ImmunoTools *special* Award 2014

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Crohn's disease associated bacteria induced immune responses in macrophages and intestinal epithelial cells

Inflammatory bowel diseases (IBD) comprising Ulcerative colitis (UC) and Crohn´s disease (CD); are chronic debilitating conditions affecting the patients' quality of life and characterized by unpredictable flares of remission and relapses. Several lines of evidence suggest that the pathogenesis of IBD is triggered by environmental factors and dysregulated local immune responses in genetic susceptible individuals.

Animal studies and metagenomic profiling of the microbiota in patients with IBD have revealed increases in Proteobacteria spps and an imbalance in the ratio of the phylum Firmicutes and Bacteroidetes. A group of Escherichia coli, so called adherent-invasive E. coli (AIEC), was identified from biopsies taken from approximately 30% of patients with CD suggesting AIEC may be involved in the pathology of a significant number of CD patients. These AIEC are distinguished from other strains of E. coli due to their unique ability to invade intestinal epithelial cells and to replicate in macrophages.

There is currently no cure for IBD, with most treatments (e.g. anti-inflammatory such anti-TNFα antibodies) primarily aiming to suppress disease severity and to maintain remission. Another cytokine, IL-1β has also been implicated in the pathology of several autoimmune diseases including IBD. Increased IL-1β levels have been reported in biopsies of CD-patients and experimental models of intestinal inflammation indicate that IL-1β may be a driver of the disease. Pattern recognition receptors (PRRs) recognise bacterial components thereby activating transcriptional factors such as NFκB, which drives the expression of TNF-α and IL-1β genes. Unlike TNF-α, IL-1β requires post-translational processing before the active cytokine is secreted. Pro-IL-1β is stored in the cytosol where is processed into a mature form by a molecular complex called the inflammasome.

Inflammasomes are a large family of multiprotein complexes playing a key role in the innate immune response against a range of different signals including bacteria. Bacteria are recognised by receptors belonging to NOD-like receptor (NLRs). Upon recognition, NLRs interact either directly or indirectly with the adaptor protein ASC and caspase-1 to promote the maturation and secretion of IL-1β and induce a type of bacterial induced cell death so called pyroptosis (which presents apoptotic and necrotic features). Recent

growing evidence has suggested that dysregulation of inflammasomes may contribute to the pathophysiology of IBD.

We have preliminary data indicating that AIEC interacts with the inflammasome in intestinal epithelial cells lines and in macrophages. Thus, the aim of this project is to characterise the immune response induced by AIEC, AIEC mutants and commensal E. coli in cells deficient in components of the inflammasome.

For this purpose we will use human intestinal epithelial cells and macrophages that are deficient in component of the inflammasome and their respective controls. The cells will be infected for with the above mentioned bacterial strains and characterised for their activation profile (CD11b, CD14, CD40, CD71, CD80, CD86), apoptosis response (CD95, Annexin V) by flow cytometry and cytokine profile (IL-6, IL-1β) by flow cytometry and ELISA at different timepoints post-infection. Cytokine cocktails will be used to mimic an activation profile associated with IBD (rh-IL-1β, rh-IL17A, rh-IL17E, rh-IFNγ, rh-TNFα).

My intended use of ImmunoTools antibodies – as stated previously we have data indicating AIEC induces the inflammasome in intestinal epithelial cells and macrophages. By using flow cytometry and ELISA, I will get an insight on how Crohn's associated bacteria regulate the inflammatory response in 2 different cell types in relation to the inflammasome.

ImmunoTools *special* AWARD for **Silvia Melgar** includes 23 reagents

FITC - conjugated anti-human CD11b, CD40, CD71, CD86, CD95, IL-6, Control-IgG1, Control-IgG2a, Control-IgG2b, Annexin V,

PE - conjugated anti-human CD80, CD95, IL-6, Control-IgG1,

APC -conjugated anti-human CD14, Control-IgG1,

recombinant human cytokines rh-IL-1β, rh-IL17A, rh-IFNγ, rh-TNFα,

human IL-6 ELISA-set (contains 3 reagents),

DETAILS