MRC Centre for Transplantation

Projects for MRes/PhD studentships

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PROJECT 1 - Dissecting the role of inflammatory peptides (C3a, C5a) in tissue injury and repair

Project Description:
Tissue repair is a dynamic process of cell proliferation and tissue regeneration following the acute injury. Impaired tissue repair results in interstitial fibrosis and inflammatory cell infiltration, which can lead to tissue destruction, chronic disease and organ failure. Understanding the factors that influence the healing process is crucial to the development of interventions that promote tissue repair. Anaphylatoxins are a group of small peptides (e.g. C3a, C5a) generated by complement activation in response to tissue stress and infection and classically involved in the process of inflammation. Our preliminary studies in mice have found that anaphylatoxins (C3a and C5a) have divergent effects on renal tissue repair following renal acute injury, whereas C3a is required for resolution of the injury, C5a leads to poor tissue repair and marked fibrosis.

The proposed study will investigate how signalling through the different receptors for C3a and C5a leads to opposite effects in the healing process, and will address the question of which cell type regulated by C3a and C5a underpins the two opposing pathways of tissue repair and fibrosis that appear to be regulated by complement (year 1 and 2). The study will also explore the therapeutic potential of targeting anaphylatoxin receptors in renal tissue repair (year 3). Understanding the influence of complement receptor signalling is expected to have transferable value in a wide area of disorders, in addition to implications for renal disease and transplantation.

Skills available will involve analytical methods to evaluate innate immune parameters in transplant and infection settings, the complement cascade, receptor signalling and cellular responses, as well as experience in cell culture and animal models of renal acute injury and genetic knockout studies. The project will also benefit from working within a larger team with an interest in innate immunity, inflammation and therapeutic protein engineering.

Centre Theme: Cytoprotective Therapy

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**PROJECT 2 - The role of collectin 11 in urinary tract infection**

**Project Description:**
The complement system is a key component of host defence against pathogens. Collectin 11 is a recently identified member of the lectin family of pattern recognition molecules (Immunobiology 217:851, 2012), and is abundantly expressed in the tissues/organs most likely to encounter foreign microorganisms including the kidney, lung and gut, suggesting that collectin 11 may play important roles in defense against microorganisms invading the urinary tract, respiratory tract, and lumen of the digestive tract (J. His. Cty. 56:243, 2008). Our preliminary studies have found that that mice deficient in collectin 11 had more severe uropathogenic E coli induced kidney infection, suggesting that collectin 11 has a protective role in urinary tract infection (UTI). This project will thoroughly investigate the roles of collectin 11 in UTI, which is an integral part of the program of complement in host defence and disease in the Centre. Using a well-established mouse model of ascending UTI leading to kidney infection and gene knockout mice, the project will confirm the protective role for collectin 11 in UTI (year 1), and examine the mechanisms by which collectin 11 protects mice from kidney infection using in vivo models and in vitro culture systems (year 2). The project will also explore the therapeutic potential of local (bladder) delivery of collectin 11 or cell membrane targeted collectin 11 for preventing UTI (year 3).

The successful student will gain molecular, genetic and cellular skills to dissect the roles of complement components involved in health and disease, which will be transferable to a number of other homeostatic and disease mechanisms. The project will also benefit from working within a larger team with an interest in innate immunity, inflammation and therapeutic protein engineering.

**Centre Theme:** Cytoprotective Therapy

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**PROJECT 3 - Role of epithelial IL-15 in kidney injury and transplant rejection**

**Project Description:**
Interleukin-15(IL-15) is a Th1 cytokine expressed by many hemapoietic cell types. However, IL-15 and its receptors are also expressed by kidney epithelial cells. Its role in the kidney is unclear: IL-15 inhibits epithelial apoptosis in cisplatin-induced nephrotoxicity, but is also known to mediate tubular interstitial fibrosis which can cause graft dysfunction. The purpose of this project is to examine the role of kidney epithelial IL-15 in kidney injury and T-cell mediated allograft rejection. The training/techniques available are shown below:

**Year 1 (MRes) and 2: Objectives: To determine expression and function of kidney epithelial IL-15 in models of kidney epithelial damage.**
Training will be provided in kidney cell-line culture. Expression of IL-15 and associated receptors on cell-lines will be determined using flow-cytometry, and PCR. In-vitro experiments will include studying effects of IL-15 in hypoxia, drug and T-cell-induced kidney cell-damage, and cell growth. If alternate IL-15 forms are discovered, these will be cloned and expressed and their in-vitro functions will be determined. In year 2, training will be provided in rodent kidney transplantation and renal damage models.

