is believed to harbor its own dedicated stem cells. These adult stem cells replace tissue that is lost due to wear and tear, or after trauma and disease. Adult stem cells are highly specialized and can only produce the tissue in which they reside. Examples are bone marrow stem cells that make all blood cells, skin stem cells and gut stem cells. Even the brain is now known to harbor its specialized stem cells.

The great danger of stem cells rests in the fact that they not only fuel tissue (re)generation, but also that they are the cells that most easily transform into cancer cells.

INTRAVITAL IMAGING OF CELLULAR DYNAMICS: SEEING IS BELIEVING

Nienke Vrisekoop

Department of Respiratory Medicine, Laboratory of Translational Immunology, University Medical Center Utrecht, 3584 CT Utrecht, The Netherlands

Although histological techniques and FACS analysis can provide important information, these only give a static image of cells and their microenvironment. To study dynamic processes, the behavior of single cells can be visualized at even subcellular resolution using two-photon intravital microscopy. Whereas initially intravital imaging was limited to a couple of hours during which the organ of an anaesthetised mouse was surgically exposed, imaging windows on the organ of interest allow for the visualization of cellular dynamics over multiple days. Through the imaging window, cells that express the photoswitchable protein Dendra2 can be photomarked by switching the color of the cells from green to red. This enables cell migration to be tracked during subsequent imaging sessions. In order to retrace intravitally imaged cells in immunohistochemical slides, both photoswitching and the technique of photo-tattooing can be employed. Using these techniques we show that the migratory behavior of mammary tumor cells of invasive lobular carcinomas is increased in the presence of T cells in the microenvironment.

We also combined two-photon imaging together with in situ as well as ex vivo analysis of cytokine production in order to compare CD4 T cell effector dynamics and responses during pulmonary mycobacterial infection versus acute influenza infection. The proportion of migration-arrested, cytokine-producing effector T cells was dramatically higher in the influenza-infected lungs due to substantial higher antigen abundance. Histocytometric analysis, a method which converts fluorescent cells in immunohisctochemistry images to xy plots for more quantitative analysis, showed that CD4 effector T cell cytokine production during influenza infection was focal, with a restriction to areas of significant Ag burden. Optimal effector function is thus constrained by the availability of TCR ligands rather than effector numbers.

Session 2: **T cells**

Chair: Debbie van Baarle

13.45 Christopher Love (Cambridge, USA)

14.30 Ton Schumacher (Amsterdam, the Netherlands)

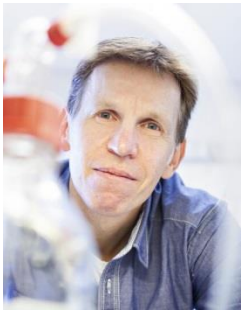
15.15 *Tea*

STAND UP AND BE COUNTED: MEASURING T CELLS IN WELLS

Christopher Love

Assessing the functional responses elicited by T cells during interventions and clinical studies of human disease rely predominantly on integrated single-cell measures, such as intracellular staining by flow cytometry or ELISpot, or on bulk assays, using cells from blood. New approaches for single-cell analysis of T cells using dense arrays of subnanoliter wells (nanowells) enable

novel classes of measurements based on dynamic responses, and accommodate small numbers of cells from limited samples like tissue biopsies. This talk will describe applications of these tools to characterize T cells in chronic human diseases. These examples, combined with corresponding computational analyses of the resulting data, provide a view towards a quantitative, systematic approach to advanced clinical monitoring for evaluating T cell responses raised by vaccines and immunotherapeutics, as well as understanding antigen-specific responses in diseases like HIV, multiple sclerosis, and diabetes.



IMMUNE SYSTEM VS CANCER. THE BATTLE WITHIN.

