

## My PhD project summary

*Toxoplasma gondii* is a pathogen that is a model for studying intracellular parasitism by a group of parasites called the Apicomplexa, which includes malaria and cryptosporidium, an emerging cause of waterborne disease. Following oral infection, the parasite crosses epithelial and endothelial cellular barriers to enter into circulation and disseminate within the organism (1). To date, it is still unclear as to how the parasite disseminates throughout the host. Previously we have found that upon *T.gondii* infection, primary dendritic cells (DCs) and microglia exhibit a hypermigratory phenotype that facilitates parasite translocation across cellular barriers (2-4). One of the key factors of this hypermigratory phenotype is gamma-aminobutyric acid (GABA), which is the main inhibitory neurotransmitter in the vertebrate brain but evidence also shows it participates in diverse functions outside the central nervous system (CNS) including cell migration, immunomodulation and metastasis (5,6). Dendritic cells have functional GABA<sub>A</sub> receptors as well as the capability to synthesize and secrete GABA. Challenge with *T.gondii* enhances GABA secretion in infected DCs and inhibition of either GABA<sub>A</sub> receptors, GABA synthesis or GABA transport abrogates the *T.gondii*-induced hypermigratory phenotype (7).

My project is to further investigate how *T.gondii* manipulates host cell functions in order to ensure intracellular survival and modulate immune responses to ultimately disseminate within restricted organs.

During *T.gondii* infection, DCs play a pivotal role as mediators of essential immune responses while simultaneously acting as parasite carriers that facilitate the dissemination of the infection (2-4). Dendritic cells act as sensors in peripheral tissues, which process and present antigens to initiate the adaptive immune response, ultimately resulting in pathogen clearance (8). The switch from an immature state to a mature state requires major alterations in the actin cytoskeleton of the DC, thereby allowing the cell to migrate from the periphery to the lymphatic circulation or from the blood into the tissue. Active invasion of *T.gondii* induces rapid cytoskeleton remodelling in DCs and induces a hypermigratory phenotype within minutes (paper I). As part of this phenotype, *T. gondii* infected DCs and microglia lose adhesive podosome structures, become rounded and integrins (CD183 and CD11c) are redistributed. These findings were also confirmed in 3D collagen matrix, where collagen is a major component of the extracellular matrix (paper II). Here, *T.gondii* infected DCs show enhanced migration, which is independent of integrins and with consistent morphological changes as observed with 2D assays (paper II).

*Toxoplasma gondii* discharges secretory proteins during cell invasion. However, parasitic effector molecules responsible for the onset of the hypermigratory phenotype have not been found. In order to address this, fractionated parasite lysates were tested for their ability to induce a hypermigratory phenotype in DCs. This parasite-derived subcellular fraction from secretory organelles contains 14-3-3 proteins that are ubiquitously expressed in all eukaryotic cells, and participate in multiple processes within the cell, including the organization of the cytoskeleton. Here, we show that

*T.gondii* 14-3-3 is sufficient to induce a hypermigratory phenotype in DCs (paper III).

Upon *T.gondii* infection, GABA receptor signaling triggers a hypermigratory phenotype in DCs by unknown signal transduction pathways. GABA<sub>A</sub> receptors are ionotropic chloride channels and their functions are regulated by cation-chloride co-transporters (CCCs). Notably in the CNS, activity of CCCs provides inward or outward directed chloride fluxes, leading to membrane hyperpolarization or depolarization. Membrane depolarization secondary to GABA receptor activation can elicit calcium responses via calcium influx, which is mediated by voltage dependent calcium channels (VDCCs) located in the plasma membrane. We demonstrate that the hypermigratory phenotype induced in DCs by *T.gondii* is dependent on the L-type VDCC subtype Cav1.3, and the activation of which is linked with GABAergic signaling (manuscript IV).