

ABSTRACT

Mast cells are critical component of the immune system. In pathological situations, they are activated and are responsible for allergic reaction. Therefore, detail understanding of mast cell activation at molecular level is important for design of new therapies of allergic diseases. Principal transmembrane receptor of mast cells is the high-affinity Fc receptor for IgE (FcεRI). FcεRI anchors IgE on mast cell surface and upon cross-linking with multivalent antigen it becomes phosphorylated at its intracellular immunoreceptor tyrosine-based activation motifs (ITAMs). This triggers signaling cascade leading to cell degranulation and cytokine production. The antigen-mediated signaling through the FcεRI is critically dependent on interplay with intracellular protein-tyrosine kinases that phosphorylate the ITAM motifs and many other components of the signaling pathway. This study was focused on better understanding of signaling events leading to mast cell activation; emphasis was put on early activation events. First, we examined the role of protein-tyrosine phosphatases (PTP) in FcεRI phosphorylation. We found that upon antigen triggering of FcεRI, PTPs undergo inhibition by oxidation of their active site located tyrosine. Studies of plasma membrane topography of inactivated PTPs showed their proximity to FcεRI receptors and actin cytoskeleton. These and other data allowed us to postulate a new model of FcεRI signal initiation. We also investigated the role of selected proteins (tetraspanin CD9, transmembrane adaptor protein PAG, serine palmitoyl transferase regulator ORMDL3) or selected chemicals as ethanol and methyl-β-cyclodextrin on FcεRI signalosome properties in the course of mast cell activation.

Major aim of this work was to identify new regulators of FcεRI signaling. For this purpose we performed high-throughput screen using RNA interference technology and identified 15 regulators of mast cell activation. We chose galectin-3 (Gal3) for detail functional analysis of its action in FcεRI signaling pathway. Mast cells with reduced Gal3 expression showed increased antigen-mediated degranulation, calcium response and phosphorylation of several signal-transduction proteins. Although phosphorylation of the FcεRI was not affected, cells with Gal3 knockdown showed impaired IgE internalization that was accompanied by decreased receptor ubiquitination. Thus, we identified Gal3 as a critical regulator controlling FcεRI plasma membrane stability and trafficking.

An important role of mast cells in health and disease also depends on their migration. To this end we examined signaling pathways controlling cell movement. We found that Gal3 positively regulates fibronectin-mediated mast cell adhesion and motility but negatively affects antigen-mediated cell chemotaxis. Our data also showed that antigen-mediated mast cell chemotaxis was regulated by CD9, PAG and ORMDL3 proteins.