MRC Centre for Transplantation

Projects for MRes/PhD studentships
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PROJECT – 1: Collectin 11 at the front line of the tissue response to stress

Project Description:
The complement system - one of the oldest systems of host defence - has generated new interest as a target for the prevention of tissue injury in common disorders of the heart, brain, eye, joints and transplanted organs (Science 337;1037, 2012). While much is known about the effector mechanisms initiated by complement activation, it is a puzzle as to what mechanism triggers complement activation after simple injury following ischaemic stress (Nature Rev Immunol 12; 431, 2012). Identification of the mechanism upstream of the release of complement effectors is of huge importance, given the possibility for prevention of tissue injury and for understanding the molecular changes in stressed tissue that start off the process of injury. Collectin 11 is a recently identified member of the lectin family of pattern recognition molecules, and is selectively expressed under adverse conditions in renal tissue, with the potential to engage with other complement components in a pathway of tissue destruction that compromises a large number of kidney transplants and has a profound effect on the life of the graft. This project will investigate the role of collectin 11 as a trigger for ischaemia-induced metabolic stress in transplanted kidney (year 1). Using gene knockout models, the project will delineate the interaction of collectin 11 with other lectin molecules including the pivotal serine protease MASP2 (PNAS 108:7523, 2011) (year 2). It will also examine the physiological and pathogenic factors that regulate collectin 11 expression in renal tubular epithelial cells, which lie at the interface between the internal and external environment (years 3).

The successful student will gain molecular, genetic and cellular skills to dissect the hierarchy of complement components involved in health and disease, which will be transferable to a number of other homeostatic and disease mechanisms. They will be part of a team working in the area of complement biology, immune regulation and protein therapeutics.

Centre Theme: Early Inflammation

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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 ongoing 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/segeg). 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 – 3: 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 transcriptional 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 – 4: Statistical Methods for Biomarker-based Prediction Models in Transplantation

Project Description:
The overall aim of this project is to develop statistical methods for the use of biomarker data in the prediction of transplantation outcomes. Through a combination of simulation and empirical data, the project will rationalise the design of an optimal ‘pipeline’ for translating biomarker data into a predictive model.

Technologies such as microarrays and new generation sequencing (NGS) now allow the collection of large highly dimensional datasets in a standardized fashion. These offer new opportunities for the discovery of biomarkers for diagnosis, screening, early stratification, prognosis, and prediction of response to treatment. But technology alone is not enough – efficient and proper statistical methods are also required to inform study design and analysis strategies. Multiple analysis options are available, and there is no single ‘best’ set of procedures to be followed.

This PhD project will provide empirical evidence to support a rational biomarker-led prediction pipeline for discovery and validation. We will use existing pilot data to derive empirical distributions and correlation structures of different types of biomarker data. We will simulate based on these, test alternative analysis strategies, and finally test selected methods on real data-sets from one of the Centre’s biomarker studies. The GAMBIT study is designed to discover markers to identify patients who have developed operational tolerance to their transplanted graft. With this purpose, we have recruited over 250 kidney transplant recipients, among which 14 are tolerant. Extensive data is available on gene-expression (PCR and microarray) at different time-points and from different tissues; flow cytometry, proteomics and NGS data are also being processed. Information on clinical characteristics of the patients is broadly available.

Specifically, this project will compare the performance of different methods for:

1) Feature Selection: Univariate preselection vs multivariate selection when different types of data are to be combined (e.g. clinical, mRNA and miRNA expression and flow cytometry). Conditions: presence and absence of missing data.

2) Internal Validation: X-fold vs leave-D-out cross-validation. Conditions: different sample sizes, and case to control ratio.


5) External validation: When the performance of a prediction model is not strictly replicated in an external sample, a number of steps can be followed to update the model to the new setting, i.e. re-calibration of model intercept, model revision, and or model extension. Conditions: resemblance of training and test sets.
This project will allow us to make the best use of the large amounts of data being generated by the MRC Centre for Transplantation, and to make the right decisions to aid a rapid translation of findings into clinical practice.

**MRES:** Statistics training. Basic descriptive analysis of clinical and genomic data sets of the GAMBIT study.

**Year 1 of PhD:** Bioinformatics training. Simulation of clinical and genomic data-sets resembling those of GAMBIT study. Methods comparisons 1 and 2.

**Year 2 of PhD:** Methods comparisons 3, 4 and 5.

**Year 3 of PhD:** Testing of selected methods on the GAMBIT data sets; write-up research for publication and thesis.

**Centre Theme:** Forecasting Impact

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PROJECT –5: Cell therapy: Have we found the key to survival after Heart Transplantation in Children

Project Description:
Coronary Allograft Vasculopathy (CAV) is the leading cause of late death or graft loss after heart transplantation in children. It is a progressive condition, caused by chronic autoimmune-mediated damage. Regulatory T-cells (Tregs) have recently been shown to prevent CAV in animal models. This has resulted in human trials, studying the development of immune tolerance using polyclonal Tregs (www.onestudy.org). The overall aim of this project is to establish techniques for Treg cell generation and for in vivo imaging to monitor inflammatory response regulation. This is a well supported project supervised by world leaders in imaging (Prof Botnar), transplant immunology (Prof Lombardi) and paediatric cardiology (Dr Hussain (KCL/GSTT) & Dr Burch (GOSH)).

Yr 1: (Techniques – tissue handling, cell culture, cell isolation and expansion). Aim to generate polyclonal Tregs from paediatric thymus. Study their expansion. Study phenotype, function and maintenance of stability.

