ImmunoTools *special* Award 2013

Helen Wright, Dr., Research Fellow

Supervisor: Professor Dr. Steven Edwards

University of Liverpool
Institute of Integrative Biology
Liverpool, UK

Regulation of neutrophil gene expression in Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a type of chronic, inflammatory arthritis which affects around 1% of the UK population. The disease progresses very quickly, leaving patients with badly damaged joints. Neutrophils are a type of white blood cell that normally protect us from infections via phagocytosis and killing of bacteria. However, in inflammatory diseases such as RA, neutrophils become inappropriately activated by cytokines, chemokines and immune-complexes, and this causes chronic inflammation. The destructive molecules that neutrophils possess to kill bacteria (proteases, reactive oxygen metabolites) instead attack healthy tissues and this leads to severe damage to joints, the hallmark of uncontrolled RA.

No-one fully understands how neutrophils become activated in RA or the full clinical consequences of this activation. One of the biggest problems facing clinicians is that different patients with RA respond better to different drug therapies, but currently there is no way to tell whether one drug is better for a patient compared to another drug. Some drugs used to treat RA (called biologics) are very expensive, and can only be given to patients with very active disease who already have badly damaged joints. This means that new patients with RA who will eventually need these drugs cannot be given them early in their disease when they may be most beneficial. In addition, some RA patients do not respond to several different drugs and often have to change therapies many times, and this means that their joints become more and more damaged.

One possible explanation for the variation in response to drugs may be that there are different ways to switch neutrophils on in RA. For example, the inflammatory milieu within the synovial joint can vary dramatically between patients, showing elevated levels of a number of different cytokines and chemokines (TNFα, IL-1β, IL-6, IL-8, G-CSF, GM-CSF). Alternatively, it is possible that genes of different patients dictate how effectively they respond to different types of treatments.

My research has shown that healthy neutrophils stimulated *in vitro* with recombinant cytokines can behave in the same way as neutrophils from RA patients. We are using RNA sequencing to fully understand the transcriptional consequences of activation by different cytokines and chemokines, and will then applying these data to compile a computational model of neutrophil gene expression in RA.

My research is trying to answer a number of questions:-

1) How are neutrophils switched on in RA, and which genes become 'activated'?

2) How are these genes switched off during successful treatment?

3) Are the same genes switched off when patients are successfully treated with different types of drugs?

4) Is it possible to identify genes that will allow clinicians to predict which patients are likely to respond to different types of RA drugs?

An ImmunoTools *special* award would allow me to expand the number of agonist treatments (rh IL-2, rh IL-4, rh IL-10, rh IL-12, rh IL-15, rh IP-10 /CXCL10, rh MIP-1α/ CCL3, rh TGF-beta3, rh TRAIL / CD253, rh VEGF-A/VEGF-165, rh EGF, rh FGF-a / FGF-1, rh FGF-b / FGF-2, rh HGF) that I am using in my transcriptomic experiments with healthy neutrophils, enhancing my model of gene expression. It would additionally allow me to carry out functional studies on cytokine-treated neutrophils, including measures of apoptosis (Annexin V-PE), respiratory burst, chemotaxis, degranulation (CD63-FITC) up-regulation of adhesion molecule and surface receptor expression (CD11a- PE, CD11b-PE, CD11c-PE, CD14-PE, CD15-PE, Control-IgG-PE) and chemokine/cytokine production. This will provide further insight into the regulation of inflammatory neutrophil gene expression by cytokines and chemokines.

ImmunoTools *special* AWARD for **Helen Wright** includes 22 reagents

FITC - conjugated anti-human CD63,

PE - conjugated anti-human CD11a, CD11b, CD11c, CD14, CD15, Control-IgG1, Annexin V,

recombinant human cytokines rh IL-2, rh IL-4, rh IL-10, rh IL-12, rh IL-15, rh IP-10 /CXCL10, rh MIP-1α/ CCL3, rh TGF-beta3, rh TRAIL / CD253, rh VEGF-A/VEGF-165, rh EGF, rh FGF-a / FGF-1, rh FGF-b / FGF-2, rh HGF,

DETAILS