ImmunoTools IT-Box-Cy55M-Award 2013

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Regulation of integrins during phagocytosis via the β2-subunit of Mac-1.

β2 integrins are exclusively expressed on leukocytes and activation of these integrins allow binding to ICAMs which enable cell adhesion and migration out of blood vessels to tissues. Leukocyte Adhesion Deficiency (LAD) is a rare illness which affected patients suffer from recurrent infections. LAD-1 patients have a deficiency in β2-integrins while LAD-3 patients have a deficiency of Kindlin3 and these mutations generally affect neutrophils. Our lab has generated a viable mouse model with a mutation in the kindlin-3 binding site in the beta2-integrin tail. Böttcher et al. (Nat. Cell Biol., 2012) has shown that integrin activation involves the binding of Kindlin2 to β1 integrin and Sorting Nexin 17 binds to β1-integrin after Kindlin2 disassociation to protect integrin from lysosomal degradation during integrin recycling to cell surface. The aim of this project is to find out if similar situation applies to β2 integrins; if β2 integrins requires Sorting Nexins for protection after activation and Kindlin3 disassociation to prevent their degradation during recycling in phagocytosis. As Sorting nexin proteins are a large family of proteins which is not limited to integrin degradation process, another hypothesis is that Kindlin3 interaction β2 and downstream Rho pathway is more important in phagocytosis.

An initial screening with various leukocyte populations has shown that bone-marrow derived dendritic cells and macrophages have a significant reduction in β2, αL, αM and αX integrin subunits (for dendritic cells) in our knock-in mice compared to wild-type mice. These cell types are also optimal for integrin recycling studies as they undergo rapid cell-cell interaction and actin reorganisation. The cytokines from ImmunoTools would be used to grow bone-marrow derived dendritic cells and macrophages from wild-type and knock-in mice. Integrin expression levels and western blot detection of integrin interacting proteins from these cells would be assessed and introduction of various proteasome, lysosome and endosome inhibitors would be used to rescue integrin degradation. Functional studies to be carried out would include cell adhesion and migration assays (using cytokines/chemokines provided in the kit as chemoattractants and to stimulate cell adhesion process), cytokine stimulated bone-marrow derived macrophages and dendritic cells would be

used to stimulate phagocytosis and confococal visualization for co-localization of Sorting Nexin proteins/Kindlin3 and β2-integrins in phagocytic cups.

ImmunoTools *IT-Box-Cy55M* for **Hwee San Lek**
includes 55 recombinant mouse cytokines

rm EGF, rm Eotaxin / CCL11, rm FGF-a / FGF-1, rm FGF-b / FGF-2, rm FGF-8, rm Flt3L / CD135, rm G-CSF, rm GM-CSF, rm GRO-a / CXCL1, rm GRO-b / CXCL2, rm IFNgamma, rm IL-1alpha, rm IL-1beta, rm IL-2, rmIL-3, rm IL-4, rm IL-5, rm IL-6, rm IL-7, rm IL-9, rm IL-10, rm IL-11, rm IL-13, rm IL-15, rm IL-16, rm IL-17A, rm IL-17C, rm IL-17F, rm IL-19, rm IL-20, rm IL-21, rm IL-22, rm IL-25 / IL-17E, rm IL-27, rm IL-31, rm IL-33, rm IP-10 / CXCL10, rm LIF, rm MCP1 / CCL2, rm M-CSF, rm MIP-1α/ CCL3, rm MIP-1β / CCL4, rm MIP3α / CCL20, rm MIP3β / CCL19, rm NGF-beta, rm PDGF-AA, rm PDGF-BB, rm RANTES / CCL5, rm sCD40L / CD154, rm SCF, rm SDF-1α / CXCL12a, rm SDF-1β / CXCL12b, rm TNFα, rm TPO, rm VEGF DETAILS.