**Year 3-4: Objectives: To determine effects of IL-15 in rodent models of T-cell mediated kidney allograft rejection and kidney damage.**
Based on above findings– antagonists of IL-15 or IL-15 itself will be assessed for effects in rodent kidney damage or T-cell mediated allograft rejection models. Novel cytotopic (cell-membrane tethering) agents will also be studied. The readouts will include renal function and animal survival after transplantation, and histochemical detection of kidney cell damage and T-cell infiltration.

**Centre Theme:** Cytoprotective Therapy

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PROJECT 4 - The role of B cells in mechanisms of transplant tolerance and rejection

Project Description:
The MRC Centre for Transplantation has a strong B cell immunology portfolio and an ongoing trial ("REMIND") is randomising living-donor kidney transplant recipients to pre-transplant treatment with Rituximab (anti-CD20, a B cell depleting agent) or standard therapy. This investigation will uniquely allow the study of B cell biology in humans in the context of transplantation and will provide precious samples for addressing important questions regarding B cell biology per se.

This PhD project will investigate the hypothesis that B cell depletion significantly alters T cell alloresponsiveness, rendering subjects more tolerant to transplanted organs. The student will use samples from REMIND to extend the preliminary data and investigate the functional effects of the observed T cell changes. In particular, he/she will assess T cell responsiveness against alloantigens versus third party antigens before and after B cell depletion, determine whether a tolerant B cell phenotype is produced after reconstitution, assess the effects of Rituximab on B cell phenotypes in the bone marrow and study B cell infiltration, including formation of tertiary lymphoid organs, in allografts. In parallel, the student will be involved in establishing a "humanised" mouse model of transplantation for the specific study of B cell biology during alloresponses. This PhD will provide comprehensive training in translational medicine, transplantation immunology and B cell biology. Objectives:

Year 1 (3 month rotation): analysis of existing samples from ABOUT-K study for T cell reactivity to third party antigens

Year 2: analysis of existing REMIND samples for T cell alloresponse and characterisation of B cell reconstitution

Year 3: Development of humanized mouse model for study of B cell depletion in relation to alloresponse

Year 4: analysis of bone marrow and biopsy samples and writing up

Centre Theme: Tolerance and Cell Therapy

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PROJECT 5 - The genomics of renal transplant dysfunction

Project Description:
There is a medical imperative to make transplanted kidneys last longer than they currently do. It is known that genetic mismatching of donors and recipients in HLA genes impact graft survival times, and it is presumed that, like other complex traits, a large number of other genetic factors are waiting to be discovered which will both improve our understanding of renal transplant dysfunction and may offer improved donor-recipient matching protocols. Until now, progress has been hampered by small sample sizes and the limited numbers of candidate genes investigated. To address these problems, the UK & Ireland Renal Transplant Consortium (www.UKIRTC.org) has pooled the resources of all the major renal transplant centres in the UK and Ireland and, with collaboration and funding from the Wellcome Trust Case-Control Consortium 3, has genome-wide genotyped ~2,500 (cadaveric) donor-recipient pairs and obtained graft dysfunction phenotypes including graft survival times and acute rejection in collaboration with the NHS Blood and Transplant service. This consortium is on-going and is seeking funding for additional phenotyping and genetics.

This project will continue the analysis of this large and important dataset. Unanswered questions include: (1) the role of HLA mismatching in genes other than the 3 currently recognised in mismatch scoring (HLA-A, -B and -DR); (2) the role of copy number variants in renal transplant dysfunction; (3) analysis of longitudinal creatinine data (creatinine is a biomarker widely used to gauge overall graft health); (4) analysis of additional phenotypes such as the effect of different post-transplant drug regimens.

