

# Identifying T Cell Subset Phenotype and Function in Infections

The Penridge Suite, 470 Bowes Road, London N11 1NL

Thursday, March 10, 2011

A revolution in the basic understanding of immunology occurred in the late 1980s with the discovery that CD4+ helper T cells were not a homogeneous population but could be divided into Th1 and Th2 subsets based on their cytokine profiles. 20 years later the field of T cell subset phenotype and function remains fast moving with the recently demonstrated existence of T regulatory and Th17 cells adding extra layers of complexity. The meeting will explore current ideas about the roles played by these varied T cells subsets in a variety of infections with presentations from leaders in the field

Meeting Chair: *Dr Marc Veldhoen*, Cambridge, UK

This event has CPD accreditation

9:00 – 9:45

**Registration**

9:45 – 10:00

**Morning Session - Introduction by the Chair:** *Dr Marc Veldhoen*, Cambridge, UK

10:00 – 10:30

**T helper phenotypes in wild rodents naturally infected with macro and microparasites**

*Professor Janette Bradley*, Nottingham, UK

Studies on laboratory mice have provided a wealth of information about the phenotype of the T helper response is directed to various pathogens under defined conditions. Studies on inbred strains of host or on infections by a single pathogen do not model the responses that would arise in animals exposed to many pathogens. Nor does such experiments evaluate the potential effects of variation of environmental factors such as nutritional availability, climatic stress etc. We will report the immune response profiles in the vole, *Microtus agrestis* and the wood mouse *Apodemus sylvaticus* under natural infection conditions and demonstrate the effects of micro, macroparasites and season on the phenotype of the T helper response

10:30 – 11:00

**Regulatory T cell populations in human and murine malaria**

*Professor Eleanor Riley*, London School of Hygiene and Tropical Medicine, UK

Acute, highly virulent infections are accompanied by florid - typically type 1- immune responses which need to be tightly regulated to avoid induction of immune-mediated pathology. In both murine and human malaria infections, immune regulation is mediated by IL-10 and TGF-beta. These cytokines emanate from a variety of cell types including several populations of T lymphocytes. I will present data from murine and human studies on the roles of IL-10, TGF-beta, monocytes, classical (natural or endogenous) regulatory T cells and induced (effector) T cell populations in the regulation of parasite control and immunopathology.

11:00- 11:05

**Speakers photo**

11:05 – 11:30

**Mid-morning break**

11:30 – 12:00

**CD4+ T helper subsets in *Helicobacter hepaticus* infection: Th1, Th17, and Treg cells.**

*Dr Marika Kullberg*, University of York, UK

Inflammatory bowel disease, including Crohn's disease and ulcerative colitis, is a chronic inflammatory disorder of the gastrointestinal tract that is caused in part by an inappropriate immune response to intestinal microbiota. To help our understanding of the process by which bacteria induce inflammation in the gut, our lab is using an experimental model of colitis involving infection with *Helicobacter hepaticus*. This model allows us to examine the early events following bacterial challenge, and to analyze bacterium-specific CD4 T cell responses in disease-susceptible versus disease-resistant hosts. The *H. hepaticus* colitis model also provides a platform by which to elucidate the role of bacterium-specific CD4 T cells and their cytokines in the inflammatory process in the intestine.

12:00 – 12:30

**Conditional mouse mutants as a tool to analyse Th1, Th2, Th17 and Treg subsets in parasite infection**

*Professor Werner Mueller*, Manchester, UK

The Muller lab dissects the cellular cytokine network by conditionally inactivating cytokine and cytokine receptor genes in mice. This technology allows the specific analysis of cytokine action at the cytokine secretion site as well as at the level of cytokine responsive cells. Our initial work in the cytokine Interleukin-10 (IL-10) has revealed that we can identify specific cell type specific functions of IL-10 producing and IL-10 responding cells, indicating that IL-10 is a locally produced and locally acting cytokine. In the presentation the use of the immune response against the parasite *trichuris muris* in various conditional mouse mutants will be presented.

## Selected References:

Pils, M., Bleich, A., Prinz, I., Fasnacht, N., Bollati-Fogolin, M., Schippers, A., Rozell, B. & Müller, W (2010). Commensal gut flora reduces susceptibility to experimentally induced colitis via T-cell-derived interleukin-10. *Inflammatory bowel diseases*, Full text DOI:10.1002/ibd.21587 |PubMed entry PMID:21182023

Pils, M., Pisano, F., Fasnacht, N., Heinrich, J., Groebe, L., Schippers, A., Rozell, B., Jack, R. & Müller, W (2010). Monocytes/macrophages and/or neutrophils are the target of IL-10 in the LPS endotoxemia model. *European journal of immunology*, 40(2), 443-8.

