BSc in Reproductive & Developmental Sciences &

BSc in Surgery and Anaesthesia Project Outline 2011-2012

**Project Title: The role of cell-derived microvesicles as endogenous mediators of acute lung injury during trauma and sepsis.**

**Academic Supervisor: Dr Kieran O’Dea**

**Division: Surgery and Cancer**

**Section: APMIC**

**Co-supervisor: Professor Masao Takata**

**Who will be responsible for day-to-day supervision? Dr Kieran O’Dea**

**Contact Details of Person whom Medical Student should contact for further details:**

**Name: Kieran O’Dea Email:** **k.odea@imperial.ac.uk** **Tel: 020 3315 8292**

**Group’s Research Interest:**

**Our primary interest is in the mechanism and organ injury in the critical care setting. We use a range of animal and in vitro cell culture models to dissect out inflammatory response to infection- and trauma-related stress stimuli. Our particular focus is on cell-cell interactions in inflammation and the critical role that the response environment plays in the development of acute lung injury.**

(Double click the appropriate check box to indicate your choices below)

**Is this a clinical [ ]  or laboratory [x]  project?**

**Suitable project for: Reproductive and Development**  **Sciences** Yes **[ ]** No **[x]**

**Surgery and Anaesthesia** Yes **[x]** No **[ ]**

**Synopsis of project (background/research question/methods to be used/relevant key references):**

**Background:**

The systemic inflammatory response syndrome (SIRS) in sepsis results from activation of the innate immune system in response to microbial infection (1). The pathophysiology of SIRS in the absence of infection (e.g. trauma, burns) is comparable to sepsis and therefore may be analogous to the innate system response to infection. Indeed, various molecules released from stressed or damaged cells appear capable of activating the innate system via the same receptor-signalling pathways (e.g. Toll-like receptor system) as those activated by microbial products (2, 7). Although several candidate endogenous mediators, termed ‘alarmins’ or ‘danger associated molecular pattern’ (DAMPS), of sterile inflammation have been proposed, there remains considerable uncertainty as to their in vivo, as well as, clinical relevance (5).

Microvesicles are sub-cellular particles derived from the plasma membrane and cytoplasmic contents of cells activated by stress signals or damage (3, 4). Once considered merely as cell debris, microvesicles now appear to represent a separate arm of the inter-cellular signalling process, alongside soluble mediator and cell-cell contact mediated signalling. Microvesicles have been implicated in diverse physiological (e.g. haemostasis and coagulation) and pathophysiological (e.g. cancer, pre-eclampsia) processes . A significant role for microvesicles in inflammation and non-infectious SIRS has only recently been suggested, based in part on their ability to induce to induce pro-inflammatory cytokine production in monocytes via TLR4 signalling pathway (8). Identification of the role played by this new inter-cellular communication pathway in SIRS-related organ injury will undoubtedly have a significant impact on our understanding of both infectious and non-infectious SIRS pathophysiology.

**Scientific questions:**

1. Do microvesicles elicited by cellular stress directly induce acute lung injury in mice?

2. Do microvesicles increase sensitivity of the lungs to secondary endogenous or sepsis-related stimuli?

**Methods and techniques.**

Microvesicles will be generated from in vitro cell cultures of monocyte and endothelial cells lines using standard stress stimuli. These will include extracellular ATP, which activates purinergic signalling pathways resulting in rapid (within minutes) microvesicle release from cells. After ultra-centrifugation, microvesicles will be injected intra-venously into mice and, at different intervals, tissues including lungs and blood will be isolated for analysis. Principle analysis methods include: flow cytometric determination of lung endothelial activation, leukocyte recruitment and activation; ELISA measurement of cytokine production; determination of lung oedema by wet-dry weight ratios and protein content of broncho-alveolar lavage fluid.

All techniques for analysis of lung inflammation and injury are used routinely in the lab (for recent examples see: 6 & 9). Dr O’Dea will perform all in vivo experiments and samples will be analysed by the student. If time permits, data related to lung injury will be supplemented by histological analysis.

**References**

1. **Beutler B, and Rietschel ET**. Innate immune sensing and its roots: the story of endotoxin. Nat Rev Immunol 3: 169-176, 2003.

2. **Castellheim A, Brekke OL, Espevik T, Harboe M, and Mollnes TE**. Innate immune responses to danger signals in systemic inflammatory response syndrome and sepsis. Scand J Immunol 69: 479-491, 2009.

3. **Cocucci E, Racchetti G, and Meldolesi J**. Shedding microvesicles: artefacts no more. Trends Cell Biol 19: 43-51, 2009.

4. **Distler JH, Huber LC, Gay S, Distler O, and Pisetsky DS**. Microparticles as mediators of cellular cross-talk in inflammatory disease. Autoimmunity 39: 683-690, 2006.

5. **Erridge C**. Endogenous ligands of TLR2 and TLR4: agonists or assistants? J Leukoc Biol 87: 989-999, 2010.

6. **O'Dea KP, Wilson MR, Dokpesi JO, Wakabayashi K, Tatton L, van Rooijen N, and Takata M**. Mobilization and margination of bone marrow Gr-1high monocytes during subclinical endotoxemia predisposes the lungs toward acute injury. J Immunol 182: 1155-1166, 2009.

7. **Tang AH, Brunn GJ, Cascalho M, and Platt JL**. Pivotal advance: endogenous pathway to SIRS, sepsis, and related conditions. J Leukoc Biol 82: 282-285, 2007.

8. **Thomas LM, and Salter RD**. Activation of macrophages by P2X7-induced microvesicles from myeloid cells is mediated by phospholipids and is partially dependent on TLR4. J Immunol 185: 3740-3749, 2010.

9. **Wilson MR, O'Dea KP, Zhang D, Shearman AD, van Rooijen N, and Takata M**. Role of lung-marginated monocytes in an in vivo mouse model of ventilator-induced lung injury. Am J Respir Crit Care Med 179: 914-922, 2009.

Will the research involve work done under the Animals (Scientific Procedures) 1986 Act? Yes **[x]** No **[ ]**

**If YES*,***

Will the student be required to undergo Home Office training? Yes **[ ]** No **[x]**

Are the appropriate project and personal licences in place? Yes **[x]** No **[ ]**

**Project licence**:

Licensee **Prof M Takata**

Date of issue **28/11/2008**

Number **70/6854**

**Personal licence**:

Licensee **Dr K. O’Dea**

Number **70/17164**

**Will the research involve the use of genetically modified tissue?** Yes **[ ]** No **[x]**

**If YES**

Has the work been approved by the relevant GM Committee Yes **[ ]** No **[ ]**

Date approval was granted

Reference Number

**Will the project involve work on human subjects, human tissue or access to confidential patient information?** Yes **[ ]** No **[x]**

## If YES

## has ethical approval been obtained Yes [ ]  No [ ]

## Date approval was granted

## IC REC or IRAS REC number

**Note: Approval for any of the above MUST be in place before the student begins the project.**

**A risk assessment form will be required.**

**Project Payment**: I have an F account Yes **[x]** No **[ ]**

## If you have an F account please give full account code: F36428