1. Abstract (EN)

This thesis is collection of work focused mainly on the understanding of mast cell activation and its regulation by Orm1-like (ORMDL) proteins. ORMDL family is a group of endoplasmic reticulum (ER) membrane resident proteins that are highly conserved amongst mammalian species. ORMDL proteins can be found in diverse range of organisms from plants through fungi to animals. ORMDL proteins were first discovered in yeasts and the interest in these proteins skyrocketed after the discovery that ORMDL3 is associated with childhood onset asthma in genome wide association studies. Following research connected ORMDL3 also with allergic inflammation and inflammatory bowel disease. Since mast cells are mainly known for their role in allergy and allergen induced inflammation, we decided to investigate the role of ORMDL proteins in regulation of mast cell activation and signaling.

In our first study we focused on the role of ORMDL3 in mast cell activation via the high affinity IgE receptor 1 (FcεRI). We prepared bone marrow-derived mast cells with decreased (ORMDL3-KD) or increased (ORMDL3-OE) ORMDL3 expression. We showed that ORMDL3 is a negative regulator of mast cell activation events like degranulation, cytokine release and migration, without any effect on calcium mobilization. ORMDL3 was previously described to regulate sphingolipid biosynthesis via serine palmitoyltransferase, calcium mobilization through SERCA2b and ER stress responses. At first, we decided to search for novel interacting partners of ORMDL3 in mast cells. Mass spectrometric analysis of the ORMDL3 immunoprecitates revealed 5-lipoxygenase (5-LO) as a potential interacting partner of ORMDL3. 5-LO is an enzyme responsible for production of leukotrienes in immune cells and is activated in mast cells after (FcεRI) triggering. To verify and investigate whether the ORMDL3 and 5-LO are parts of a macromolecular complex or they interact directly, we used Glutathione S-transferase (GST) pull-down assay with GST-tagged proteins prepared in bacteria and ^{35}S-Met-labeled proteins prepared in *in vitro* translation system. Pull-down experiments proved the direct interaction of the proteins. Furthermore, we identified 5-LO activating protein (FLAP) as another interacting partner. Upregulated levels of leukotriene C_4 in ORMDL3-KO mice proved that ORMDL3 is not only regulator of sphingolipid biosynthesis but also a negative regulator of 5-LO in FcεRI activated mast cells.

The extent of expression of ORMDL proteins was found to be associated with many diseases. It has been described that ORMDL3 expression is reduced by miRNA in inflammatory bowel disease. We investigated expression of ORMDL proteins in DSS-induced colitis and found that expression of ORMDL3 mRNA is downregulated in colon of DSS-treated mice but total ORMDLs protein amount is, in fact, increased upon DSS treatment *in vivo* as well as *in vitro*. Further studies on intestinal epithelial cell lines Caco2 overexpressing myc-tagged ORMDL proteins revealed that acute DSS treatment causes ORMDL upregulation on both mRNA and protein levels. Mass spectrometry

analysis of lipids showed that *De novo* sphingolipid biosynthesis is not altered in DSS-treated mice, while sphingosine and ceramide, products of salvage pathway, are increased in colitis. Mucus layer is an important part of colonic epithelium that is disrupted in DSS-induced colitis. When we analyzed expression of mucins in Caco2 cells we found that increased expression of all ORMDL proteins leads to downregulation of Muc2 and Muc5AC expression and this effect is prevalent also after induction of mucin expression via DSS. Hence, we showed novel mechanism how alterations in ORMDLs expression may be involved in pathophysiology of IBD.

During our studies we characterized different regulatory mechanisms of FcεRI receptor. localizes to membrane microdomains containing cholesterol-sphingolipids-and proteins. Since ORMDL3 is a regulator of sphingolipid biosynthesis it bears a potential to affect these membrane microdomains. Lipid investigation requires suitable solvents, ethanol often being solvent of choice. Therefore, we investigated how acute ethanol treatment affects FcεRI-mediated mast cell activation. Indeed, we found that ethanol inhibits mast cell activation via FcεRI at non-toxic concentration on the level of calcium mobilization, ROS production, cytokine release and migration. Our observation that cholesterol increase prevents inhibitory effects of ethanol shows that lipids and membrane rigidity play important role in FcεRI activation. Early receptor signaling of FcεRI is accompanied by mobilization of intracellular calcium stores. We investigated how calcium release is affected by C-terminal Src kinase (CSK). CSK is a regulatory protein responsible for phosphorylation of inhibitory tyrosine in Src family kinases (SFKs). We found that CSK negatively regulates FcεRI induced calcium mobilization with impact on mast cell degranulation and migration. Furthermore, we investigated, how miltefosine an inhibitor of mast cell mediator release affect calcium mobilization via FcεRI. However, we found no differences in calcium response between miltefosine treated and non-treated mast cells.