RTG 1830
GERMAN CANADIAN
COMPLEX MEMBRANE PROTEINS
IN CELLULAR DEVELOPMENT AND DISEASE

P10

Membrane transport in human natural killer cells (Barbara Niemeyer / Markus Hoth)

Natural killer (NK) cells belong to the innate immune system. A wide array of germline-encoded activating and inhibtory receptors control their Cytotoxic functions. NK cell malfunction is implicated in several diseases including cancer, autoimmune diseases or hemaphagocytotic lymphohistiocytosis (FHL). Our long-term major aim is to elucidate the potential role of ion channel malfunction in NK cells for disease. At present very little is known about membrane transport of ions in NK cells apart from the role of ORAI1 and STIM1 for cytotoxicity. We will thus first characterize endogenous ion channels in primary human NK cells using imaging, patch-clamp and detailed molecular analysis (qRTPCR, Western Blot) to determine the relative contribution of potassium, calcium (including all STIM/ORAI subfamily members and voltage-operated calcium channels) and non-selective cation channels such as members of the TRP channel family. All these channels may contribute to local and global NK cell calcium signals required for lytic granule release at the NK cell/target cell contact site and cytokine release. We will analyze the relevance of the different ion channels for calcium signals and NK cell cytotoxicty using a combination of imaging-, electrophysiological, molecular biological tools (siRNA) and cytotoxicity assays.

To link membrane transport with NK cell function, we will modulate ion channel activity and correlate it with calcium signals, with lytic granule transport and release, and the killing of target cells and the immune synapse. siRNA, overexpression and pharmacological inhibition of ion channels will be used to modify ion channel activity. NK cells will be stimulated through their membrane receptors or by target cell contact (which we can induce through target cells hooked up to a pipette based micromanipulator system). For total internal reflection fluorescence microscopy (TIRFM), we will coat NK cell activating antibodies on glass coverslips and let NK cells settle on those. Movement and release of lytic granules can be tracked by total internal reflection fluorescence microscopy (TIRF) or by epifluorescence imaging (if needed 4D with deconvolution). Similar to our epifluorescence-based imaging system, our TIRF system also allows the parallel measurements of different calcium indicators (including Fura-2 for ratiometric measurements) and lytic granule fluorescence. In addition to TIRF and fast 4D epifluorescence microscopy, we will also use high-resolution microscopy (SIM and potentially PALM) to quantify the localization and movements of lytic granules. We have developed single cell and population killing assays for NK cells to quantify their killing competence (compare preliminary work).

Combining the different techniques outlined above, we are able to correlate ion channel function and calcium signals with NK cell function with rather good time and spatial resolution. Finally we aim to link membrane transport in NK cells with NK cell malfunctioning relevant for disease.