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Development of a novel assay for single cell nociceptin/orphaninFQ (N/OFQ) release from leucocyte subtypes for use in sepsis research

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Nociceptin/OrphaninFQ is an opioid-like peptide that acts on the nociceptin receptor (NOP). There is a substantial body of evidence that supports the role of nociceptin in modulating the sepsis response.

Through development and use of a novel assay, we plan to determine the concentration of nociceptin released from stimulated immune cells and so identify the primary immunocyte involved in nociceptin release.

Individual types of immunocytes will be isolated from the whole blood of 20 volunteers. Isolation will be achieved through a combination of density gradients, flow-activated cell sorting (FACS) and magnetic-activated cell sorting (MACS). Each cell line will be layered on modified Chinese Hamster Ovary (CHO) cells expressing the nociceptin-sensitive chimeric G-protein $G\alpha_{qi5}$, enabling them to increase intracellular Ca^{2+}. Confocal microscopy of Fluo-4 (a fluorescent calcium indicator dye) loaded cells will then be used to observe increases in CHO cell Ca^{2+} (fluorescence) in response to immunocyte stimulation with lipopolysaccharide or staphylococcal enterotoxin B. We will therefore be able to indirectly measure N/OFQ release from individual cells in real time.

We intend to identify the immunocyte most associated with nociceptin release in response to a standard *in vitro* sepsis stimulus.

This information will help us better characterise the role of individual immunocyte types in the nociceptin system. Combined with other ongoing work identifying the type most associated with nociceptin receptor (NOP) expression in sepsis, we will make a substantial contribution to the understanding of N/OFQ in sepsis. This has the potential for providing a novel therapeutic target.