EPSRC Centre for Doctoral Training (CDT) in Molecular Sciences for Medicine (MoSMed)

Improving the outcome of organ transplantation by analysis of chemokine modifications in ischaemia reperfusion injury

Translational and Clinical Research Institute, Faculty of Medical Sciences, Newcastle University

Supervisory Team

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Project overview/context

Ischaemia reperfusion injury (IRI) is associated with serious clinical manifestations including myocardial infarction, stroke and transplant rejection. Oxidative stress associated with IRI induces expression of inflammatory chemokines as well as their posttranslational modifications. This affects chemokine interactions with G protein-coupled receptors and glycosaminoglycans.

We will characterise post-translational modifications on donor organ derived chemokines and optimize methods to quantify modified chemokines. We will examine effects of these modifications on chemokine-glycosaminoglycan and G protein-coupled receptor interactions and relate these molecular interactions to chemokine function in vitro and in vivo including in IRI models. This will allow to explore novel strategies to reduce IRI-induced inflammation.

Research Project

Ischemia reperfusion injury (IRI) is a critical medical condition and major therapeutic challenge. IRI is unavoidable after organ transplantation and its severity significantly impacts short and long-term outcomes. Due to shortage of donors there is an urgent, unmet clinical need to prolong organ survival after transplantation, and to increase the transplants performed with marginal organs. However, the use of marginal organs is associated with significant IRI, delayed graft function and poor outcomes. Furthermore, informed use of marginal organs is severely hampered by a lack of validated tests to accurately assess organ damage before and after transplantation.

Initial IRI causes production of reactive oxygen and nitrogen species, and subsequent recruitment and activation of inflammatory cells. Chemokines are key components in this complex network. Stress-induced proteomic changes following transplantation have been previously described, and although most chemokines have been identified, post-translational modifications (PTMs) affecting their activity are still being discovered. PTMs alter chemokine function, both enhancing or abrogating their potency or altering their receptor specificity. Although the chemokine system is a major focus of drug development, due to complexities, few therapies have entered clinical use.

Hypothesis: “Unravelling the molecular and biomedical *mechanisms of modified chemokine – Glycosaminoglycan–GPCR interactions in an interdisciplinary approach will enable us to understand*

the disease in sufficient detail to develop better-targeted therapies"

Aims & Experimental approach:

1. Identify PTMs of chemokines and chemokine-modifying enzymes during IRI (JM): Proteins will be purified through a combination of affinity, ion exchange or reversed phase chromatography. The presence of chemokines in the eluted fractions will be determined by ELISA, and purified modified proteins will be biochemically characterised by mass spectrometry in combination with Edman degradation. We will access patient tissue/blood/plasma/serum/urine and ex vivo organ perfusate already collected through the Newcastle Institute of Transplantation's BioBank.

2. Examine the effects of posttranslational modification on chemokine activity in vitro and in vivo. Recombinantly produced (EP) modified chemokines will be purified to homogeneity for in vitro and in vivo testing.
    a. Study their interaction with GAG, chemokine receptors and atypical receptors.
    b. Examine their ability and specificity to recruit leukocytes in vitro.
    c. Evaluate the activity of modified chemokines in signal transduction experiments. This will include measurement of intracellular calcium, phosphorylation of second messengers. This will allow to identify modified chemokines with biased signalling properties through specific receptors.
    d. Examine in vivo: air pouch and IRI model (heart, Kidney).
    e. In vivo imaging, characterization and quantification of leukocyte migration in response to PTM chemokines by intravital confocal microscopy.

3. Biophysical and structural characterisation of modified chemokines with a focus on GAG binding. This will unravel the molecular and structural basis of PTMs by a combination of biophysical experimental techniques and computational methods.

4. Use of chemokine-derived peptides as inhibitors of IRI. Production and optimisation of chemokine-based peptide inhibitors (lead molecule is available that inhibits IRI).

Training & Skills

We have designed our proposal around innovative training programme, which, in addition to providing comprehensive training, will also allow unique interdisciplinary (Mass Spectrometry (Teesside) protein crystallography (Durham) and intersectoral (private sector) experience.
The combined expertise of our multidisciplinary team of Biologists, Chemists and Biophysicist will make major advances in the understanding of the role of post-translational modification of chemokines, bringing us to a point of developing new approaches to ameliorate IRI.
The student will get training in advanced research skills particularly in areas of unmet national need e.g. interdisciplinary training in translational medicine.

Further Information

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How to Apply

You must apply through the University's online postgraduate application system

You will need to:

- Insert the **programme code 8207F** in the programme of study section
- Select '**PhD in Molecular Sciences'** as the programme of study
- Input (only) the **studentship reference code (e.g. 21_08)** that you are applying for in the studentship/partnership reference field when prompted (all codes are outlined in the individual project adverts found on the MoSMed website: https://research.ncl.ac.uk/mosmed/phdstudentships/).

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- Attach all documents that are requested including a CV and cover letter. The cover letter must **clearly** state the project reference code, the full title of the studentship and set out how your interests and experience relate to the project
- Attach degree transcripts and certificates and, if English is not your first language, a copy of your English language qualifications
- Email: mosmed.cdt@ncl.ac.uk once you have submitted your application to confirm the project you have applied for. Please include the studentship reference code and full project title.

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