

Analysing the Phenotype & Function of Regulatory T cells

The BioPark Hertfordshire, Welwyn Garden City , AL7 3AX

7th March 2008

- 9:00 – 9:40 **Registration**
- 9:40 – 9:45 **Introduction by the Chair:** *Dr Catherine Derry*, Science Communicator
- 9:45 – 10:10 **Human Regulatory T cells- generation and maintenance**
Dr Milica Vukmanovic-Stejic, UCL, UK
- 10:10 – 10:35 **Natural and induced regulatory T cells in rheumatoid arthritis**
Professor Michael Ehrenstein, University College London, UK London
TNF-alpha is a major pro-inflammatory cytokine and an important therapeutic target in rheumatoid arthritis (RA). We have previously shown regulatory T cells (T regs) from patients with RA have defective suppressor function. Following infliximab (anti-TNFalpha) therapy this defect is reversed, and is accompanied by a significant increase in peripheral Foxp3⁺ T regs. Manipulation of a pro-inflammatory environment could represent a therapeutic strategy for the induction of Treg and the restoration of tolerance.
- 10:35 – 11:00 **How is Treg suppression overcome during the onset of spontaneous diabetes??**
Dr Lucy Walker, Institute of Biomedical Research, University of Birmingham, UK
The activity of regulatory T cells (Treg) is widely accepted to play a central role in preventing pathogenic immune responses against self antigens. However it is not clear why such regulation breaks down during the onset of spontaneous autoimmune disease. In a TCR transgenic model of diabetes we show that islet-specific Treg actively prevent disease early in life but that this tolerance mechanism fails over time. To explore the molecular basis for failed Treg suppression we performed quantitative PCR on pancreatic LN. We found elevated levels of the newest member of the class I cytokine family, IL-21, and demonstrate that this cytokine can counteract Treg suppression.
- 11:00– 11:10 **Speakers photo**
- 11:10 – 11:35 **Mid-morning break**
- 11:35 – 12:00 **FOXP3+ regulatory T cells as biomarkers in human malignancies**
Dr Alison H. Banham, University of Oxford, UK
The FOXP3 transcription factor is essential for the development of CD4+CD25+ regulatory T cells (Tregs). Using a panel of anti-FOXP3 monoclonal antibodies we have immunophenotyped human Tregs. Demonstrating FOXP3 expression primarily, but not exclusively, in CD4+CD25+ T cells. FOXP3 has diagnostic utility for identifying a subgroup of patients with adult T-cell leukaemia/lymphoma. While high FOXP3+ Treg numbers identify poor prognosis carcinoma patients, we have found the reverse in lymphoma patients. The contribution of individual FOXP3 antibodies to the publication of conflicting data will be discussed
- 12:00 – 12:15 **The phenotype of Foxp3+ cells after *in vitro* expansion and at an inflammatory site**
Dr Richard.C. Duggleby-Anthony Nolan Trust Research institute, Royal Free Hospital, Pond Street, London
FOXP3 expression has been an extremely useful tool in identification and characterization of regulatory T cells (Tregs) but recent studies in humans have suggested that FOXP3 can be induced in non-Tregs. The functional properties of these cells are as yet undetermined but it does suggest that FOXP3+ cells may not represent a homogenous population. By studying expanded regulatory and effector cell lines we have found a phenotype that seems to be associated with induction of FOXP3 in effector cells. Interestingly this phenotype is highly prevalent in inflamed arthritic joints; suggesting that there maybe a functional change in the FOXP3+ cells
- 12:15 – 12:40 **The role of regulatory T cells in acute Dengue infection**
Dr Kerstin Luhn, University of Oxford UK
Regulatory T cells control immune responses and limit immunopathology. As immunopathogenesis occurs in Dengue virus infection we investigated T_{regs} in acute patients. CD4+CD25^{high}FoxP3+ T_{regs} showed a conventional phenotype. Unexpectedly, they invariably suppressed T cell proliferation and IL-10 secretion. Even the production of vasoactive cytokines was unaltered after Dengue-specific stimulation. T_{reg} frequencies and also T_{reg}/T_{effector} ratios were increased. As this ratio was only significantly increased in mild but not in severe cases a relative rise could be beneficial for disease outcome. We suggest that although T_{regs} expand and function normally in Dengue infection their relative frequencies are insufficient to control the immunopathology of severe disease.
- 12:40 – 13:00 **Tour of the BioPark**
- 13:00 – 14.00 **Lunch and Poster Viewing**