This is a statistical genetics analysis project, and an aptitude in statistics and/or computer programming is required. The successful application will benefit from the excellent training and support provided by the KCL Statistical Genetics Unit (www.kcl.ac.uk/medicine/research/divisions/gmm/Departments/clusters/statisticalgenetics.aspx), one of the largest such groupings in the country. The Unit organises weekly seminars, journal clubs and research meetings, as well as convening the South of England Genetic Epidemiology Group (http://smarturl.it/seseg). Training in renal transplant biology and medicine will be provided by the second and third supervisors on this project, enabled by the supportive umbrella of the MRC Centre for Transplantation.

Year 1 of PhD: HLA Genetics and its role in renal transplant dysfunction.

Year 2 of PhD: CNV and mismatch analysis. Novel phenotypes.

Year 3 of PhD: Writing papers and thesis.

Centre Theme: Immune monitoring
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PROJECT 6 - Understanding transplantation tolerance: clinical use of New Generation Sequencing

Project Description:
The general aim of this project is to further understand the mechanisms underlying transplantation tolerance operating in kidney transplant recipients.

In the context of a study that aimed at discovering biomarkers of transplantation tolerance, we have previously described that tolerant kidney transplant recipients have an expansion of B cells in peripheral blood (Sagoo, et al; JCI 2010), and differential expression of a set of genes, a number of which are mainly found in B lymphocytes. When a cross-validation was performed on these set of biomarkers and validated in a similar cohort from the USA (Newell, et al; JCI 2010), we found that the expression of TLR5, PNOC and SH2D1B as a combination would give the least errors on continuous replication of the analysis.

We are currently conducting a study that is validating the described set of biomarkers of tolerance (The GAMBIT study). For this we have recruited an independent set operationally tolerant kidney transplant recipients from the UK, Spain and Sweden (n=14). As control groups we have recruited age and gender matched healthy controls (n=12); and a set of kidney transplant recipients: age, gender and time post-transplantation matched stable patients (n=12), patients on low dose monotherapy (n=13) and patients with chronic rejection (n=12). Preliminary data from this study, indicate that the expression in peripheral blood of the same 3 genes above indicated by RT-PCR has high sensitivity and specificity to identify the Tolerant recipients. Their role in tolerance and activation pathways are still largely unknown.

We would like to test the hypothesis that the expression of these 3 genes is central to the process of transplantation tolerance.

We have peripheral blood collected in Tempus tubes and frozen cells available from all of the patients included in the GAMBIT study and a large cohort of stable and patients with CR.

Data is available for RNA expression (Agilent Whole Human Genome Microarray (8x60K format)) on isolated PBMC, Monocytes and B cells from 5 patients from each of the above groups (except monotherapy). These arrays have already been performed by Miltenyi, Cologne. The student will perform Pathway analysis of the 3 mentioned genes in the arrays. Additional in vitro assays will indicate relevant proteins or nuclear factors that may explain their differential expression in tolerant recipients.

We propose to perform next generation RNA sequencing (NGS) in PBMC from 6 individuals of each of the following groups: tolerant recipients, healthy controls, stable patients and patients on chronic rejection. This technique allows a global survey of the usage of genes and their alternative splice sites. Student will prepare the samples and NGS will be performed in the Genomic Centre of the NIH-BRC at Guy’s Hospital. Initial analysis will be initiated. Dr Irene Rebollo-Mesa can help supervise. Funding has been submitted for this part of the study to ROTRF.
MRES: Pathway analysis in the mRNA expression arrays.

Year 1 of PhD: Laboratory training, experimental design, sample preparation and doing NGS assays

Year 2 of PhD: Bioinformatics training, bioinformatics processing and statistical analysis of sequencing data. Mechanistic studies will be initiated.

Year 3 of PhD: Mechanistic laboratory studies based on results of selected candidates in the previous years.

Centre Theme: Immune monitoring

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PROJECT 7 - Defining molecular and cellular signatures of clinical utility in kidney transplant recipients

Project Description:
Transplantation is the optimal treatment for patients with kidney failure and patients thus require immunosuppression for life. The intensity of immunosuppression each patient requires is empirically defined and currently not well tailored to the patient needs. Furthermore these drugs have unwanted side effects. The end result is an unsatisfactory long term graft survival that has seen little improvement in the last 10 years.