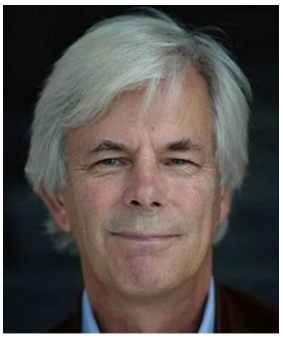
Ton Schumacher

Human tumors contain large numbers of mutations, of which many hundreds can be present within expressed genes. As the resulting altered protein sequences are foreign to the immune system, immune recognition of such 'neo-antigens' is likely to be of significant importance to the activity of clinically effective cancer immunotherapies. However, the vast majority of the mutations in human cancers are unique to individual patients and, because of this, broadly applicable approaches to link the consequences of DNA damage in human cancer to tumor-specific T cell activity have long been lacking.

Over the past few years we have developed technologies for the analysis of T cell recognition of the mutations that are present within individual tumors. A key observation made in this work has been that T cell recognition of the consequences of DNA damage is a common feature in human melanoma. Furthermore, based on the distribution of mutation loads in other major human cancer types, we consider it plausible that also in many other human tumors, the repertoire of mutant antigens provided by DNA damage (the 'neo-antigen space') will suffice to allow T cell recognition.

These data provide a strong incentive for the development of a new generation of 'personalized immunotherapies' that exploit cancer genome information to target patient-specific mutant antigens.

Session 3:	**B Cells**
Chair:	Rudi Hendriks
16.00	Hergen Spits (Amsterdam, The Netherlands)
16.45	Michael Reth (Freiburg, Germany)
17.30	*Drinks and Dinner*



Utilization of B cell responses in elite responders in Infectious disease and Cancer

Hergen Spits

It is well established that certain individuals have superior immune responses against "foreign" substances such as infectious disease targets or cancer cells. Such individuals are called elite responders. In my presentation I will

discuss a platform to analyze the B cells responses in such elite responders. I will show some examples of monoclonal antibodies developed from these B cells and discuss the utilization of such antibodies as therapeutics.

ON THE DOORSTEP TO SYNTHETIC IMMUNOLOGY

Michael Reth

BIOSS Centre for Biological Signalling Studies, University of Freiburg, Dept. of Molecular Immunology, Biology III, Faculty of Biology, University of Freiburg and MPI of Immunobiology and Epigenetic, Stuebeweg 51, D-79108 Freiburg, Germany

Immunology is a strange science. Whereas normal science starts with curiosity driven questions leading to experiments and an increasing knowledge that finally is translated to useful applications, immunology did it the other way round starting with an application. Indeed vaccination protocols were established in the 18th and 19th century long before any basic knowledge about composition and function of the immune system existed. What immunologists did the last 100 years was trying to understand why vaccination is working and how the immune system is composed and regulated.

In the meantime we have learnt quite a lot about the players and their play in the immune system but strangely enough this knowledge only rarely got translated into new vaccines and procedures for immune modulation. This is due to the enormous complexity of the system and the delicate balances of immune regulation. Indeed, the generation of super vaccines that maximally stimulates the immune system may rather result in autoimmunity than protection. Nevertheless, in the near future I can foresee that with the help of synthetic biology and its increasingly sophisticated toolboxes new procedures will become available for a better induction and regulation of immune processes.

In my presentation I will describe how in my laboratory we applied synthetic biology approaches for a better study of the mechanism operating during B cell development and activation and outline how in the future synthetic biology can be used for the remote control of immunity.

Schamel, WW. and **Reth, M**. (2012). Synthetic immune signaling. **Curr Opin Biotechnol** 23(5): 780-784.

Reth M. Matching cellular dimensions with molecular sizes. **Nat Immunol**. 2013 Aug;14(8):765-7. doi: 10.1038/ni.2621.

Klasener K, Maity PC, Hobeika E, Yang J, **Reth M.** (2014). B cell activation involves nanoscale receptor reorganizations and inside-out signaling by Syk. **Elife** 3: e02069.

Evening lecture

Chair: Esther de Jong

20.00 Marca Wauben (Utrecht, The Netherlands)

21.00 *Party*

EXTRACELLULAR VESICLES: VEHICLES SHAPING IMMUNE SYSTEM FUNCTION BEYOND THE CONFINES OF THE ORGANISM?

