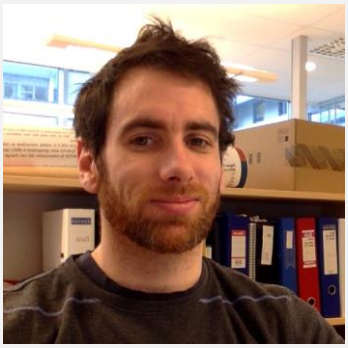
ImmunoTools *special* Award 2014



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Signaling cascades and immunological networks in Sjögren's syndrome

Sjögren's syndrome (SS) is a systemic autoimmune disease characterized by lymphocytic infiltrates of the salivary and lacrimal glands. The hallmarks of the disease are a dryness of the mouth (xerostomia) and the eyes (keratoconjunctivitis sicca). This dryness and other clinical manifestations result in a significant decrease of life quality of those affected by the disease. Currently diagnosis of SS is difficult, with major symptoms (dryness, fatigue and pain) frequent in the general population through a variety of causes including side effects of numerous drugs, anxiety and aging. Additionally management of SS is limited to the relief of symptoms, with no cure or effective treatment as yet available.

SS has been associated with polymorphisms in genes encoding proteins involved in cell signaling. Additionally broad scale disruptions in cytokine and gene expression profiles, in particular those associated with type I interferon (IFN) has been detected at increased levels in labial salivary glands, plasma and peripheral bloods cells in SS patients. These features suggest that intracellular signaling pathways may be involved in disease pathogenesis. Dysfunctional intracellular signaling mechanisms may influence the immunological response of a cell to a given stimulus, resulting in aberrant regulation of gene expression and cytokine production. Aberrant regulation may result in inappropriate immunological responses causing fatal patho-physiological injuries via dysregulated production of inflammatory cytokines.

Dysfunctional signaling pathways have been shown to promote disease development in cancer-based studies, however little is known on potential defective signaling pathways in SS as well as other autoimmune diseases. Signaling dysfunctions therefore present an interesting target for diagnostic and classification biomarkers for

SS. Additionally, identified dysfunctions may provide platforms for the development of new therapies targeting these intracellular mechanisms in SS.

The goal of the project is to establish and characterize distinct signaling pathways in response to activation of certain pattern recognition and cytokine receptors in several types of immune cells of patients with SS and compare this to healthy controls. Immune cells react to activation and stimulation of their receptors by mediators through initiation of signalling cascades. Many of these proteins involved in intracellular signalling events are being phosphorylated upon activation. The phosphorylation state can thus be measured to establish the extent of pathway activation. To achieve our aims we will use single cell phosphor-specific flow cytometry (phosphoflow). The method enables the simultaneous measurement- at a single cell level, of extracellular surface markers and the phosphorylation status of intracellular signaling proteins. MAPK/ERK and JAK/STAT signaling networks in human peripheral blood cells will be analyzed by measuring intracellular phosphorylation states through the use of a number of well-characterized phosphoprotein-specific antibodies including pERK 1 / 2, pP38, pNF-κB, pSTAT1, pSTAT3 and pSTAT4. The baseline levels as well as cytokine and pathogen-associated molecular patterns stimulated levels of phosphoproteins across a subset of immune cells (Monocytes, T cells, Natural Killer cells, Natural Killer T cells and B cells) will be measured. The obtained results will help to gain insights in possible misdirected signaling pathways in patients with autoimmune diseases and thus support the development of new diagnostic tools and improve current therapies.

As we are interested in the cell signaling response of immune cells to a number of recombinant human cytokines the ImmunoTools Award would provide an important contribution to my PhD project in allowing us to analyze the response of larger range of immune cells to a greater range of cytokines.

ImmunoTools *special* AWARD for **Richard Davies** includes 19 reagents

FITC - conjugated anti-human CD4, CD8, CD33,

PE - conjugated anti-human CD11b, CD27,

APC -conjugated anti-human CD22,

recombinant human cytokines rh BAFF/sCD257, rh Flt3L /CD135, rh IL-1beta /IL-1F2, rh IL-4, rh IL-6, rh IL-10, rh IL-12, rh IL-17A, rh IL-17F, rh IL-21, rh SDF-1α / CXCL12a, rh SDF-1β /CXCL12b, rh TGF-beta3

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