ImmunoTools *special* Award 2016

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Regulation of dynamic membrane processes by neutral sphingomyelinase for a balanced T cell response

T cells are important lymphocytes in the immune response against invading viruses, bacteria and other pathogens. Generally speaking, their response requires activation, migration to the sites of infection and finally a termination of their activation.These functions require receptors and their organization on the cellular surface. As the cell membrane consists of various lipids and proteins it is of interest to investigate how the lipid composition influences T cell responses.

Our major interest lies in investigating ceramide and sphingomyelin localizations. Ceramide-enriched membrane microdomains act to segregate membrane receptors and their associated signalosomes, but also have the potential to affect membrane curvature thereby promoting exo- or endocytosis of membrane vesicles.

One part of our project addresses the visualization of ceramides in the membrane and their organization into different compartments upon T cell activation. We therefore synthesized azido-functionalized ceramides (N_3-C_6-ceramide) and explored their suitability for incorporation and importantly, for analysing their stimulated redistribution in T cells. We established that our new azido-functionalized ceramides are non-toxic (Annexin V to analyse cell viability) and are efficiently incorporated into membranes also of activated living T cells where they can be detected as early as 5 min following labelling. N_3-C_6-ceramide localized to plasma membrane microdomains and proximal vesicles and segregated into clusters following TCR-, and especially CD28 ligation indicating efficient sorting into plasma membrane domains associated with T cell activation. To characterize these domains in more detail, colocalization studies with directly conjugated antibodies in living cells are planned.

In studies using Dynabeads or dendritic cells contacted with labelled T cells, N_3-C_6-ceramide was efficiently excluded from the IS centre and rather accumulated at the periphery of a functional IS indicating that this analogue can be used for ceramide membrane domain compartmentalization studies in living cells. In these experiments is important to ensure that the introduction of these chemically modified lipids does not interfere with cellular functions. Therefore, we need to analyse by flow cytometry the expression of various surface molecules (e.g. CD3, CD5, CD44).

In the second part of the project we are analysing the impact of neutral sphingomyelinase ablation on the migration of T cells. The neutral sphingomyelinase is located in the inner leaflet of the plasma membrane and converts sphingomyelin to ceramide. We hypothesise the function of this enzyme influences the ability of T cells to migrate at all and to sense the direction along a chemokine gradient. We therefore want to silence the neutral sphingomyelinase by chemical inhibitors or by siRNA knockdown in primary human T cells and investigate how these cells are migrating on collage or fibronectin towards various chemokines, e.g. ImmunoTools recombinant human cytokines SDF1a, CCL21, CCL19 and others. If these experiments show interesting results, we will analyse the chemotaxis of mouse T cells *in vivo*. Additionally, the characterization of T cells regarding their cytokine profile with or without neutral sphingomyelinase ablation is of major interest to us (human IFN-gamma, IL-4, IL-6, IL-10, IL-12p40, TNF-a).

Needless to say your reagents will greatly benefit my project and make important advances towards the publication of my second manuscript in regards to discovering the impact of the neutral sphingomyelinase on T cell functions. Furthermore, it will improve my CV in terms of publications and help me in future interviews for postdoc labs within the area of immunology.

ImmunoTools *special* AWARD for **Lena Collenburg** includes 25 reagents

FITC - conjugated anti-human CD5

APC - conjugated anti-human CD44

recombinant human cytokines: rh Exodus-2 / CCL21, rh IP-10 /CXCL10, rh MIP-1b / CCL4, rh MIP-3b / CCL19

human ELISA-set for 96 wells: human IFN-gamma, human IL-4, human IL-6, human IL-10, human IL-12p40 total, human TNF-a (each 3 reagents)

recombinant mouse cytokines: rm SDF-1a / CXCL12a

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