Marca Wauben

The regulated release of vesicles in the environment is a phenomenon shared by cells of all organisms. These extracellular vesicles (EVs) contain bioactive proteins, lipids and nucleic acids incorporated in a highly regulated fashion. Besides balancing the needs of the cell of origin, it is now generally accepted that EVs play an integral role in intercellular communication in (patho)physiological processes. Ample evidence has been obtained that EVs play a role in immune (dys)regulation. Furthermore, the wide spread functional conservation of vesicle release indicates that EV-mediated communication could play a role in interspecies, cross-kingdom communication. These findings sparked the idea that EVs are vehicles to support the immune system function within a larger context than the restricted confines of the organism. Understanding these means of intercellular communication will shed new light on the working mechanism of the immune system and will shift immunology from characterizing immune reactions within an organism to a next level of contextualizing responses within the environment in which they occur.

Friday March 27

8.15 'Meet-the-speaker' breakfast sessions

Session 4: High resolution
Chair: Martijn Nolte
9.00 Dan Davis (Manchester, UK)
9.45 Paul Parren (Utrecht, The Netherlands)
10.30 *Coffee*

USING SUPER-RESOLUTION MICROSCOPY TO WATCH IMMUNE CELLS KILL

Daniel M. Davis

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Cell-contact dependent regulation of immune cell responses plays a vital role in balancing the need for rapid and efficient responses to a wide variety of pathological challenges, while at the same time maintaining self-tolerance. Over the last decade, much research has studied how immune cell interactions are often accompanied by the segregation of proteins into micrometer- and submicrometer-scale domains at an immune synapse. The emerging new paradigm is that interactions between immune cell receptors, kinases and adaptors are at least in part controlled by transient interactions between supramolecular assemblies. This is a significantly different concept from a linear cascade of individual protein-protein interactions depicted in textbook diagrams of immune receptor signaling pathways. However, understanding what happens at the cell surface during immune cell interactions has been hampered by the resolution of light microscopy. Here, I will present new data using high- and super-resolution imaging techniques that reveal novel insights into molecular recognition by human Natural Killer cells and how specific effector functions are realized. Our findings indicate that immune cell activation involves a nanometre-scale reorganisation of surface receptors which can in turn impact the thresholds for cellular activation and inhibition.

Refs:

1. Cartwright A, Griggs J, Davis DM, The immune synapse clears and excludes molecules above a size threshold, Nature Comm., 5, 5479 (2014).
2. Pageon S. et al, Super-resolution imaging reveals nanometre-scale reorganisation of inhibitory NK cell receptors upon activation via NKG2D, Science Signaling, 6, ra62 (2013).
3. Davis D.M. The Compatibility Gene, Penguin paperback, (2014).

HOW TO GROW IDEAS INTO NOVEL MEDICINES

Paul Parren

Translating science into practical applications requires strong data, teamwork and perseverance. The concept that antibodies require clustering in order to activate complement was published 50 years ago. Nevertheless, only last year, we were able to visualize the ordered antibody complex required for complement activation using high resolution structure-function analyses. The molecular and mechanistic insights obtained were leveraged to develop a novel technology platform for antibody potentiation. This platform allows the generation of therapeutic antibodies of the future.

Session 5:	**Neuronal control**
Chair:	Sander Tas
11.00	Peter Pickkers (Nijmegen, The Netherlands)
11.45	Paul-Peter Tak (Stevenage, UK)
12.30	*Lunch*

"JUST THINK ABOUT IT: INFLUENCING YOUR INNATE IMMUNITY WITH YOUR BRAIN, WOULDN'T THAT BE GREAT?"