YR 2: (Techniques – transplant models, SPECT, magnetic resonance imaging, molecular imaging). Aim to generate & trial graft-specific Tregs using a transplant humanised mouse model. In vivo cell tracking by SPECT. This will help to visualise in vivo modus operandi of Tregs. Molecular MRI imaging to detect outcome in transplant mouse models after graft-specific Tregs administration. Inflammatory monitoring using 19F labelled nanoparticles and specific molecular-binding contrast agents to determine outcome in vivo.

YR 3: (Techniques – Histology, transplant models, SPECT, MRI, molecular imaging, clinical trials). Histological validation of SPECT, MRI and molecular imaging techniques. Write-up of findings. Determine implications on generation, delivery and monitoring of Tregs for refining on-going clinical trials.

Centre Theme: Investigating the inflammatory and immune response by external imaging

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PROJECT –6: The role of microRNA-142 in modulating the alloresponse to implanted organs by expression in B cells

Project Description: Overview
MicroRNAs have emerged as critical regulators of a wide range of biological processes including development and function, and of these microRNA-142 (miR-142) is amongst the most highly expressed miRNA in B cells. B cells have been strongly implicated in transplant rejection.

Work leading up to this proposal
We have established a mouse model for conditional deletion of mir-142 in B cells. Interestingly, these mice display lower numbers of transitional 2 B cells (T2), which have recently been ascribed a regulatory role through their production of IL-10. We have shown T2 B cells to be increased in patients with long-term kidney allograft tolerance. In comparison, chronic rejection of kidney transplants is characterised by low levels of T2 B cells and concomitant low levels of IL-10 production. Importantly, miR-142 has been shown to be overexpressed in the peripheral blood of tolerant renal transplant patients.

This project aims to establish the role of mir-142 in peripheral B cell subsets in the modulation of alloresponses to implanted organs. Using a combination of state of the art genetically modified pre-clinical models of transplantation, we will dissect the contribution of this microRNA in transplant rejection and tolerance. We will also examine the expression of miR-142 in different subsets of B cells from tolerant transplant recipients.

Year 1: Establish transplant models
Year 2: experiments in conditional knockout mice
Year 3: detailed analysis of mechanistic pathways in humans and mice

Centre Theme: Immune modulation

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PROJECT –7: Characterisation of phenotype and function of human intestinal NKG2D positive T cells and the consequences of therapeutic NKG2D blockade in transplantation on intestinal mucosal homeostasis

Project Description:
Our laboratory has determined that NKG2D stress-surveillance regulates the immunogenicity of skin grafts across MHC mismatches (1). Thus, an NKG2D-blocking antibody showed synergy with sub-optimal concentrations of rapamycin, mandating further investigation into its therapeutic application (1). Human T cell expression of NKG2D is highest on intestinal intraepithelial T cells, however they remain poorly characterised. This study will provide the first state-of-the-art characterisation of these cells, and define the response modes to NKG2D activation that could be compromised by clinical NKG2D-targetting.

Year 1. The cellular and molecular phenotypes of NKG2D(+) duodenal, ileal, and colonic T cells will be defined. Simultaneous gastroscopy and colonoscopy is commonly performed when investigating anaemia, providing healthy tissue from controls, under Dr Irving’s supervision on the Guy’s site. A novel culture system for human skin T cells (2) permits outgrowth of large numbers of tissue-associated T cells, facilitating de novo phenotyping. By novel application of this system to human gut obtained at endoscopy, this Aim can provide a definitive reference characterisation of gastrointestinal T cells.

Year 2. How do T cells respond to NKG2D activation, with or without cytokines and T cell receptor activation? This Aim will demonstrate the potential functional compromise of inhibiting NKG2D on intestinal mucosal homeostasis.

Year 3. Mouse models will be established in which transplant rejection is inhibited by NKG2D blockade while coincidentally challenging the gut with infections or toxins pertaining to the functions of NKG2D+ T cells identified previously. This Aim will seek to demonstrate a therapeutic window for a novel immunosuppressant. (1) Sofra et al, in preparation (2) Woolf, Kyle, Hayday - in preparation

Centre Theme: Overcoming early inflammation after transplantation using cell protective therapy

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PROJECT –8: Mesenchymal stem cell modulation of immune responses during tissue repair

Project Description:
Mesenchymal stem cells, (MSCs), occur in most organs where they provide a quiescent reservoir of cells that can be mobilized following tissue damage to differentiate into specialized cells to facilitate repair. When MSCs are injected systemically they are capable of modulating immune responses and are increasingly being used as a cellular immunotherapy for the treatment of graft v host disease (GvHD) and autoimmune conditions such as Crohn’s disease. Our In vitro studies implicate tryptophan depletion via the action of the intracellular enzyme indoleamine 2,3-dioxygenase (IDO) as the primary mechanism of T-cell immunomodulation by MSCs. Significantly however, on the basis of in vivo studies using the humanized mouse model of GvHD, we believe that following systemic administration the direct interaction of IDO+ MSC with pathogenic T-cells does not account for the observed therapeutic effect in vivo. These findings pose two fundamental questions regarding the immunological function of MSC. Namely, what is the in vivo mechanism of immunomodulation by MSC in a therapeutic setting, i.e. following systemic administration? Secondly, what is the relevance of endogenous IDO expression in the normal biological function of MSC?

Objectives:
i) Year 1. Investigate the therapeutic immunomodulatory function of MSC in the humanized mouse model of skin transplantation
ii) Year 1/2. Develop models of MSC-specific IDO deletion in using bone marrow chimerism in IDO deficient mice.
iii) Year 3. Investigate the importance of IDO expressed by MSC in vivo using skin transplant and skin wound healing models.

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