

Human autoimmune disease: learning from models

The BioPark Hertfordshire, Welwyn Garden City, AL7 3AX: 2nd July 2010

“More than 3% of the population suffer from some form of autoimmune disease and this meeting will bring together experts working with well-validated models of human autoimmunity. The emphasis of the meeting will be on the use of animal models to elucidate pathogenetic mechanisms of relevance to human disease and to develop novel therapeutic strategies” **Chair:** *Dr. Richard Williams*, Imperial College London, UK.

This event has CPD accreditation and a discussion panel session.

9:00 – 9:45 **Registration**

9:45 – 10:00 **Introduction by the Chair:** *Dr. Richard Williams*, Imperial College London, UK

10:00 – 10:30 **Immunotherapy of rheumatoid arthritis: lessons learnt from animal models**

Dr Stephen J Thompson, KCL School of Medicine at Guy's, King's and St Thomas' Hospitals, London, UK

Stress proteins are upregulated at the site of inflammation such as that found within the joints of patients with rheumatoid arthritis. Initially these proteins were identified as autoantigens and hence targets for immune attack. However, subsequent studies both in man and mouse have characterised these antigens as either stimulators of anti-inflammatory mediators or are themselves in fact targets of regulatory T cells. Both these observations make stress proteins attractive candidates for the development of novel immunotherapeutics. The translational research aimed at developing such biologics for the treatment of inflammatory arthritis will be discussed.

10:30 – 11:00 **VITILIGO: An Enigmatic Auto Immune Disease?**

Emeritus Professor P.K. Das, University of Amsterdam, The Netherlands

Vitiligo is a disease where the epidermal pigment cells [melanocytes], the main defense mechanism against UV mediated damage, are lost randomly. The disappearance of melanocytes shows ugly appearance of skin with varying degree of white maculae. As such, vitiligo is neither life threatening nor a debilitating disease as compared to other well known autoimmune diseases such as lupus, rheumatoid arthritis, autoimmune diabetes etc. Further, despite the demonstration of the presence of mild inflammatory infiltrate in the skin paralleling the loss of melanocytes, vitiligo can not be categorized as an inflammatory dermatoses. Interestingly, the disease is often associated with the presence of classical autoimmune disease symptoms in the same patient.

Various types of theories on the etiology of vitiligo are often being discussed and debated endlessly among the investigators. Since last decade, however, the autoimmune mechanism is being advocated by the investigators. Indeed, our own work meticulously demonstrate that the loss of melanocytes in “a certain type of generalized vitiligo” is caused by autoreactive melanocyte specific T cells. In addition, in some patients auto-antibodies against melanocytes can also be demonstrated. Nevertheless, vitiligo as such is not always perceived in the category of the classically known autoimmune disease. Since the autoimmune pathology, though remains obscure, it is believed that the perturbation of homeostatic immune physiology of host, (which is maintained via the interacting antigen presenting cells [APC]-T cells and B cells), leads to the precipitation of any autoimmune disease including vitiligo. Such perturbation immune homeostasis can be illustrated clearly in vitiligo pathology. One bonus point for studying vitiligo as a model autoimmune disease is that the therapeutics for melanoma, a deadly skin cancer, can possibly be designed, by studying vitiligo. Against the above scenario, this presentation will argue the necessity for undertaking vitiligo research within the umbrella of both autoimmunity and cancer. For further reading the followings could be referred for further reading.

11:00 – 11:30 **Morning break and poster presentations**

11:30 – 12:00 **Does the T_h1/T_h17 reactivity to neuroantigen predict disease onset and progression in autoimmune encephalomyelitis?**

Dr. Stefanie Kuerten, University of Cologne, Germany

Due to the very limited numbers of PBMCs that can be obtained from the blood of individual mice, so far there has been no possibility of measuring antigen-specific immune responses without sacrificing the animal. Therefore, the question whether central disease parameters such as onset, progression and severity correlate with a variable magnitude and cytokine quality of the T cell response in experimental autoimmune encephalomyelitis (EAE) has remained unanswered. Here we introduce an ELISPOT-based PBMC test system in which as little as 150 μ l of murine blood obtained from the tail vein are sufficient allowing to bleed mice repeatedly while continuing to observe

the clinical course of EAE. Having this technique at hand, we demonstrate that longitudinal measurements of antigen-specific IFN- γ and IL-17 clonal sizes are a highly suitable approach to predict the disease outcome in remitting-relapsing PLP:139-151- and chronic MOG:35-55-induced EAE of SJL/J and C57BL/6J mice, respectively. Our data propound cytokine monitoring as valuable tool in the quest for more efficient diagnostic and prognostic options in human multiple sclerosis and other autoimmune diseases.

