1 ABSTRACT (EN)

This thesis is focused mainly on understanding mechanisms of regulatory roles of C-terminal Src kinase (CSK) and phosphoprotein associated with glycosphingolipid-enriched microdomains (PAG) in the high-affinity IgE receptor (FcεRI)-mediated signaling of murine mast cells. FcεRI activation is initiated by aggregation of the receptor by complexes of multivalent antigen with IgE, followed by activation and enhanced activities of protein tyrosine kinases, phosphatases, adaptor proteins and number of other signal transduction molecules. The signaling events result in mast cell degranulation and release of variety of proinflammatory mediators, responsible for initiation of allergy and other inflammatory diseases. Understanding the function of key regulatory molecules controlling FcεRI-mediated mast cell activation, degranulation, and cytokines production could have therapeutic impact.

CSK is a major negative regulator of Src family tyrosine kinases (SFKs) that play a critical role in various immunoreceptor signaling events. However, its function in mast cell activation has not been completely understood. Because of its cytoplasmic localization, CSK was assumed to be brought to the vicinity of the plasma membrane-bound SFKs via binding to membrane-bound adaptors and PAG was a major candidate. To determine the roles of CSK and PAG in FcεRI signaling, we used as a model bone marrow-derived mast cells (BMMCs) from wild-type or PAG knockout (PAG-KO) mice with reduced amount of CSK (CSK-KD) or with reduced amount of both proteins (CSK-KD/PAG-KO). We found that antigen-activated cells with CSK-KD exhibited significantly higher degranulation, calcium response and chemotaxis. Unexpectedly, PAG played an opposite role in these processes and cells with CSK-KD, similarly as PAG-KO, displayed impaired phosphorylation of the transcription factor STAT5. This was probably caused by enhanced enzymatic activity of Src homology region 2 domain-containing phosphatase-1 (SHP-1), which resulted in inhibition of proinflammatory cytokines and chemokines production. Our data also showed distinct involvement of adaptor proteins LAT and NTAL in CSK- or PAG-dependent signaling in BMMCs. Several lines of evidence suggested, that CSK binds not only to PAG, but also to some other anchors, which could serve better than PAG for positioning of CSK in the vicinity of SFKs and thus more efficiently contribute to their inactivation. Based on these data we postulated a new model of CSK-PAG interplay in mast cell activation.

Since these studies required sensitive detection of various cytokines released or utilized by mast cells, part of the thesis was dedicated to development of such assay. In collaborative studies we used this method, called nano-iPCR, for detection of clinically relevant markers in cerebrospinal fluid of patients and compared it with routinely used enzyme-link immunosorbent assays (ELISAs).

We also used this method for elucidating the role of ORMDL3 protein in mast cell signaling. The key finding was determination of ORMDL3 as a negative regulator of proinflammatory cytokines production, mediated via AKT and NF-κB-directed signaling pathways. Our results also showed that mast cell chemotaxis is regulated by ORMDL3 and CD9. Furthermore, we examined mode of action of widely used dissolving agent, ethanol, and cellular cholesterol remover, methyl-β-cyclodextrin, on FcεRI activation. Our data indicated that ethanol has an inhibitory effect on function of FcεRI signalosomes in which cholesterol plays an indispensable role. These data were corroborated in mice *in vivo* using passive cutaneous anaphylaxis.