- 14:00 – 14:25 **Comparing the phenotype and function of CD4 versus CD8 regulatory T cells**
Dr Alistair Noble, King's College London, UK
 Differential expression of MHC class I and class II molecules necessitates regulation in both CD4 and CD8 T cell compartments. CD8 T cells differ from CD4's in that they do not contain an endogenous Foxp3+ CD25+ population, and their differentiation is naturally skewed towards the type 1 pathway. However CD8 cells can develop into potent contact dependent IL-10-secreting Treg in the presence of cytokines. The phenotype and function of CD4 vs CD8 Treg subsets will be discussed.
- 14:25 – 14:50 **Regulatory T cells in Allergy and Asthma**
Dr Catherine Hawrylowicz, King's College, London
 The role of naturally occurring and IL-10-secreting regulatory T cell (Treg) populations in the maintenance of respiratory health and therapeutic potential in allergic and asthmatic disease will be discussed. We have investigated the role of glucocorticoids and the active form of vitamin D (calcitriol) to promote IL-10 synthesis and promote a Treg phenotype in asthma patients. Our current work is investigating (i) specific markers of IL-10 secreting Treg; (ii) the potential steroid-enhancing role of calcitriol in severe asthma; (iii) the effects of calcitriol on human regulatory versus effector T cell balance.
- 14:50 – 15:15 **Regulatory T cells- Adoptive T cell therapy to induce longterm allograft survival**
Dr Yakup Tanriver, Kings College, London.
 CD4+CD25+ regulatory T (Tregs) cells play an important role in regulating immune responses. Here we investigated the possibility to expand them in vitro and transfer antigen specificity by T cell receptor transduction. Transduction did not interfere with their regulatory capacity and subsequent transplant experiment showed that those modified Tregs were capable of inducing tolerance to fully mismatched heart allografts .
- 15:15 – 15:40 **Afternoon Tea/Coffee and Last Poster Viewing**
- 15:40 – 16:05 **The Impact of Tregs on Immune Responses to Tumours**
Dr Gareth Betts, Cardiff University, UK
 Foxp3+CD4+ T cells (Tregs) are found at high frequency within tumours in rodent and human and results of some studies indicate that the cells promote disease progression. We addressed whether Tregs impinge upon effective anti-tumour immunosurveillance. Tumour development, induced by the carcinogen methylcholanthrene (MCA) was evaluated in the presence and absence of Tregs. The results of this study were striking indicating that even a partial and transient depletion of Tregs was sufficient to result in a significant reduction in MCA-induced tumours. This study, which indicates that Tregs do indeed impinge upon immunosurveillance, provides a model whereby useful anti-tumour effector cells can be identified.
- 16:05 – 16:20 **Listeriolysin-o expressed in a bacterial vaccine suppresses CD4 CD25high regulatory t cell function in vivo**
Ming-Shen Dai, University of London, UK
 The regulatory T cells are recognised as playing important roles in autoimmune, chronic infectious or neoplastic disease. An ideal vaccine for cancer or infection would stimulate specific cytotoxic responses and reduce/suppress Treg function. Our study showed that *Escherichia coli* vaccine expressing listeriolysin-O and OVA (*E.coli*-LLO/OVA) demonstrated remarkable levels of protection against OVA-expressing tumour cells and induces high-avidity specific CTLs. Also, LLO expression vaccine suppress Treg cell function and may have important implications for enhancing antitumor vaccination strategies in humans.
- 16: 20 – 16:45 **Interactions between CD4+CD25+ regulatory T cells and monocytes/macrophages: implications for the immune response**
Dr Leonie Taams, King's College London, UK
 The suppressive effects of CD4+CD25+Foxp3+ regulatory T cells (Tregs) on the adaptive immune system and on CD4+ T cells in particular have been well documented; however the effects on the innate immune system are still relatively unexplored. We will present evidence for a novel function of CD4+CD25+ Tregs, namely their ability to induce alternative activation of monocytes/macrophages. Following interactions with Tregs, monocytes/macrophages display reduced pro-inflammatory potential but enhanced phagocytic/scavenging ability. Our results suggest that the interactions between Tregs and monocytes/macrophages may contribute to the maintenance of immune homeostasis in the tissue
- 16:45 – 17:00 **Chairman's summing up & close.**

This meeting was organised by Euroscicon (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 Hertfordshire' (www.biopark.co.uk), a new 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

About the Speakers

Dr Leonie Taams, King's College London, UK - 1995-1998: PhD Immunology, Utrecht University, the Netherlands (title thesis 'Anergic T cells as active regulators of the immune response'), 1999-Sept 2000: Postdoctoral research fellow Dept Clin Immunology, Royal Free and University College Medical School, London (with prof Arne Akbar), Oct 2000-Jan 2003: Postdoctoral research fellow Dept Rheumatology & Clin Immunology, University Medical Centre Utrecht, NL (with prof Hans Bijlsma), Jan 2003-present: Lecturer/Senior Lecturer, Dept Immunobiology, King's College London

Dr Yakup Tanriver - Medical Training in Germany/Berlin (1994-2001), Thesis on chronic allograft dysfunction in a rat model of kidney transplantation, working as a medical doctor from 2002-2005 in the department of Nephrology and Transplantation (Charite/Berlin), since 2005 Post-Doc in Robert Lechler's group investigating the possibility to harness regulatory T cells to promote transplantation tolerance.