Peter Pickkers

In the recent decade, it has become increasingly clear that there is considerable interplay between the autonomic nervous system and the immune system. Both the parasympathetic (via the vagus nerve) as well as the sympathetic nervous system exert a regulatory function on the immune response. Animal studies have shown that electrical stimulation of the vagus nerve dampens inflammation via the release of the vagal neurotransmitter acetylcholine and subsequent activation of the alpha7 nicotinic acetylcholine receptor present on macrophages and other immune cells. Furthermore, several alpha7 nicotinic acetylcholine receptor agonists have been shown exert similar anti-inflammatory effects *in vitro* and in animals. This so-called "cholinergic anti-inflammatory pathway" could therefore represents a possible novel therapeutic modality to limit inflammation in various conditions. However, human data is very scarce. In this talk I will give an short introduction of the cholinergic anti-inflammatory pathway and discuss several animal and human studies we have performed in the recent years.
In the second part of my talk, I will address sympathetic nervous system-immune system interactions. Previous work has shown that infusion of the sympathetic neurotransmitter adrenaline attenuates the inflammatory response via beta-receptor stimulation. Therefore, it might be envisioned that endogenous activation of the sympathetic nervous system exerts immunosuppressive effects as well. However, it is generally accepted that the sympathetic nervous system, being part of the autonomic nervous system cannot be influenced voluntarily. In contrast, we have recently shown that through practicing techniques developed by "iceman" Wim Hof, it is indeed possible to voluntarily activate the sympathetic nervous system and that this is results in a

profound suppression of the immune response in humans *in vivo*. I will address our studies into the techniques of the iceman and discuss future plans and perspectives regarding this extraordinary manner of influencing the immune system.

VAGUS NERVE STIMULATION: A NEW BIOELECTRONICS APPROACH TO TREAT RHEUMATOID ARTHRITIS?

Paul-Peter Tak

Department of Clinical Immunology and Rheumatology, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands. Currently also: Cambridge University, Cambridge, UK, Ghent University, Ghent, Belgium and GlaxoSmithKline, Stevenage, UK. paul-peter.x.tak@gsk.com.

There has been a marked improvement in the treatment of rheumatoid arthritis (RA), but most patients do not achieve disease remission. Therefore, there is still a need for new treatments. By screening an adenoviral short hairpin RNA library, we discovered that knockdown of the nicotinic acetylcholine receptor type 7 (α7nAChR) in RA fibroblast-like synoviocytes results in an increased production of mediators of inflammation and degradation. The α7nAChR is intimately involved in the cholinergic anti-inflammatory pathway (CAP). This led us to study the effects of α7nAChR activation in an animal model of RA, and we could show that this resulted in reduced arthritis activity. Accordingly, stimulation of the CAP by vagus nerve stimulation improved experimental arthritis. Conversely, we found aggravation of arthritis activity after unilateral cervical vagotomy as well as in α7nAChR-knockout mice. Together, these data provided the basis for exploration of vagus nerve stimulation in RA patients as a novel anti-inflammatory approach.

Closing lecture

Chair: Maaike Ressing

13.15 Thorsten Mempel (Charlestown, USA)

INTRAVITAL IMMUNOLOGY: SEEING T CELLS MAKE DECISIONS IN VIVO

Thorsten R. Mempel, Francesco Marangoni, Edward Y. Kim, Christian A. Bauer, Esteban Carrizosa

Center for Immunology and Inflammatory Diseases, Massachusetts General Hospital and Harvard Medical School, Boston

Development, homeostasis, and effector function of T cells are regulated through cues that they receive during their contact-dependent interactions with antigen-presenting cells (APC) in lymphoid and non-lymphoid tissues. While encounters of T

cells with dendritic cells in secondary lymphoid tissues induce their differentiation into effector T cells, their function continues to be regulated and edited upon migration into peripheral tissues. The acquisition of a dysfunctional state by tumor-infiltrating cytotoxic T cells (CTL) is paradigmatic for this continued susceptibility to signals that local either reinforce or attenuate the capacity of T cells to execute their immunological effector functions.
Our lab uses multiphoton intravital microscopy to examine the cellular interactions and the signals exchanged between CTL, various APC, as well as regulatory T cells in tumor tissue, which organize their functional interplay and restrain the ability of CTL to control or reject tumors. By dynamically visualizing in individual cells the activation of signaling pathways that control gene expression programs in CTL and Treg we seek to understand the context in which cellular and molecular processes take place that ultimately determine the outcomes of tumor tolerance or immunity.

References:
Marangoni F et al., *Immunity* 38(2): 237-49 (2013)
Bauer CA, Kim EY et al., *J Clin Invest* 124: 2425-40 (2014)
Martinez GJ et al., *Immunity* 42: 265-78 (2015)

Closure

14.15