ImmunoTools *special* Award 2014

Joanne Hay, PhD student

Supervisor: Dr. Anne Astier

MRC Centre for Inflammation Research, C2.20, QMRI,
The University of Edinburgh, 47 Little France Crescent,
Edinburgh EH16 4TJ

The role of glycosylation on the CD46 pathway in T cells

Immune homeostasis is important for the regulation of inflammation and autoimmunity. A role of CD46, a ubiquitously expressed transmembrane protein, in immune homeostasis has recently been described. Its coligation with CD3 and consequent cleavage from the T cell surface serves as a costimulatory stimulus for human T cell activation, leading to T cell proliferation and differentiation to a regulatory Tr1 phenotype (in the presence of IL-2), characterised by secretion of high levels of anti-inflammatory IL-10 and low levels of IFN-gamma. The CD46 regulatory pathway is defective in patients with autoimmune conditions such as multiple sclerosis and rheumatoid arthritis, as IL-10 production is impaired by CD46 costimulated T cells from patients. This highlights the need to investigate the molecular mechanisms involved in the regulation of the CD46 pathway. Directly related to CD46 function is the protein structure. CD46 is a highly glycosylated protein with three N-glycosylation sites and up to 18 O-glycosylation sites.

Our data show that T cell activation changes the glycosylation of CD46 and it is hypothesised that these changes in turn affect the CD46 pathway. Preliminary data obtained after expressing CD46 glycosylation mutants in total $CD4^+$ T cells strongly support this hypothesis. The downregulation of CD46 expression usually seen on T cells after CD3/CD46 costimulation, T cell proliferation, and cytokine production was differentially affected depending on whether O or N-mutants were expressed in $CD4^+$ T cells. The aim of my project is to further determine the effect that glycosylation of CD46 has on human T cell activation. Due to the previous work on CD46 glycosylation being done on total $CD4^+$ T cells and the fact that total $CD4^+$ T cells

include naïve, memory and regulatory T cells, it is of interest to determine if the effect of glycosylation on the different cell types is differential. For this reason $CD4^+CD45RA^+$ (naive) and $CD4^+CD45RO^+$ (memory) T cells will be isolated from the peripheral blood of healthy human donors. The cells will be transfected with GFP-tagged CD46 glycosylation mutants to allow for isolation of the transfected cells. Expression of T cell activation markers, proliferation and IL-10 and IFN-gamma cytokine secretion will be measured using flow cytometry, cell proliferation dye and ELISA/secretion assay, respectively. I will also compare the phenotypic profiles of activated T cells isolated from healthy donors and patients with MS and relate this with CD46 glycosylation status. Together, the data will highlight novel molecular mechanisms controlling human T cell activation and this may provide novel targets to design future drugs restoring the regulatory function of CD46 in MS.