The use of biomarkers that improve guidance to clinicians as to whether a patient is at higher risk of Acute Rejection (AR) or alternatively he/she is developing donor-specific tolerance, would allow us to personalise post-transplant care and prolong the life of a transplanted organs.

The discovery of biomarkers in certain clinical situations allows for improvement in our knowledge of novel pathways of immune injury.

Preliminary data from flow cytometry studies allows the identification of a specific subset of T lymphocyte overrepresented in AR samples.

AIMS:
The study will have time dependent focus:
(i) validation of biomarkers detected in the mentioned studies of AR
(ii) the identification of novel biomarkers that allow early prediction of AR may reveal novel pathways of immune injury
(iii) a specific transcriptional signature will be detected in lymphocyte subsets that are significantly over or under-represented in AR.

We will exploit a sample collection already available in the department from studies currently running. The KALIBRE study is a prospective study, currently running, where we are collecting blood and urine samples for up to 30 timepoints during the first year post-transplantation. Currently 280 recipients have been recruited.

Deep immunophenotyping of lymphocyte subsets using novel 10-colour flow cytometry panels. Isolation of relevant subsets of lymphocytes from peripheral blood and biopsies (this needs to be developed in collaboration with Miltenyi) will be undertaken. mRNA and microRNA expression profiling of specific lymphocyte subsets obtained from peripheral blood, urine sediment and biopsy samples. The transcriptonal regulation of the expression of relevant genes in AR will be assessed in different subsets of T lymphocytes.

MRES: Training in 10 colour flow cytometry. Analysis of banked samples.
In Year 1: the team will continue the prospective collection of blood/urine samples from up to 400 kidney transplant recipients in the first year post-transplantation. The fellow will master all the knowledge and skill needed for biomarker development programme: sample processing and storage, lymphocyte preparation and culture, tracking of clinical samples, clinical and research database development to support translational studies. The focus will
be in deep immune phenotyping of lymphocyte subsets using multiparametric flow cytometry and combining flow cytometry and mass-spectrometry, and 2) mRNA and microRNA expression profiling of peripheral blood cells. An industry stay in Becton Dickinson for development of multiparametric flow cytometry combined with mRNA expression and data analysis will be included.

**In Year 2:** The team will have validated molecular and cellular signatures associated to acute rejection in the early post-transplant period. The fellow will investigate mechanistic alterations underlying the expression of specific genes or proteins related to immune activation of relevance in transplant rejection or worse survival. A short stay in Miltenyi Biotec for development of cell separation methods from tissue samples will be included.

**In Year 3:** The group will have evaluated the performance of the acute rejection signature. The fellow will further expand the mechanistic studies to focus on a novel immune activation pathway of relevance in AR.

**Centre Theme:** Immune monitoring

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PROJECT 8 - Effects of acute kidney injury on immune function

Project Description:
Background: Acute kidney injury (AKI) affects 30-65% of critically ill patients and is associated with significant morbidity and mortality which has been partially attributed to the effects on other organs. Animal research suggests that systemic inflammatory mediators, leukocyte trafficking, cytokine-chemokine expression and apoptotic dysregulation as well as haemodynamic and neurohumoral alterations are involved in this organ-crosstalk.

Hypothesis: An overriding pro-inflammatory immune response underlies AKI in humans and pharmacological modification of these pathways will result in benefits for patients.
Objective: Evaluation of immune function in hospitalised patients with AKI

Skills training:
2. Immuno-phenotypic characterisation and functional assays of leukocyte subsets using multiparameter flow cytometry at the BRC core facility and standard tissue culture techniques.
3. Measurement of miRNA levels using arrays in a set of patient samples followed by validation measurements in an independent cohort (by RT-PCR) and correlation with clinical data
4. Full involvement in all aspects of translational research (transforming information from basic laboratory data to informed clinical decisions, critical analysis of data in the context of clinical utility, ethical considerations for research, statistics, etc)

Objectives:
MRES: Training in all laboratory techniques needed, start sample collection process. Analysis of the currently available miRNA data.

Year 1: Continue sample collection process. Analysis of early data on flow cytometry, ELISA, RT-PCR and tissue culture techniques. Identification of targets for mechanistic studies.

