Mast cells are well known effector cells in immune system. They have been implicated in such important processes as host defense against bacteria, toxins or parasites. However, in some cases they can develop improper reaction against harmless environmental antigens and thus causing allergies. It is therefore essential to understand signaling events that lead to activation of these cells in order to develop new treatment strategies.

Newly prepared rat monoclonal antibody of IgG1 subtype raised against murine mast cells was characterized and found suitable for flow cytometry, immunoblotting and immunoprecipitation. Employing of optimized procedure for immunopurification in combination with mass spectrometry led to identification of its target cluster of differentiation (CD)9 protein. CD9 is a member of large protein family called tetraspanins. Functional studies showed that binding of this antibody to mast cells induced degranulation and early activation events such as increased tyrosine phosphorylation and enhanced levels of free cytoplasmic calcium. Interestingly, subsequent activation of these cells via antigen-mediated aggregation of the high-affinity IgE receptor (FcεRI) led to decreased degranulation, calcium response and tyrosine phosphorylation of several substrates. Importantly, anti-CD9 antibody did not inhibit these responses in cells activated by thapsigargin, an inhibitor of endoplasmic reticulum calcium ATPases, indicating that downstream signaling events were not affected. The observed effects of the antibody were not caused by blocking binding of the antigen to FcεRI, coupling of CD9 to FcεRI by antibody nor by engagement of Fcγ receptors.

Involvement of CD9 in FcεRI-mediated activation events was supported by experiments employing cells with enhanced or decreased expression of CD9 by transfection with CD9 cDNA or silencing RNA, respectively. Cells with enhanced expression of CD9 exhibited more, while cells with decreased expression exhibited less anti-CD9-mediated effects. Surprisingly, degranulation of cells with decreased CD9 expression was comparable to wild-type cells which probably reflects of compensation by other tetraspanin family member. The combined data suggest that CD9 acts as a regulator of FcεRI-mediated activation. Antibody-mediated aggregation of CD9 leads to cell activation events. This might also activate negative feed-back mechanism which dampens FcεRI-mediated activation resulting in impaired degranulation.