Fasnacht N, Greweling MC, Bollati-Fogolin M, Schippers A, Müller W. (2009). T-cell-specific deletion of gp130 renders the highly susceptible IL-10-deficient mouse resistant to intestinal nematode infection. *European journal of immunology*, Full text DOI:10.1002/eji.200838710 |PubMed entry PMID:19593768

Rubtsov YP, Rasmussen JP, Chi EY, Fontenot J, Castelli L, Ye X, Treuting P, Siewe L, Roers A, Henderson WR, Muller W, Rudensky AY. (2008). Regulatory T Cell-Derived Interleukin-10 Limits Inflammation at Environmental Interfaces. *Immunity*, 28(4), 546-558.

Siewe L, Bollati-Fogolin M, Wickenhauser C, Krieg T, Müller W, Roers A. (2006). Interleukin-10 derived from macrophages and/or neutrophils regulates the inflammatory response to LPS but not the response to CpG DNA. *European journal of immunology*, 36(12), 3248-55.

Roers A, Siewe L, Strittmatter E, Deckert M, Schlüter D, Stenzel W, Gruber AD, Krieg T, Rajewsky K, Müller W. (2004). T cell-specific inactivation of the interleukin 10 gene in mice results in enhanced T cell responses but normal innate responses to lipopolysaccharide or skin irritation. *The Journal of experimental medicine*, 200(10), 1289-97.

12:30 – 13:30

### **Lunch and Poster Viewing**

13:30 – 14:30

### **Discussion panel session**

Please submit questions to Euroscicon staff during the event. These questions will be asked to the panel of speakers at this panel session. Plus you are free to ask additional questions during the session

14:30 – 15:00

### **A Reflection on the Balancing act of T cell orchestra in the dynamic pathology of Leprosy**

*Professor Pranab Das, University of Amsterdam, Netherlands*

Leprosy is presently coined as a disease of the past, although in certain parts of the globe, like (Brazil, India, Bangladesh, and Africa) the disease is still prominently present. As such leprosy can probably be mentioned under the category of the neglected diseases. Therefore, the disease as such attracts negligible attention from the main stream of the core health care system, even in countries like Brazil and India.

However, the spectral pathology of the disease superimposed by the changeable hosts' immunity to the parasite *Mycobacterium leprae* (*Ml*) during the evolution of the disease, offers an opportunity for studying the aspects of immune dynamics in a human disease model. Leprosy is an infectious disease caused by the infection with a very slow growing organism, *Mycobacterium leprae* and as this organism can not be grown *in vitro* the research on host-parasite interaction largely concentrates in patients' oriented exercise. The spectral nature of the disease is closely associated with the differences in the hosts' immune reactivity to pathogen's antigenic determinants and in the variations of the regulatory mechanisms that control the pathogen specific immunities.

In this respect, discrete T cell subsets and relevant mycobacterium antigenic determinants appear to control the clinical and immunological spectrum of leprosy. T cells (probably together with the participation of antigen presenting cells and B cells), within the adaptive immunity, play the pivotal role in both protective immunity and also in dictating the pathology. In order to address this fact, the T cells from the skin lesions of patients as a prospective longitudinal study were isolated, cloned and their cytokine profiles were established.

The results showed that Type-1, *Ml* specific T cells producing IL-2, IFN-gamma, and TNF-alpha sustains the effective CMI to contain the spread of intracellular infection where as Type-2 *Ml* specific T cells with secreting profile of IL-4/IL-5 and IL-13 favor antibody formation but without the containment of intracellular bacterial growth. Interestingly, some of these T cells showed production of IL-10/IL-6 and some not. Unfortunately, the status of these T cells in the context of the production of IL-17 and TGF-beta remains to be evaluated. Nevertheless, the skewing of T cell subsets to the polarized Type-1 T cells coincided with the patients undergoing a reversal reaction (RR) or Type-1 leprosy reaction (T1LR), a severe clinical symptom showing strong bactericidal and tissue damaging T cell reactivity, when only the effective therapy is immune suppression by steroid treatment. However, it is also recognized that many patients suffering from T1LR, spontaneously recover. Recently, the group of Sallusto showed that some polarized Th1 clones also produce IL-17, termed as TH-17. Some of the latter also produce IL-10 and some not, which shows some similarity to the present sets of T cell clones. The questions arise whether or not, these T cell subsets presently described could play a balancing act both in exacerbation and remission, whereby Th1(Type-1)/Th17 cells either being IL-10 negative or positive may prove to be crucial.

**Concluding Comment:** Last but not the least, since nerve damage resulting into permanent disability is the major problem in the course of *Ml* infection, due to an immune reaction, leprosy will remain a challenge to experimental immunologists in the next millennium. The main emphasis therein will be on the early diagnosis of leprosy reaction in one hand and on the other, for identification of an early case of leprosy in the risk of developing reaction. Since the T cell characteristics are largely guided by their cytokine profile, it is possible that by monitoring the cytokine profile of the patients together with immune-pathological analysis of the patients would be helpful in preventing the reactions at an early stage.

