Positive & Negative Regulation of FcεRI Signaling in Mast Cells

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Abstract

The interplay of positive and negative signaling shaping the degranulation responses in mast cells across different ligand doses was examined using integrated experimental and modeling approaches. We describe a new structurally defined trivalent ligand (DF3) that crosslinks DNP-specific IgE bound to FcεRI. We have characterized FcεRI aggregation properties, as well as differential recruitment of Syk (positive) and SHIP (negative) to FcεRI across different DF3 doses. We present a computational model for DF3-IgE-FcεRI interaction, as well as results of equilibrium binding assays, electron microscopy, proximity ligation and biochemical techniques. The impact of regulatory loops involving Lyn, Csk and SHP-1 on early FcεRI events are considered by rule-based modeling and validated experimentally.

***Keywords* — FcεRI, IgE receptor signaling, mast cells**

I. Purpose

Mast cells and basophils trigger allergic reactions when polyvalent antigens crosslink IgE-FcεRI complexes on the cell surface. Signaling begins with phosphorylation of FcεRIβ/γ ITAMs (immunoreceptor tyrosine-based activation motifs), which in turn recruit downstream regulatory proteins for signal transduction [1]. FcεRI signaling is known to be a function of receptor aggregate properties [2], but mechanisms of signal initiation and its regulation by negative players remains in question. Our goal is to build a mathematical model for FcεRI signal initiation and propagation, [3] based on experimentally derived parameters utilizing a new structurally defined ligand (DF3). Further, we integrate modeling and experimentation to predict and validate the behavior of positive and negative regulatory proteins like Syk, Lyn, SHIP, Csk and SHP-1 in mast cell signaling.

II. RESULTS

A. To address critical questions regarding the influence of aggregation state on FcεRI activation, we designed a new structurally-defined trivalent ligand termed DF3, based upon the stable trimeric assembly of DNP conjugated foldon domain of fibritin. We demonstrate the ability of DF3 to trigger robust dose-dependent mast cell responses.

B. Equilibrium binding assays and diffusion measurements provided parameters for building a mathematical model for DF3-IgE-FcεRI interactions. Based on the model, significant antigen-IgE-FcεRI aggregation was observed at both sub-optimal and supra-optimal DF3 doses, which was confirmed experimentally by electron microscopy techniques. The model predicted dose-dependent differential kinetics of FcεRI aggregation that were validated by fluorescence-based imaging analysis.

C. Dose-dependent differences in the balance of Syk and SHIP recruitment to FcεRI were observed through simulations, and measured experimentally using proximity ligation assay and biochemical assays.

D. Control of FcεRI signal initiation and regulatory loops by Lyn, Csk and SHP-1 was predicted by the model and validated by siRNA and over-expression studies.

III. Conclusion

These results suggest that the degranulation response is a function of both ligand-induced receptor aggregation and a balance of negative and positive signaling by Lyn, Syk, Csk, SHP-1 and SHIP.

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