RCoA Research, Education & Travel Grants 2015

Award: The Ernest Leach Fund

Applicant: Dr Christopher Hebbes

Project Title: Does the immunocyte release profile of nociceptin differ in critically ill septic patients and healthy volunteers

Project Description:

The peptide nociceptin is thought to have roles in pain tolerance, behaviour and immune modulation. This link has been strengthened by studies demonstrating the presence of RNA precursors for nociceptin (Anesth Analg. 2007;105{4):998-1005), and its receptor on immunocytes, and polymorphonuclear cells, and later the demonstration of the nociceptin receptor in mixed immunocyte populations. The expression of free plasma nociception is known to increase during the septic response (Br J Anaesth 2011; 106:566-72.) and post cardiopulmonary bypass, further adding to the suggestion that it has a role in control or modulation of the immune system. There is also evidence that critically ill septic patients with a greater plasma concentration of nociceptin had an increased mortality (PLoS One. 2013; 8(10):e76682}, suggesting a role either in priming an overwhelming septic response, or immunosuppression.

Our group has developed and validated an assay to semi-quantitatively detect single cell release of nociceptin from immunocytes using a live cell confocal fluorescence microscopy assay using a biosensor layer of Chinese Hamster Ovary (CHO) cells transfected with the Nociceptin receptor and the $G_{\alpha iqs}$ chimera, and loaded with the FLU0-4 Calcium sensitive dye. Once layered with immunocytes extracted from whole blood and separated into immune populations using density gradient, and magnetic bead separation, this enables nociceptin release (after cell degranulation) to be positively localised to individual immune cell types due to the fluorescence of the surrounding biosensor cells. This validated assay can detect concentrations of nociceptin to 10^{-9}M and is reproducible, allows direct visualisation of the releasing cells, and provides a functional link lacking in genomic studies. The ability to image and positively identify single immunocyte cell lines is a significant development over and above existing studies in mixed cell populations.

We propose to use our novel biosensor assay to determine the differences in nociceptin release from leucocyte populations in non-stressed healthy volunteers, and critically ill septic patients {10 each group), with a sample taken on the day of admission to ICU (when concentrations of plasma nociceptin are highest).

This will enable characterisation of the source for the previously identified peak in nociceptin, add to the current in-vitro knowledge of the link between the nervous and immune systems, and allow a clinical, functional link and identification of new therapeutic targets for modulation of the immune response to sepsis response.