15:00 – 15:30 **Afternoon Tea/Coffee and Last Poster Viewing**

15:30- 16:00 **Multiplexed Immunoassays; The Technology, Benefits and Applications**

*Dr Stephen David*, Biorad, UK

Talk will provide an overview of the Bioplex technology and its applications as well as a look at the planned developments for 2011.

16:00 - 16:30 **The development and interactions of regulatory and effector T cell responses during helminth infections**

*Dr Matthew Taylor*, University of Edinburgh, UK

Human helminth infections are synonymous with suppression of the host immunity resulting in parasite survival and the maintenance of chronic infections. Using a murine model of filariasis, *Litomosoides sigmodontis* infection of susceptible (BALB/c) and resistant (C57BL/6) mice, we have shown that T cell regulation occurs at two levels; through a CD4<sup>+</sup>Foxp3<sup>+</sup> regulatory T (Treg) cell response and the development of CD4<sup>+</sup> effector T (Teff) cell hypo-responsiveness. The CD4<sup>+</sup>Foxp3<sup>+</sup> Treg cell response is initiated by the infective L3 stage rapidly upon contact with the host, with increased CD4<sup>+</sup>Foxp3<sup>+</sup>CD25<sup>+</sup> Treg cell proliferation *in vivo* resulting in a dominant expansion of CD4<sup>+</sup>Foxp3<sup>+</sup>CD25<sup>+</sup> T cells. Depletion of CD25<sup>+</sup> Treg cells prior to infection enhances parasite clearance indicating that the Treg cell response inhibits protective immunity and is mainly recruited from the pre-existing pool of natural CD4<sup>+</sup>Foxp3<sup>+</sup> Treg cells. The second level of T cell regulation develops as infection establishes and the CD4<sup>+</sup> Teff become intrinsically hypo-responsive to antigenic stimulation. This is associated with enhanced expression of CTLA-4, GITR, and PD-1. Once established, infection-induced suppression can be overcome by depleting CD25<sup>+</sup> Tregs, but only if combined with restoring Teff cell responses by providing co-stimulation through GITR, or blocking co-inhibition through CTLA-4. As yet, it is not known what factors drive the initial bias towards a Treg response or the later Teff cell hypo-responsiveness. Our hypothesis is that the balance of co-stimulatory/ inhibitory signals during T cell priming and maintenance determines whether regulatory or effector responses prevail, with a lack of co-stimulation or a bias towards co-inhibition resulting in immune suppression. Initial work shows an important role for GITR in Th2 cell priming as blocking GITRL in resistant C57BL/6 mice ablates the Ag-specific Th2 response (IL-4, IL-13) and results in a Th1 phenotype (increased IFN- $\gamma$ ). Additionally, co-stimulating susceptible BALB/c mice with an agonistic anti-GITR mAb enhances their Ag-specific Th2 response. We are currently investigating whether a bias towards co-inhibition favors a regulatory environment using blocking antibodies against PD-1 and its ligands. To further delineate Th2 responses following infection and treatments, as well as interactions between Treg and Teff cells, we are using BALB/c 4get IL-4gfp mice to track and quantify Th2 cells. Overall we believe that the initial T cell priming to filarial helminths is critical in determining whether the host will succumb to or resist parasite immunomodulation. Co-stimulatory/inhibitory signals play a role in the development of T cell responses against *L. sigmodontis* and therapeutic manipulation of these pathways could be used to enhance immune priming and restore protective immunity.

16:30 - 17:00 **Chairman's summing up.**

*This meeting was organised by Euroscicon ([www.euroscicon.com](http://www.euroscicon.com)), a team of dedicated professionals working for the continuous improvement of technical knowledge transfer to all scientists. Euroscicon believe that they can make a positive difference to the quality of science by providing cutting edge information on new technological advancements to the scientific community. This is provided via our exceptional services to individual scientists, research institutions and industry. The event was hosted by 'BioPark ([www.biopark.co.uk](http://www.biopark.co.uk)), a research and development centre in Welwyn Garden City providing specialist facilities and support for bioscience and health technology businesses to grow, and to develop new products and technologies*

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### About the Chair

*Marc Veldhoen* trained in Medical Biology at the Faculty of Medicine, Utrecht University, and continued his scientific career at the Division of Molecular Immunology at the National Institute for Medical Research in Mill Hill, London. He was the first to describe the de novo differentiation of the new Th17 as well as the Th9 subsets of T helper cells. He went on to show the importance of Th17 cells in the initiation of autoimmune responses and, via the identification of the Aryl Hydrocarbon Receptor in Th17, established a link between environmental toxins and auto-immunity.

