ImmunoTools *special* Award 2015

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Investigating the mechanisms underlying induction of IL-17A by corticosteroids and pollution.

IL-17A is a pro-inflammatory cytokine that is essential for defense against mucosal pathogens. However in excess, IL-17A is associated with various autoimmune diseases and asthma. Our lab has shown that elevated IL-17A synthesis occurs in T cells isolated from severe corticosteroid-refractory asthma patients as compared to cells from steroid sensitive asthmatics. Furthermore, corticosteroids, the primary treatment for asthma, increase IL-17A synthesis *in vitro* with supporting evidence in patients where inhaled corticosteroid dose positively correlates with IL-17A synthesis (*Nanzer et al. 2013, JACI*). In contrast active vitamin D ($1,25(OH)_2D3$) inhibits IL-17A synthesis, including corticosteroid-induced IL-17A, which is likely to represent a contributory mechanism by which vitamin D helps to protect against immune-mediated diseases like severe asthma (*Nanzer et al. 2013 & 2014, JACI*).

Asthma currently affects 300 million people worldwide and is increasing in prevalence at a rate that surpasses evolution, highlighting the involvement of environmental factors. Accumulating epidemiological evidence shows a substantial role for particulate matter (PM) within air pollution in both the incidence and severity of asthma. The mechanisms that underlie this relationship are poorly understood, but PM has been reported to drive Th17 differentiation in mice (*Van Voorhis et al. 2013, PLoS One*). Moreover, in children with allergic asthma, serum levels of IL-17A were 6 times in those exposed to high levels of PM compared to those exposed to lower levels (*Brandt et al. 2013, JACI*).

Inhaled PM is sequestered by myeloid dendritic cells (mDCs) that then traffic to the lung and stimulate memory $CD4^+$ T cells. We have developed an *in vitro* model wherein mDCs are pre-treated with PM and then co-cultured with T cells prior to assessing expression of mRNA, surface markers and cytokines. Amongst other effects, PM pre-treatment of mDCs was found to enhance $CD4^+$ T cell synthesis of IL-17A (*Matthews, Mann et al., in preparation*).

Taken together, we have independently shown that corticosteroids and PM can increase $CD4^+$ T cell synthesis of IL-17A whereas $1,25(OH)_2D3$ has the opposite effect. We

hypothesise that there is an additive effect of PM and corticosteroids on expression IL-17A and other Th17-associated molecules that is detrimental in severe asthma, but that vitamin D can help protect against this.

Using our *in vitro* model we will assess the effects of PM, corticosteroids and/or $1,25(OH)_2D3$ on the mRNA and protein expression of various Th17-associated molecules. Mechanisms for any observed effects will be investigated by employing ELISAs and siRNA knockdown experiments. Of particular interest is the role of IL-23 because this cytokine can drive the differentiation of a pathogenic subset of Th17 cells (*Lee et al. 2012, Nat Immunol*).

For this project to be possible, multiple antibodies and recombinant proteins are required as outlined below:

- Checking purity of isolated human mDCs and memory $CD4^+$ T cells – CD4 (PerCP), HLA-DR (FITC), CD1a (FITC), CD11c (APC), CD45RA (FITC)
- Recombinant proteins for cell culture – rh GM-CSF, rh IL-23, rh sCD40L
- Surface phenotyping of mDCs – CD80 (PE), CD86 (FITC), CD40 (APC), CD45 (PerCP), HLA-ABC (FITC), Annexin V (PerCP)
- Intracellular cytokine staining – IFN-gamma (PE)
- ELISAs to assess differential IL-23 production – human IL-12p40 total and human IL-12p40 differential

A subset of asthma patients have severe symptoms that are uncontrolled by current therapies resulting in considerable morbidity and an economic burden. These individuals have elevated IL-17A but whether this is cause or effect of disease is not clear. By teasing apart the mechanisms driving IL-17A synthesis, we hope to ultimately generate more efficacious therapeutic targets with reduced side effects. The paradigms uncovered are likely to be highly pertinent to other chronic inflammatory diseases where corticosteroids represent the first line of treatment.