12:00 – 12:10

Speakers photo

12:10 – 13:00

Lunch and poster presentations

13:00– 14:00

Question and Answer Session

Delegates will be asked to submit questions to a panel of experts. Questions can be submitted on the day of the event

14:00 – 14:30

Inflammatory mediators and lupus autoimmunity

Professor Rizgar A Mageed, William Harvey Research Institute, St Barts and the Royal London, UK

It is established that the immune and inflammatory responses cross-regulate each other. In this study we show that manipulation of the immune system in murine lupus by administration of recombinant TNF α , or blocking endogenous TNF α with antibody profoundly influences lupus autoimmunity. The studies have also shown that in this setting TNF α /anti-TNF α act directly on T and B-lymphocytes and profoundly affect their proliferation, cytokine production and a number of other vital functions with consequent effects on autoimmunity. We explore the pathways through which these responses are effected. Further, the relevance of the studies to human diseases will be discussed.

14:30 – 15:00

Latest advances in helper T cell subset analysis in autoimmunity

Dr Hermann Bohnenkamp, Miltenyi Biotec Ltd, Germany

In autoimmune research CD4⁺ Th1 and Th17 cells are currently the focus of much interest. In EAE, both Th1 and Th17 cells responsive to myelin autoantigens appear throughout the course of the disease, but the specific roles of these cell populations, in particular the stability of their cytokine production phenotype, are still being elucidated. To pursue this question it is vital to generate pure cell populations producing the cytokine of interest, devoid of any contaminating cells. The detection and isolation of viable IFN- γ and IL-17-producing T cells direct from blood and spleen using the IL-17 secretion assay system will be discussed. The phenotypes and functions of natural IL-17-producing cells will be demonstrated along with the possibility of splitting the IL-17 and IFN- γ secreting populations based on two-colour cytokine secretion.

IFN- γ produced by T helper cells plays also a prominent role in rheumatoid arthritis. These effector-memory Th1 cells are abundant in inflamed tissues. However, it is not clear how these cells get activated at the site of chronic inflammation in the absence of pathogens and, thus, without stimulation of the T cell receptor. To allow for an accurate analysis of T cells the isolation of viable IFN- γ secreting cells from synovial tissue will be discussed.

15:00 – 15:30

Afternoon Tea/Coffee and Last Poster Viewing

15:30 – 16:00

What knockout mice have taught us about the pathogenesis of lupus

Dr Liliane Fossati-Jimack, Imperial College, London, UK

Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease characterised by the production of an extraordinary array of autoantibodies reactive with nuclear antigens. Interaction of these autoantibodies with their cognate antigens leads to widespread inflammatory injury and underlies the pathogenesis of SLE. The aetiology of SLE is unknown, as well as the factors that influence the severity of disease manifestations. In mice and humans, expression of autoimmunity is under complex genetic control. A strategy to analyse the contribution of individual alleles to a multigenic trait has been the development of animals carrying genetic manipulations of specific genes implicated in the pathogenesis of SLE. This approach allows an *in vivo* assessment of the impact on the immune system of severe modifications in the expression (deficiency or overproduction) of genes suspected to play a role in the development of an autoimmune response. Genetically manipulated models have proved to be very useful to dissect effector mechanisms involved in disease pathogenesis and/or to delineate genetic mechanisms that may lead to systemic autoimmunity. Several important observations have emerged from the genetically engineered models. First, whether a particular gene or mutation causes a disease depends on the host: both disease susceptibility and the disease phenotype that result from the alteration of a single gene depend on other genes. Second, some genetic defects may share common pathogenic pathways. As a result, one could reasonably predict the possibility of developing common therapeutic strategies to treat this multifactorial complex condition. Finally, the development of genetically manipulated animals has led to the discovery of new roles for genes with known immune functions. The

