An unbiased proteomic approach to identifying cell surface markers for innate inflammatory cells

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Neutrophils are crucial effector cells of the immune system and during infection are actively recruited to the site of inflammation. Migration to the inflammation site coincides with a gradual process of activation and culminates in a microbial killing program involving phagocytosis and degranulation (*Summers et al., 2010*). Resolution of this inflammation is also an active process and is essential to limit auto-reactive pathological damage to the host. The failure to effectively clear neutrophils can result in chronic inflammation that is linked with severe immunopathology as seen in chronic obstructive pulmonary disease, cystic fibrosis and rheumatoid arthritis (*Cowburn et al., 2008*). Most information regarding neutrophil activation has been obtained in *in vitro* studies and little is known regarding this process *in vivo* and in patients with chronic inflammatory diseases. This project will address this limitation with a specific focus on the identification of novel neutrophil cell surface markers.

The traditional approach to identify cell surface markers has been based on either a 'best guess' or 'transcriptional profiling systems' approach, which does not necessarily reveal which proteins are expressed at the cell surface, nor their abundance. The EBPOD fellow will make use of an entirely new functional quantitative proteomics approach developed by PJL's group and termed 'Plasma Membrane Profiling' (*Weekes et al 2013 Science; 2014 Cell*). This approach will be applied to the identification of cell surface markers of primed/activated neutrophils *in vitro* and also those purified from inflammatory sites *in vivo*, and will be directly compared with the total proteome and transcriptome (RNAseq) of these cells. This project has major relevance to our understanding of, and the potential to treat, the immune-paretic state seen in patients with systemic inflammatory response syndrome and the non-resolving hyper-immune state characteristic of many chronic inflammatory diseases.

PJL's group has developed a proteomics approach to quantify the relative protein expression of 800 - 1200 cell surface proteins, and 6 – 8000 total proteins. This approach has been successfully used to determine how cell surface proteins are deregulated during viral infection (*Weekes et al 2013 Science; 2014 Cell*). Using this method it was possible to visualise how viruses remodel the cell surface landscape of the infected cell, and to identify cell surface markers down-regulated in human cytomegalovirus (HCMV) infection. This finding was exploited by demonstrating that removal of infected cells, based on expression of the MRP1 cell surface marker, eliminates latent HCMV-infected cells, an approach that could be applied to the removal of HCMV-infected donor cells prior to infusion into bone marrow transplants. PJL has further improved this technology such that that it is now possible to multiplex (10-plex) a single mass spec run on the new instrument awarded to Lehner as part of a Wellcome Trust PRF award.

This approach, which we have shown to be effective on human neutrophils, provides a unique and totally unbiased view of cell surface receptors and how their expression changes as a consequence of infection, inflammation or indeed, malignant transformation. We plan to use this technology to determine the cell surface proteome of (i) purified human neutrophils treated *in vitro* with specific inflammatory stimuli, notably

TNFα, GM-CSF and hypoxia, and (ii) neutrophils recovered from patients with a variety of specific inflammatory disease states. For part (ii), we will build on ECs expertise in isolation of human neutrophils from alveolar airspace of patients with acute lung injury/ARDS and compare the cell surface proteome with simultaneously obtained RNAseq and whole cell proteome data.

We will focus on the TNFα and GM-CSF pathway which plays a key role in establishing the immune paretic state. This is characteristic of patients with systemic inflammatory response syndrome/acute lung injury (SIRS/ALI) and makes these patients extremely infection prone. The efficacy of TNFα and GM-CSF-receptor blockade in more chronic inflammatory diseases, most notably rheumatoid arthritis, also supports a focus on these two inflammatory pathways (*Greven DE et al., 2014*).

Under the supervision of PB the fellow will be responsible for the integration of the proteomic and genomic information. Changes in membrane protein composition can occur via multiple different mechanisms such as: changes in gene expression, protein expression and protein localization. The fellow will integrate the experimental data for gene expression, membrane and cellular protein expression upon neutrophil activation to distinguish between these different mechanisms. As previously demonstrated by PJL (*Piper RC, Lehner PJ. Trends Cell Biol 2011*) ubiquitylation plays a major role in determining receptor turn-over and localization at the membrane. The fellow will make use of human ubiquitylation data and methods developed by PB (*Beltrao P et al.,2012; Swaney DL et al.,2014*) to analyse the role of this post-translation process in changes in membrane composition during neutrophil activation. In addition, known or predicted regulatory interactions will be used to predict candidate drivers (e.g. transcription factors, kinases, E3 ligases) responsible for the observed changes. Based on the data derived from these time courses, the fellow will then analyse the inflammatory state of cells derived from control patients with those derived from patients with chronic inflammatory conditions. The differences/similarities between the in-vitro activation and patient cells will be determined. The focus of this analysis will be to determine potential cell surface markers for *in vivo* neutrophil activation to suggest potential antibody targets for therapeutic applications. In addition the fellow will also predict which drivers of the activation process are relevant *in vivo* and will therefore be targets of small-molecule inhibitors.

We note that the experimental and computational methods developed during the course of the project are immediately applicable to related and ongoing collaborations with other groups at the BRC, EMBL and Sanger. For example, these include collaborations on Plasmodium infection with the Sanger Malaria Programme, Salmonella infection with Nassos Typas (EMBL-Heidelberg) and HCMV infection with John Sinclair (BRC).

References:

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