Year 2: Mechanistic analysis on a limited set of identified targets

Year 3: Completion of mechanistic analysis in at least a subset of lymphocytes and one or more molecular pathways of lymphocyte activation.

Centre Theme: Immune monitoring
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PROJECT 9 - Defining sites of regulatory T cell trafficking and suppression in models of human transplantation using in vivo imaging

Project Description:

Highly suppressive CD4+CD25hiFoxP3+ “regulatory” T cells (Tregs) are critical to the prevention of autoimmune diseases (AID), with defects in these cells evident in AID of mammals. Human Tregs are ideal candidates for cell-based clinical therapy to treat AID and prevent rejection of transplanted organs, especially as they can now be expanded ex vivo for specified antigen specificities. Important, unresolved, questions include the longevity of Tregs in the body once injected in vivo, their sites of trafficking in relation to sites of antigen expression and the main target cells of suppression in vivo. In this PhD, the student will inject human Tregs of defined antigen-specificity with engineered cell “tracking” markers into pre-clinical “humanized” murine models of transplantation (immunodeficient mice reconstituted with human immune cells followed by transplantation of allogeneic human skin). Fate and function of injected Tregs will be assessed by in vivo imaging techniques, including PET/SPECT/MRI and correlated with clinical outcomes (transplant survival and tissue histology). Specific objectives and time-frames will be:

<table>
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<th>Year 1</th>
<th>Optimise labelling of human Tregs for PET/SPECT/MRI</th>
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<tr>
<td>Year 1/2</td>
<td>Visualise Tregs with different specificities in transplant models</td>
</tr>
<tr>
<td>Year 3</td>
<td>Dissect target cell(s) of Tregs at sites identified above by intravital microscopy</td>
</tr>
</tbody>
</table>

These experiments will be critical for Treg cell-based therapy in human transplantation by determining the importance of antigen specificity to Treg function and trafficking. The student will receive training in cutting-edge animal models, Treg biology and in vivo imaging techniques. All models, reagents and expertise for successful completion of this project are already in place.

Centre Theme: Tolerance and Cell Therapy

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PROJECT 10 - Dissecting the mechanisms of Treg suppression to facilitate the optimization of these cells in the clinic

Project Description:

CD4+CD25+FoxP3+ regulatory T cells (Tregs) control immune responses and maintain immunological tolerance. A greater understanding of how these cells function will help define the protocol for Treg based cellular therapy for clinical use. Recently, we observed that Tregs control immune responses via the production of exosomes, which are small, secreted membrane vesicles of endocytic origin. Although we identified CD73 as a mediator of exosome induced suppression other mechanisms by which these structures function may exist. Several regulatory molecules have been associated with exosomes derived from CD4+ T cells, including miRNAs. Transferred miRNAs, within T cell-derived exosomes, are acquired by APCs following T cell antigen recognition and are functional within these cells, suggesting that T cell-derived exosomes can alter immune responses of the target cells. It is feasible that a similar mechanism contributes to the suppressive nature of Treg-derived exosomes and this proposal aims to address this point. We have performed a miRNA array and identified several highly expressed miRNAs in our Tregs lines. Specific objectives and time-frames will be:

<table>
<thead>
<tr>
<th>Year 1</th>
<th>Optimise protocols to profile miRNA/RNA expression within the exosomes produced by Tregs and CD4+ T cells.</th>
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<tbody>
<tr>
<td>Year 1/2</td>
<td>Investigate transfer of miRNA to different target cells (e.g. APCs) and studying the consequences of this transfer using reporter genes and in vitro assays.</td>
</tr>
<tr>
<td>Year 3</td>
<td>Identify whether exosomes play an important role in Treg function in vivo, by quantitation of Treg specific exosomes produced during transplantation using Nanosight and visualisation of these structures using PET/SPECT and intravital imaging.</td>
</tr>
</tbody>
</table>

These experiments will be critical for Treg cell-based therapy in human transplantation. The student will receive training in cutting-edge animal models, Treg biology and in vivo imaging techniques. All models, reagents and expertise for successful completion of this project are already in place.

Centre Theme: Tolerance and Cell Therapy

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