ImmunoTools *special* Award 2018

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Investigating the Heme Oxygenase system as a Therapeutic intervention for Inflammatory Bowel Disease

Inflammatory bowel disease (IBD) affects approximately 2.2 million people in Europe, and 20,000 individuals in Ireland. IBD describes two conditions; Ulcerative Colitis and Crohn's disease. Both of these conditions are characterised by chronic relapsing inflammation of the gastrointestinal tract, and patients with IBD also have increased risk of the development of colorectal cancer. While the exact cause of IBD is unknown, there is strong evidence to support the involvement of the immune system in the pathogenesis of the disease, with high levels of pro-inflammatory cytokines and T cells seen in the gut of affected individuals compared to healthy controls. As a result, many therapies for IBD have attempted to exploit this important pathogenic factor by trying to modify the over-active immune response seen. These treatments include certain immunosuppressive drugs, however they have several undesirable side effects and many patients are refractory to them. Therefore, there is an urgent need for novel, beneficial anti-inflammatory therapies, with increased long term efficacy, tolerability and less adverse side effects.

Heme Oxygenase (HO) is a stress response protein, which is the rate limiting step in heme catabolism. The inducible form of HO, HO-1, is upregulated in response to a vast array of stimuli and has been shown to have potent anti-inflammatory and anti-oxidant properties. Pharmacological inducers of HO-1 have been proposed as possible treatments for many inflammatory diseases, including IBD. However, clinical implementation of HO-1 based therapies faces numerous challenges, especially as most inducers of HO-1 which have been tested for treatment of these disorders are associated with high levels of toxicity, and despite the great efficacy they show, they are unsuitable for clinical use. For that reason, there is a solid rationale to identify safer HO-1 inducers, with less toxicity, and the same level of clinical efficacy.

The aim of my project is to assess the immunomodulatory properties of naturally derived heme oxygenase inducers first in healthy primary human innate and adaptive

immune cells, before examining their potential in IBD patient blood and biopsy samples. We will also carry out a pre-clinical in vivo murine colitis model.

The compounds in question include the plant derived polyphenols, Curcumin and Carnosol and also a novel compound secreted by the parasite, Trypanosoma Brucei. Curcumin is widely available as a health supplement, and is derived from the spice Turmeric, which is traditionally used in Asian medicine for its anti-inflammatory properties. Carnosol is derived from the herb Rosemary and has been shown to have anti-proliferative and pro-apoptotic properties and has been mostly studied as a potential anti-cancer treatment. Trypanosoma Brucei is a parasite which causes Sleeping sickness in humans. Similar to other parasites, T. Brucei uses various methods to dampen the host immune response and this, in turn, enables the parasite to persist unharmed. Therefore factors produced by parasites are being exploited as a treatment for a number inflammatory diseases and autoimmune diseases. Using western blotting, we have found that all 3 compounds of interest are strong inducers of HO-1 in primary human immune cells.

Key elements of my project involve isolation of PBMCs from human blood. $CD14^+$ monocytes will then be positively selected for, and differentiated into DC, using GM-CSF and IL-4, or Macrophages, using M-CSF. DC and Macrophages will then be pre-treated with the potential HO-1 inducers, before stimulation with TLR ligands to drive activation of the cells. After 24 hours the supernatants will be removed and examined for the levels of the pro- and anti-inflammatory cytokines (IL-6, IL-10, IL-12, TNF-alpha, IL-1, IL-23) by ELISA. The maturation status of the innate cells, particularly the DC, will then be tested by flow cytometry, by staining for the maturation markers CD40, CD80 and CD86. The viability of the cells when treated with the HO-1 inducing compounds will also be examined by flow cytometry by staining with Annexin V to determine the toxicity of the compounds on the cells. CD4 T cells will be pre-treated with the HO-1 inducing compounds before stimulation with anti-CD3 and anti-CD28 for 4 days. DC-T cell co-culture experiments will also be carried out, in which the DCs have been pre-treated with the compounds of interest. For both of these experiments cell supernatants will be examined by ELISA for levels of the cytokines IL-17A, IFN-gamma, GM-CSF and M-CSF. The ability of the compounds of interest to induce Treg cells will also be investigated by flow cytometry by staining with FoxP3.