ImmunoTools *special* Award 2014

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Integrin-mediated regulation of leukocytes in immunity

Integrins are heterodimeric cell surface proteins that regulate cellular adhesion and migration. Expression of the beta2-integrin subfamily (alphaL/beta2, LFA-1; alphaM/beta2, Mac-1; alphaX/beta2; alphaD/beta2) is restricted to leukocytes, where they play roles in homing, activation and effector responses. Integrin function and regulation is therefore vital for the generation of immune responses, the control of inflammation, and effective protection of the host from invading pathogens. The importance of integrins in vivo is demonstrated in humans by the disorders called Leukocyte Adhesion Deficiency, caused by mutations in beta2-integrins or the integrin cytoplasmic regulator kindlin-3.

In resting leukocytes, integrins are maintained in an inactive state, and undergo a conformational change upon cell stimulation to an active high affinity state. Subsequent ligand binding results in signal transmission into the cell, which leads to actin cytoskeleton rearrangements, integrin clustering and other downstream effects. Integrin affinity and signaling is controlled at the plasma membrane by the binding of cytoplasmic proteins, such as talin and kindlin-3, to the intracellular domain of the beta2-integrin subunit. A threonine triplet (TTT-motif) at positions 758-760 in humans (759-761 in mice), has been identified as being vital for downstream 'outside out' signaling in beta2-integrins and for normal T cell function, and is the main kindlin-binding site in integrins. We are interested in the involvement of beta2-integrin-kindlin-3 interactions in the function of leukocytes and immune responses, particularly focusing on $CD8^+$ T cell responses.

Dendritic cells are the main antigen-presenting cells necessary for the priming of $CD8^+$ T cells. $CD8^+$ cytotoxic T cells are responsible for the killing of host cells infected with intracellular pathogens, such as viruses and some bacteria, as well as for the killing of cancer cells. $CD8^+$ T cells are therefore key in maintaining host defense against invading intracellular pathogens and tumours. However, cytotoxic T cells are also implicated in pathological conditions such as transplant rejection, and autoimmune disorders, e.g. diabetes. Beta2-integrins have been implicated in $CD8^+$ T cell cytolytic activity. However, the role of beta2-integrins in the regulation of dendritic cells, which present antigen to T cells and are crucial for $CD8^+$ T cell activation, is currently unclear. Also, the role of $CD8^+$ T cell integrins in activation and effector cytolytic responses is unknown.

We aim to explore the fundamental mechanisms of beta2-integrin involvement in $CD8^+$ T cell priming and effector responses using *in vitro* and *in vivo* models. To do this, we will use ImmunoTools growth factors such as GM-CSF, Flt3-ligand and M-CSF for the *in vitro*

differentiation of wild type and beta2-TTT/AAA integrin knock-in immune cell subsets, including dendritic cells and macrophages. These antigen-presenting cells can then be co-cultured together with wild type and/or knock-in $CD8^+$ T cells. These T cells will be purified from mouse spleens, and their purity will be verified by flow cytometry using ImmunoTools fluorescently-labelled antibodies e.g. anti-CD4 FITC, anti-CD8 PE. After purification and/or culture, T cell activation will also be measured using anti-CD62L APC and anti-CD44 FITC. Recombinant murine cytokines will be utilized for in vitro stimulation of dendritic cells and T cells, as well as for the quantitation of cytokine production by ELISA.