Professor Michael Ehrensteins research focusses upon immunoregulation in autoimmune rheumatic disease. He is an Honorary Consultant Rheumatologist at University College London Hospitals

Dr Alison H. Banham, BA, MA and DPhil degrees from the University of Oxford. Since 1995, based in the Nuffield Dept of Clinical Laboratory Sciences at the John Radcliffe Hospital in Oxford. Research focus is to identify and characterise clinically relevant lymphoma-associated proteins that have fundamental roles in the molecular pathogenesis of human malignancies. Director of a Leukaemia Research Fund Specialist Programme Grant investigating the role of FOXP forkhead transcription factors in lymphoma, to facilitate the identification of high-risk patient groups and novel targets for therapeutic intervention

Dr. DAI is from the Haematology/Oncology Department of National Defense Medical Center, TAIWAN. He is doing his postgraduate research about the application of recombinant E. coli as cancer vaccine in the Molecular Oncology Unit, Institute of Cancer, Queen Mary University of London.

Dr Kerstin Luhn, University of Oxford UK. 1997-2001: PhD in Biology at the Institute of Cell Biology, Center for Molecular Biology of Inflammation (ZMBE), Muenster University, Germany; 2001-2003: Postdoctoral Researcher at the Max Planck Institute of Molecular Biomedicine, Muenster, Germany, with Professor Dietmar Vestweber, working on Leukocyte Adhesion Deficiency II; 2003-present: Postdoctoral Research Fellow at the MRC Human Immunology Unit, Weatherall Institute of Molecular Medicine, Oxford, with Professor Sarah Rowland-Jones, working on immunology of Dengue infection

Dr Alistair Noble, gained a 1st Class Honours degree in Biotechnology from King's College London in 1988 and then joined UMDS Guy's Hospital, where he gained a PhD in Immunology in 1994. He completed postdoctoral training in the Dept of Pathology, Harvard Medical School before returning to the UK to take up a faculty position at King's College London in 1996. Dr Noble's group works primarily on the immunoregulatory mechanisms involved in allergic disease.

Dr Milica Vukmanovic-Stejic, UCL, UK, 2000 PhD Immunology, King's College Medical School; Post-doctoral Research fellow at Department of Immunology, UCL Medical School, with Professor Arne Akbar, working on human regulatory T cells.

Dr Richard.C. Duggleby, Anthony Nolan Research Institute, UK After completing a PhD in Biochemistry at Southampton University on the mechanism of action of Ca²⁺ Pumps, I changed direction by studying the immune response to Collagen fragments after digestion with metalloproteinases (Dept of Rheumatology Newcastle University). I followed this with a 6 year tenure at the University of Cambridge, Dept of Medicine, studying tolerance mechanisms in arthritis (to heat shock proteins). This led to investigating the role of regulatory T cells in this system. I am now continuing my studies of regulatory T cells in the field of cellular therapy in transplantation at the Anthony Nolan Research Institute.

Dr Catherine Hawrylowicz, trained in Immunology at the National Institute for Medical Research, Mill Hill with Dr Gerry Klaus and then at Washington University, St Louis, USA, with Professor Emil Unanue. On returning to the UK she worked first with Professor Marc Feldmann and then went on to university posts at Imperial College (St Mary's Hospital Medical School) and then as part of the MRC and Asthma UK Centre for Allergic Mechanisms in Asthma at King's College London (Guy's Hospital campus).

Dr Lucy Walker has a longstanding interest in costimulatory molecules and T cell tolerance. During postdoctoral training with Professor Peter Lane she identified roles for CD28 and OX40 in directing T cell help for B cell antibody production. Funded by a Wellcome Trust Traveling fellowship, she generated a transgenic model to study antigen-specific regulatory T cells in Professor Abul Abbas's lab at University of California, San Francisco. On her return to the UK she was awarded an MRC Career Development Award to study the role of regulatory T cells in the control of autoimmune diabetes.