complement deficient animals that will be presented in more details are a typical example of this. There is overwhelming evidence that deficiency of classical pathway complement proteins causes the development of SLE in humans and mice. Complement is implicated in the pathogenesis of SLE in several ways and may act as both friend and foe. Recently it has been suggested that one of the main activities of the classical pathway is to promote the resolution of inflammation by enhancing the clearance and uptake of dying cells by macrophages. We have developed a series of murine models of complement deficiency and SLE and found that these mice develop a lupus-like disease and have an impaired clearance of apoptotic cells. We have observed a similar phagocytic defect in macrophages derived from C1q-deficient humans cultured in autologous serum. This defect was rectifiable with purified human C1q. Consistent with these findings, we have data showing that macrophages from two lupus-prone murine strains have an impaired phagocytosis of apoptotic cells when compared with two non-autoimmune strains. Collectively these data strongly support the hypothesis that deficiency in complement predisposes to the development of lupus through inefficient removal of potentially pathogenic apoptotic cell debris. However, impaired clearance of such cells is, on its own, insufficient to produce autoimmunity. The data available from knockout mice emphasize that susceptibility to an autoimmune disease might depend on many factors in addition to the defective removal of dying cells. In summary it is clear that the traditional view of the role of complement in autoimmunity needs revision. Complement activation in lupus has been viewed as a major cause of tissue injury. Instead, evidence is emerging that complement may play a protective role rather than an exclusively pro-inflammatory role in tissue injury.

16:00 – 16:30 **Pre-clinical models of autoimmune connective tissue diseases**

Professor David Abraham, University College London, UK

Connective tissue diseases have complex pathogenic mechanisms encompassing host genetics, vascular manifestations, aberrant inflammation and autoimmunity leading to enhanced tissue repair resulting in scarring and replacement fibrosis. Contemporary approaches use reporter transgenesis to track and target pathogenic cells and knock-in and -out technologies to manipulate the cells and key molecular events involved. These are utilised within existing naturally occurring disease models and those induced by modulating the environment. Developing useful systems to model and study human disease processes in vivo represents a major biomedical challenge, as does their interpretation and utility as pre-clinical models to reliably access novel therapeutics

16:30 – 17:00 **Animal models for autoimmune diabetes**

Dr Lucienne Chatenoud, Hôpital Necker, France

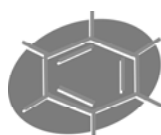
17:00 **Chairman's summing up.**

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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' (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

This meeting is sponsored by



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About the Chair

Richard Williams has been working for many years at the Kennedy Institute of Rheumatology on the immunotherapy of rheumatoid arthritis using animal models. His work contributed to the successful development of TNF blocking biologics, which are now widely used in the treatment of rheumatoid arthritis and other autoimmune diseases.

About the Speakers

Rizgar Mageed was awarded his PhD at London University in 1985. His interests are in understanding causes of autoimmunity and relationship with chronic inflammation. He worked at the Department of Immunology University of Birmingham until 1990 when he joined the Kennedy Institute of Rheumatology Division at Imperial College as a senior lecturer. In 2000, Prof. Mageed was awarded an Arthritis Research Campaign Fellowship and appointed Senior Lecturer at the Centre for Rheumatology at University College London. He was appointed to his current post at the Bone and Joint Research Unit at the William Harvey Institute in December 2002.

Professor P.K. 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

Stephen Thompson received his B.Sc. in Cellular Pathology from the University of Bristol in 1984. He received his PhD in 1989 for studies characterising the role of House Dust Mite allergens in children with asthma and eczema. After these studies he began working on murine models of inflammatory arthritis, most notably pristane-induced arthritis, where he and his group were one of the first to characterise the immunopathology of this disease. Through the award of Arthritis Research Campaign post-doctoral fellowships (both in the UK and at the Southwestern Medical Center in Dallas, USA) he developed his interests in the role of stress proteins as inducers or targets of regulatory T cells and their potential use as anti-inflammatory immune modulators. He is currently based at Kings College London, Department of Rheumatology at Guys Hospital where he and his colleagues continue translational research to evaluate novel immunotherapeutic strategies for the treatment of rheumatoid arthritis.