### About the Speakers

**Eleanor Riley** graduated from Bristol University with degrees in Cellular Pathology and Veterinary Science. After an internship in Veterinary Pathology at Cornell University (USA) she studied for a PhD in immunology and parasitology in the Department of Veterinary Pathology at the University of Liverpool. She began working on the immunology of malaria in 1985, as a member of the senior scientific staff at the Medical Research Council Laboratories in The Gambia, West Africa. In 1990, Eleanor moved to the University of Edinburgh as a Wellcome Trust Senior Research Fellow. Eleanor moved to the London School of Hygiene and Tropical Medicine in October 1998 where she is Professor of Infectious Disease Immunology and Head of the Immunology Unit.

**Professor Jan Bradley** did her PhD at the London School of Hygiene and Tropical medicine on Trypanosomes. After a Post doctoral fellowship at Scripps Clinic and Research Centre in California working on malaria she returned to the UK to work in the lab of Prof Rick Maizels at Imperial College to work on filariasis. After 10 years at Imperial college she took up the position of Senior lecturer then Reader and Professor at Salford University. Still working on helminth parasites she is currently Professor of Parasitology at the University of Nottingham where her research is focused on the complex interactions between host and parasite. In particular, she is interested in the effects that helminths exert on the outcome of other infectious diseases via the host immune system.

Following 6 years as a 2D - DIGE application specialist at GE Healthcare and 4 years in business development **Stephen David** joined Biorad in 2010 as the southern UK instrument specialist for the Bioplex technology.

**Matthew Taylor** graduated from the University of Edinburgh with a BSc (Hons) in Immunology. His PhD at the University of Manchester studying immune responses to intestinal nematodes introduced to him to the world of parasitology that has grabbed his attention ever since. His post-doctoral work at the University of Edinburgh continued this interest studying immune subversion by filarial parasites. At the end of 2006 Matthew received a MRC Career Development Award to establish his own group at the university of Edinburgh investigating T cell responses to helminth parasites.

**Pranab Das** is retired as Associate Professor in Immunology and Head of the Research Group of Experimental Dermato Immuno Pathology, Department of Pathology, Academic Medical Center University of Amsterdam(AMC-UvA), Netherlands,(however still active as a Free Lance Research Consultant at AMC-UvA and also continuing as Professor of Chronic Inflammation/Immunodeficiency, at the Institute of Paediatrics, Faculty of Medicine, University of Brescia, Italy. I am a UK citizen, studied Biochemistry with a Ph.D.degree from London University and worked as an University Academic in London University, Hong Kong University, McGill University(Canada), University of Hamburg(Germany) until settling down in Netherlands. Since last 30 years worked as an Immunologist then as immunopathologist in University of Amsterdam until the retirement at the age of 65 years. Since last twenty five years devoted the research career in immunopathology of Tropical Diseases like Leprosy, Schistosomiasis, Tuberculosis and relevant inflammatory tissue reaction. He has authored more than 200 publications in various Peer reviewed journals and chapters of Book and popularising articles. His scientific publications scan the field of Enzymology, Human Genetics, Neuro science and since last 30 years in the field of inflammation, immunity, infection and immunodeficiency. Supervised more than 20 \Ph.D. students of different Nationality. His current interests among others is in the development of tropical medical research and education with a relevance to global health in an active co-operation with Brazil and India, in an Honorary Capacity

**Marika Kullberg** obtained her BSc in Chemistry and Microbiology from Stockholm University, Sweden. She went on to do a PhD in Immunology at Stockholm University and at the National Institutes of Health (NIH, USA), focusing on immunoregulation during *Schistosoma mansoni* infection. After a post-doc year at Stockholm University, Marika returned to the NIH in 1997 for a post-doc at the National Institute of Allergy and Infectious Diseases where she spent 8 years as a Visiting Fellow and a Research Fellow. Here, she established a new model of bacterial-induced colitis involving infection with *Helicobacter hepaticus*, and characterized both the pathogenic and the disease-protective arms of the immune response to this bacterium. In 2005, Marika moved to the University of York where she is a Lecturer in Immunology at the Centre for Immunology and Infection and the Hull York Medical School.

**Werner Muller** received his doctoral degree at the University of Cologne, Germany, in 1987 in the lab of Klaus Rajewsky. He continue to work in Klaus Rajewsky's lab until 2000 when he moved to the Helmholtz Centre of Infection research, Braunschweig opening a new department on experimental Immunology. In 2006 he accepted the Bill Ford Chair at the University of Manchester and finally moved completely to the University of Manchester in 2008. He is widely known in the immunological community for the Interleukin-4 deficient (published 1991) and the Interleukin-10 deficient mouse mutant (published in 1993).