

# RENAL SCIENTISTS' NEWSLETTER

## *Renal Research in Cardiff*



*Wales Millennium Centre and the Senedd building in Cardiff Bay*

### *Research Focus*

This month's newsletter is the first in a series focusing on aspects of renal research in different Universities or Institutes within the UK. This issue centres on the renal research being conducted within Cardiff University and is compiled by Dr. Ceri Fielding.

*-Dr. Julie Williams*

*Newsletter Editor RSWP*

### **Introduction**

The School of Medicine is the centre for Cardiff University's renal research, predominantly within the Institute of Nephrology and Section of Nephrology (both headed by Prof. Aled Phillips) and the Department of Medicine (Head of Department: Prof. John D. Williams), but also in association with other research groups within the merged Department of Infection, Immunity and Biochemistry (formerly the Departments of Medical Microbiology and Medical Biochemistry and Immunology).



*University Hospital Wales and Heath Park*

The Institute of Nephrology is located on the University Hospital Wales (UHW) site near Heath Park, having moved here in 1999 from Cardiff Royal Infirmary (CRI). Research is carried out within the Institute by clinicians and basic scientists with active PhD and MD programs, drawing students from throughout the world. The international nature of the Institute is illustrated by its links with other institutions in China (with Nanjing University, through the ISN sister centre program), Taiwan, Japan and Ethiopia. In addition, the Institute is the focus of the Global Fluid and Peritoneal Biopsy Registry studies, relating to long-term effects of peritoneal dialysis treatment. Researchers in the Department of Infection, Immunity and Biochemistry are located mainly within the Tenovus building and the Henry Wellcome Research Institute. The Department's research is focussed on the investigation of the innate and adaptive immune system in infectious and autoimmune disease, with traditional strengths in the study of complement (Prof. Paul Morgan and Dr. Claire Harris), inflammation (Profs. Nick Topley and Simon Jones) and viral immune evasion (Prof. Gavin Wilkinson, Drs. Eddie Wang and Peter Tomasek).

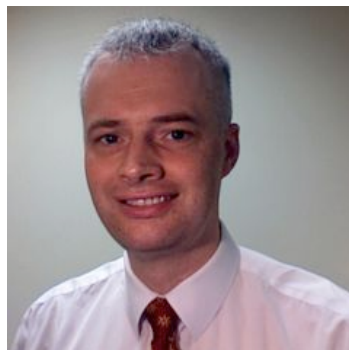
There are a broad range of renal research projects currently running in the University; from molecular studies into the regulation of transforming growth factor beta (TGF- $\beta$ ) expression (Dr. Donald Fraser and Prof. Aled Phillips) and hyaluronan synthesis (Drs. Robert Steadman, Tim Bowen, Soma Meran and Prof. Aled Phillips) to investigations of immune function in peritoneal dialysis patients (Prof. Nick Topley and Drs. Matthias Eberl and Gareth Roberts, in collaboration with Prof. John Williams) and the role of inflammation in peritoneal fibrosis (Profs. Nick Topley and John Williams and Dr. Ceri Fielding). There are also a number of on-going clinical studies with the Institute of Nephrology from trials investigating potential novel therapies and programs to improve patient outcome.

### Dr. Donald Fraser, Institute of Nephrology

As a clinical academic in The Institute of Nephrology, I share the department's long-term interest in the mechanisms underlying progressive loss of kidney function in chronic kidney disease (CKD). The key themes within my research group are the role of TGF- $\beta$  in renal fibrosis, and post-transcriptional regulation of gene expression.

TGF- $\beta$  appears central to progression of CKD. We have previously shown independent regulation of TGF- $\beta$  synthesis at the levels of transcription and translation, and ongoing work seeks to clarify the precise molecular mechanisms. Increasing evidence points to the more general importance of post-transcriptional regulation, by mechanisms including microRNAs (miRs). We have characterised miR expression in PTC, and are studying their role in progressive CKD, including expression studies in renal biopsy tissue. Our preliminary data demonstrate changes in miR expression in proximal tubular cells *in vitro* in response to TGF beta, and in renal biopsies from patients with diabetic nephropathy with histopathological characteristics of progressive disease. We are particularly excited about our renal biopsy data, as this suggests that miR analysis in routine, formalin fixed paraffin embedded renal biopsy tissue may be of widespread utility in nephrology research. A re-

cently awarded grant from Kidney Research UK (KRUK) provides us with the opportunity to perform an in depth analysis of two miRs, to determine their role in determining proximal tubular cell phenotype.



