Focus of the PhD-project (Prof. Dr. Markus Hoth/Dr. Eva Schwarz)

Local calcium signalling at the immunological synapse

T-lymphocytes are activated by antigen presenting cells (APC) or target cells. Upon contact with matching APC or target cells, an immunological synapse (IS) is formed between the cells. T-killer cells (= mature $CD8^+$ T-lymphocytes) form two different types of IS with their target cells: a stimulating IS, which activates maturation and proliferation of T-cells, and a killing IS, which induces target cell death via exocytosis of perforin and granzymes at the IS. While it is well-established that a rise in the cytosolic calcium concentration is necessary for signalling processes at both synapses, nothing is known about the role of local calcium signalling at the synapses. In the future we have the following goals:

1. Analyze the spatio-temporal characteristics of localized calcium signals at the stimulating and killing synapse in $CD8^+$ T-lymphocytes.
2. Analyze the (different?) functional implications of localized calcium signals for signal transduction at the stimulating and killing synapse.
3. Analyze the calcium dependence of perforin and granzyme release at the killing synapse and the calcium dependence of target cell killing.
4. Identify the SNARE proteins responsible for granule fusion at the killing synapse.

Methods:
To achieve our goals we will combine sophisticated imaging technology (confocal microscopy, deconvolution microscopy, total internal reflection microscopy) and patch clamp techniques with molecular, biochemical, siRNA and knock-down techniques. All experiments will be carried out with primary human or mouse T-cells.

Selected recent publications:

Quintana A, Schwindling C, Wenning AS, Becherer U, Rettig J, Schwarz EC, Hoth M (2007). T cell activation requires mitochondrial translocation to the immunological synapse, *PNAS* 104, 14418-23.

Schwindling C, Quintana A, Krause E, Hoth M (2010). Mitochondrial positioning controls local calcium influx in T cells. *J Immunol* 184, 184-190.

Bogeski I, Kummerow C, Al-Ansary D, Schwarz EC, Koehler R, Kozai D, Takahashi N, Peinelt C, Griesemer D, Bozem M, Mori Y, Hoth M, Niemeyer BA (2010). Differential redox regulation of ORAI channels: a mechanism to tune cellular calcium signaling. Science Signaling 3 (115), ra24.

Wenning AS, Neblung K, Strauß B, Wolfs M-J, Sappok A, Hoth M, Schwarz EC (2011). TRP expression pattern and the functional importance of TRPC3 in primary human T-cells. *BBA Molecular Cell Research* 1813, 412-423.

Pattu V, Qu B, Marshall M, Becherer U, Junker C, Schwarz EC, Matti U, Schwarz EC, Krause E, Hoth M, Rettig J (2011). Syntaxin is required for lytic granule release from cytotoxic T lymphocytes. *Traffic* 12, 890-901.

Qu B, Pattu V, Junker C, Schwarz EC, Marshall M, Matti U, Becherer U, Bhat S, Kummerow K, Neumann F, Pfreundschuh M, Rieger H, Rettig J, Hoth M (2011). Docking of lytic granules at the immunological synapse in human CTL requires Vti1b-dependent pairing with TCR endosomes. *J Immunol* 186, 6894-6904.

Quintana A, Pasche M, Junker C, Al-Ansary D, Rieger H, Kummerow C, Nuñez L, Villalobos C, Meraner P, Becherer U, Rettig J, Niemeyer BA, Hoth M (2011) Calcium microdomains at the immunological synapse: how ORAI channels, mitochondria and calcium pumps generate local calcium signals for efficient T-cell activation. *EMBO J* 30, 3895-3912.

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Qu B, Al-Ansary D, Kummerow C, Hoth M, Schwarz EC (2011). ORAI-mediated calcium influx in T cell proliferation, apoptosis and tolerance. *Cell Calcium* 50, 261-269.

Niemeyer BA, Hoth M (2011). Excitable T cell: Ca(v)1.4 channel contributions and controversies. *Immunity* 35, 315-317.