ImmunoTools *special* Award 2015

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Role of sphingolipids in the regulation of anti-viral T cell responses in a measles virus model

Sphingolipids are an important class of lipid molecules in cell membranes but despite their structural role sphingolipids also act as bioactive signaling molecules. Moreover, the local segregation of sphingolipids and their metabolites directly affects biophysical properties of the membrane at a local basis regulating membrane deformation, vesiculation, and fusogenicity. In this work the focus is on sphingomyelin, a subgroup of sphingolipids consisting of phosphocholine, which is a sphingosine and a fatty acid, and ceramide. The enzymes sphingomyelinases (ASMases, NSMases) hydrolyze sphingomyelin to generate ceramide. Cermides themselves are important for T-cell stimulation. And vice versa, activation of T cells in the immune system induces an increased activity of acid sphingomyelinases. Resulting ceramides are involved in forming microdomains in membranes which are essential for clustering of receptors and signalling platforms in the plasma membrane. Ceramides accumulating as a result of membrane SM breakdown are therefore also essential for pathogen entry and the modulation of signalling pathways such as the phosphatidylinositol-3-kinase (PI3K) pathway.

The aim of this project is to analyze the role of sphingolipids for T-cells. So far it is known that the metabolism of sphingolipids has pronounced consequences for the immune response against viruses and the course of viral infections but the underlying mechanisms are not well defined. For analysis of the role of sphingolipids a knockout animal was created. These Sphingomyelinase-deficient mice develop pathologies resembling Niemann-Pick disease or osteogenesis imperfecta, respectively, in humans. Data from other groups showed that sphingomyelinase knock-out animals have deficiency in IL-2, IFNγ and cytotoxic granula secretion resulting in delayed viral clearance in the case of lymphocytic choriomeningitis virus (LCMV)-infected ASMase-deficient mice. We will do now *in-vivo* experiments using the CNS infection model with rodent-adapted recombinant measles viruses carrying a GFP protein for

detection. In these experiments the anti-viral immune response in the acute and chronic phase will be analyzed including the influence of Sphingolipid metabolism on the control of the measles virus. Effector functions of $CD4^+$ and $CD8^+$ T cells known to be important for virus clearance include cytotoxic activity, production of effector cytokines, and localization to tissue sites of virus infection. Therefore we will analyze functions of $CD4^+$ conventional and regulatory T cells ex vivo. Additionally, we will investigate the differentiation of $CD4^+$ T cells into T-Helper cells and do functional assays with regulatory T cells. On the $CD8^+$ T cells we will check effector functions of antigen-specific $CD8^+$ T cells with regard to their dependency on sphingomyelinase activities using knockout animals and sphingomyelinase inhibitors in WT mice.

ImmunoTools anti-mouse antibodies for flow cytometry would be very useful for the basic characterization of T cells concerning frequencies of subpopulations and activation markers. For sorting of cell populations also these antibodies are required. In addition these antibodies will be used for evaluation of many functional assays like Treg cell suppression assays, proliferations assays and detection of produced cytokines. ImmunoTools recombinant mouse cytokines would be used for differentiation assays. Additionally, as I'm working with Treg cells I'm using a lot of IL-2 for cell cultures.

ImmunoTools *special* AWARD for **Claudia Hollmann** includes 24 reagents

FITC - conjugated anti-mouse CD4, CD8a, CD19, CD25, CD44, CD45, CD45R, CD62L,

PE - conjugated anti-mouse CD3e, CD4, CD8a, CD19, CD25, CD44, CD62L,

APC - conjugated anti-mouse CD3e, CD4, CD8a, CD25, CD62L,

recombinant mouse cytokines: rm IFNgamma, rm IL-2, rm IL-4, rm IL-10

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