Dr. Donald Fraser

Other projects are currently investigating why differentiated epithelial cells, such as PTC, exhibit varying responses to TGF- $\beta$  depending on cellular

context. This involves

examining the mechanisms underlying modulation of TGF- $\beta$  signalling in the kidney, including the interaction between pro-inflammatory signals and TGF- $\beta$ , and the effects of cytokines, which may be reno-protective e.g. members of the Bone Morphogenetic Protein family. I am currently supported by a Welsh Office of Research Development (WORD)/Medical Research Council (MRC) Clinician Scientist Fellowship, and also receive ongoing funding from KRUK.

### Dr. Timothy Bowen, Institute of Nephrology

My research focuses on gene expression and forms part of an ongoing series of studies at the Institute of Nephrology that aims to determine the pathophysiological mechanisms underlying renal fibrosis and the progression of chronic kidney disease (CKD). Fibrosis is associated with increased cortical synthesis of the extracellular matrix glycosaminoglycan, hyaluronan (HA). Previous analyses of renal biopsy samples from diabetic nephropathy patients carried out at the Institute have shown that HA is a correlate of interstitial fibrosis *in vivo*. Our *in vitro* findings have suggested that transcriptional induction of HA synthase (HAS) gene *HAS2* and subsequent *HAS2*-driven HA synthesis may impact on fibrosis via the modulation of renal proximal tubular epithelial cell (PTC) phenotype and fibroblast-to-myofibroblast differentiation.

To understand the transcriptional mechanisms regulating *HAS* gene expression, we began by demonstrating promoter activity of the genomic DNA

sequences upstream of each *HAS* reference mRNA sequence. Further studies identified the *HAS2* transcription start site and showed that Sp1 and Sp3 mediate constitutive *HAS2* transcription, while our most recent data suggest that these ubiquitous transcription factors play a gatekeeper role in cytokine-stimulated *HAS2* induction. We have also identified transcription in PTC of *HAS2AS*, a natural antisense RNA to *HAS2*, and are evaluating its role in the post-transcriptional regulation of *HAS2* expression in these cells.

The work outlined above has been carried out in collaboration with fellow Institute members John Williams, Aled Phillips and Robert Steadman, and has been funded by Kidney Wales Foundation and The Wellcome Trust. Donald Fraser and I have recently received funding from KRUK to analyse the regulation of expression of target microRNAs (miRs) in PTC and to evaluate the role of these miRs in CKD.

**Dr. Robert Steadman, Institute of Nephrology**

Renal fibrosis is characterised by the accumulation of extracellular matrix which promotes disease progression. This extracellular matrix is laid down by myofibroblasts which differentiate from either resident or infiltrating cells. The focus of my group is the role of the extracellular matrix, particularly glycosaminoglycans, in controlling the development of this phenotype. A specific interest is the role of hyaluronan and its degradative enzymes in modulating the effect of pro-fibrotic (e.g. transforming growth factor  $\beta_1$ ) and pro-inflammatory cytokines (e.g. interleukin-1). Our aim is to identify specific changes in matrix components that can be mimicked in order to antagonise differentiation and halt or even reverse phenotypic change.

Working with Professor Aled Phillips (*Nephrology*), and with Professors Phil Stephens and Dave Thomas (*School of Dentistry*) we have identified hyaluronan synthesis as a major permissive factor promoting transforming growth factor $\beta_1$ -induced differentiation. In complimentary collaborative studies

with Dr. Marian Ludgate (*Centre for Endocrinology and Diabetes Science*), we have found that hyaluronan production is also a major factor controlling the lineage-specific differentiation of pre-adipocyte mesenchymal stem cells. Understanding the mechanism underlying the effect of hyaluronan is our current priority.

**COLLABORATORS-**

Professor Robert Stern,  
*University of California, San Francisco, USA*  
Dr Dusty Miller,  
*Fred Hutchinson Cancer Research Center, Seattle, USA*  
Professor Vince Hascall  
*Lerner Research Institute, Cleveland, Ohio, USA*  
Professor Bryan Toole,  
*University of South Carolina, Charleston, SC, USA*  
Professor Aled Phillips, *Institute of Nephrology*  
Professor Phil Stephens and Professor Dave Thomas,  
*School of Dentistry, Cardiff University*  
Professor John Gallagher and Malcolm Lyon,  
*Christie Hospital, Manchester, UK.*

**Dr. Soma Meran, Institute of Nephrology      Dr. Gareth Roberts, Institute of Nephrology**

I am a specialist registrar and a Walport Academic Clinical Lecturer in Adult Nephrology. My research to date has centred on the cell biology of wound healing and progressive fibrosis, and falls under the remit of the institutes long-term goals in delineating the mechanisms leading to progressive renal disease.