Professor Abraham is research director in the Centre for Rheumatology at UCL. Initially trained at the Kennedy Institute of Rheumatology in London, he then became a Medical Research Council Travelling Fellow in Molecular Genetics and Transgenics at the Jackson Laboratory in the USA, and has worked at the MRC National Institute for Medical Research as a senior scientist in genes and development. Major research interests include the biology of tissue repair, and pathology of scarring and fibrosis, and the development of *in vivo* pre-clinical models of human disease as to study the pathogenesis and treatment of autoimmune connective tissue diseases.

Professor P.K. Das is retired as 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

Hermann Bohnenkamp is Business Area Manager for oncology, infections and tolerance at Miltenyi Biotec, Germany. He studied chemistry and received his Ph.D. degree from the University of Bonn and the Helmholtz Research Center Juelich. Continuing translational approaches for immunological strategies for the treatment of breast cancer he worked at Guy's Hospital and was funded by Cancer Research UK. Disease mediated neoangiogenesis in solid tumours and autoimmune diseases was one of the topics driving

his interest as immunologist and project leader at Munich based MediGene AG. In 2009 he joined Miltenyi Biotec as Global Product Manager immunology.

POSTERS

DOES THE T_H1/T_H17 REACTIVITY TO NEUROANTIGEN PREDICT DISEASE ONSET AND PROGRESSION IN AUTOIMMUNE ENCEPHALOMYELITIS?

S. Kuerten¹, K. Addicks, M. Tary-Lehmann and P.V. Lehmann²

Department of Anatomy I, University of Cologne, Joseph-Stelzmann-Str. 9, 50931 Cologne, Germany.

Due to the very limited numbers of PBMCs that can be obtained from the blood of individual mice, so far there has been no possibility of measuring antigen-specific immune responses without sacrificing the animal. Therefore, the question whether central disease parameters such as onset, progression and severity correlate with a variable magnitude and cytokine quality of the T cell response in experimental autoimmune encephalomyelitis (EAE) has remained unanswered.

Here we introduce an ELISPOT-based PBMC test system in which as little as 150 μ l of murine blood obtained from the tail vein are sufficient allowing to bleed mice repeatedly while continuing to observe the clinical course of EAE. Having this technique at hand, we demonstrate that longitudinal measurements of antigen-specific IFN- γ and IL-17 clonal sizes are a highly suitable approach to predict the disease outcome in remitting-relapsing PLP:139-151- and chronic MOG:35-55-induced EAE of SJL/J and C57BL/6J mice, respectively.

Our data propound cytokine monitoring as valuable tool in the quest for more efficient diagnostic and prognostic options in human multiple sclerosis and other autoimmune diseases.

LONG-LASTING PREVENTIVE AND THERAPEUTIC EFFECT OF GRAFTED TEMPERATURE-SENSITIVE IMMORTALIZED FIBROBLASTS PRODUCING LATENT TGF β 1 ON EAE

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To determine whether conditionally immortalized syngeneic fibroblasts producing latent transforming growth factor β 1 (TGF β 1) can safely prevent or ameliorate experimental autoimmune encephalomyelitis (EAE), temperature-sensitive fibroblasts (tsF) have been obtained by electrofusion of primary rat fibroblasts engineered to express latent TGF β 1 precursor and fibroblasts immortalized by retrovirus encoding a temperature-sensitive non-SV40 origin-binding U19 mutant of the SV40 large T Ag. Out of 23 clones of tsF-TGF β 1 6 clones displayed maximal latent TGF β 1 production of 4.7-8.2 ng/ml per 10^6 cells per 24 h and were selected for further *in vivo* studies.

Intraperitoneal administration of tsF-TGF β 1 into DA rats during the priming phase of EAE resulted in significant reduction in EAE severity. The protected animals remained asymptomatic through the duration of the experiment (80 days), whereas 5 out of 7 control rats relapsed at least once during this period. Rats treated with tsF-TGF β 1 during the acute phase of the disease exhibited a lower incidence rate of relapse and significantly reduced mean clinical score during the relapse phase comparing to control tsF-treated animals.

Rats treated with either tsF-TGF β 1 or control tsF cells were followed for more than 10 months to assess long-term side effects such as tumour formation or excessive fibrosis due to exogenous TGF β 1 expression. No tumours were observed due to proliferation of transplanted immortalized cells in any of the grafting experiments. This fact proves that the risk of malignancy caused by the immortalized cells is minimal. No signs of increased fibrosis were found in the lungs, liver or kidney of rats under study.