During my PhD, my studies focussed on the role of the matrix polysaccharide, hyaluronan in tissue fibrosis by assessing its synthesis, distribution and turnover in fibroblasts. Substantial work was directed towards characterisation of differences in function between scarring and non-scarring fibroblast phenotypes. In addition, my work was the first to establish a causal relationship between hyaluronan and TGF- $\beta_1$  dependent functions in fibroblasts. As a post-doctoral fellow, my work has focussed on the role of the principle HA receptor, CD44, in regulation of fibroblast phenotype and immune activation. In addition, I have recently been awarded a grant from Kidney Research UK to investigate the role of epithelial-mesenchymal crosstalk in the regulation of renal proximal tubular cell function.

I am a specialist registrar in Nephrology. My research has focussed on the effects of peritoneal dialysis treatment on the local immune response, with Profs. Nick Topley, John Williams and Dr. Matthias Eberl.

My PhD studies investigated local peritoneal T-lymphocyte responses in peritoneal dialysis (PD) patients compared with peripheral blood. The cavity of PD patients were enriched with functional effector memory T-lymphocytes capable of mounting long-term antigen recall responses. These responses were predominantly of a T-helper cell 1 (TH1) phenotype and were greater than those observed with peripheral blood. Additionally, peritoneal T-cells had shorter telomere lengths, suggesting a highly differentiated local population. In summary, we speculate that this local memory T-cell population forms part of the first line of defence against invading pathogens in PD patients. This work was carried out in collaboration with Drs. Chris Pepper (*Haematology*), Kathleen Gallagher (*Infection, Immunity and Biochemistry*) and Duncan Baird (*Pathology*) and was recently accepted for publication in the *Journal of the American Society of Nephrology*.

**Dr. Ceri Fielding, Department of Infection, Immunity and Biochemistry**

I am interested in how the body's response to infection or injury, inflammation, is altered by repeated infection and leads to the development of peritoneal fibrosis and encapsulating peritoneal sclerosis (EPS). Cytokines are soluble protein messengers, which are key regulators of normal acute inflammation and other immune processes. It is well established that they can also have detrimental consequences in inflammatory diseases. Recurrent peritonitis in peritoneal dialysis (PD) patients is a risk factor associated with the development of peritoneal fibrosis and PD treatment failure. EPS is a rare but often fatal complication of PD treatment, characterized by cocooning of the small intestine by the peritoneum. It is unclear why EPS develops but long-term PD treatment, recurrent peritonitis and pre-existing peritoneal fibrosis are implicated.

In collaboration with Profs. Nick Topley, Simon Jones and John Williams, my work has identified specific cytokines as being important factors in the

fibrosis process, including interleukin-6 (IL-6), interferon- $\gamma$  (IFN- $\gamma$ ) and the STAT1 signaling pathway. These studies were supported by a Career Development Fellowship from KRUK (2006-2009, CDF2/2006) and have identified potential targets for preventing the development of peritoneal fibrosis.

Recently grant funding was secured from the Kenyon Gilson EPS Research Fund (2009-2011), which will allow further investigation of the underlying mechanisms leading to the development of fibrosis and EPS.



*Dr. Ceri Fielding*

**Dr. Matthias Eberl, Department of Infection, Immunity and Biochemistry**

I am a RCUK Fellow in Translational Research at the Department of Infection, Immunity and Biochemistry. My team currently consists of five researchers who investigate different aspects of human V $\gamma$ 9/V $\delta$ 2 T cell responses *in vitro* and *in vivo*. V $\gamma$ 9/V $\delta$ 2 T cells represent a unique lymphocyte population in peripheral blood of humans and higher primates. These cells respond to HMB-PP, a molecule shared by the majority of bacterial pathogens, and are quickly drawn to sites of acute inflammation, where they encounter invading microbes in the context of other immune cells, mainly granulocytes and monocytes. Recent data demonstrate that peritoneal V $\gamma$ 9/V $\delta$ 2 T cell numbers increase in PD-associated peritonitis caused by HMB-PP producing species. The interaction of V $\gamma$ 9/V $\delta$ 2 T cells with monocytes will lead to attraction of further effector cells, enhanced activity of scavenger cells, and development of microbe-specific immunity. These findings provide insight into the complex cellular interactions in early infection and may also suggest novel approaches for therapeutic intervention.



*Dr. Matthias Eberl*

I have been successful in obtaining funding from the Welsh Assembly Government for my work on PD-associated infections, together with Prof. Nicholas Topley.

I have also managed to secure US\$375,000 from Baxter Healthcare's 'Renal Discoveries' programme, together with Prof. John Williams, as the sole recipients for the Europe/Middle East/Africa/Russia region in 2008.

The Baxter grant includes a three-year visiting fellowship for Dr. Chan-Yu Lin, a nephrologist from Chang Gung Memorial Hospital, Taiwan.

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