IgE receptor signaling encodes dynamic memories of antigen exposure

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***Short Abstract* — Mast cells drive allergic reactions in response to allergen-mediated crosslinking of the high-affinity IgE receptor. The relationship between patterns of allergen exposure and cellular responses is unclear. To investigate this issue, we used a microfluidics platform to expose cells to pulses of stimulatory multivalent antigen interrupted by intervals of inhibitory, excess monovalent antigen. We measured secretion of inflammatory mediators. Results were analyzed with the aid of a mathematical model. The secretory response to a repeated antigen pulse was diminished when the inhibitory interval was <1 hr, which we were able to attribute to a desensitization process involving the lipid phosphatase Ship1. After an interval of ~1 hr, response to repeated stimulation became similar to the initial response. Hyperactive responses were then observed for longer intervals of up to 4 hrs, suggesting that Ship1 is slowly deactivated after termination of signaling and eventually (on an even slower time scale) becomes refractory to (re)activation. Hyperactivity is linked to proteosomal degradation of a Ship1 co-factor, the adaptor protein Shc1.**

***Keywords* — cell signaling, microfluidics, modeling**

I. Introduction

ALLERGIES afflict ~25% of people in the developed world. Central players in allergic reactions include mast cells and basophils, which release histamine and other mediators of inflammation upon allergen interaction with cell-surface IgE-FcεRI complexes. Aggregation of FcεRI leads to activation of several kinases, including the protein tyrosine kinase Syk. Like most receptor-initiated signaling cascades, signaling by FcεRI is held in check by desensitization processes. The dynamic interplay between these positive and negative signaling axes likely govern how a cell responds to complex inputs.

Study of cellular outputs in response to complex inputs has been accelerated by the advent of microfluidics devices, which allow for precise manipulation of fluids at small length scales from micrometers to millimeters. These new and distinctive capabilities enable the study of fundamental cellular behaviors, including cellular information processing and decision-making. Microfluidics facilitates controlled exposure profiles to single cells including pulsatile, ramp, square-wave and oscillatory signals. In this work, we used a microfluidic platform to examine desensitization linked to pulsed stimulation of FcεRI. This work represents an investigation of the system's frequency response properties.

II. Results

A. Short- and long-term molecular memory

Two surprising phenomena were observed in experiments: rapid desensitization, or short-term molecular memory, and hypersensitization after a delay, or long-term molecular memory. A model reproducing the observed responses to pulsed stimulation and capturing (fast) positive signaling by Syk and (slow) negative signaling by Ship1 was developed and used to guide investigation of short- and long-term memory. Enzyme activity assays confirmed that Syk signals decay quickly, whereas Ship1 signals decay slowly, after IgE-FcεRI aggregate breakup is induced by excess monovalent antigen.

B. Ship1 and the proteasome regulate memory

The model, as initially formulated, predicted that inhibition of Ship1 phosphatase activity would abrogate short-term memory, whereas inhibition of Ship1 degradation would abrogate long-term memory. The former prediction was confirmed experimentally but not the latter. Although proteosome inhibition eliminated long-term memory, Ship1 levels stayed constant over time. The model was able to explain the available observations when it was modified to include a Ship1 co-factor that is degraded in response to multivalent antigen inputs.

C. Shc1 plays a role in delayed hypersensitivity

We hypothesized that the Ship1 co-factor in the model might correspond to one of the several known Ship1 binding partners, such as Shc1. The model predicted that co-factor knockdown would result in accelerated hyperdegranulation. This prediction was confirmed experimentally in cells with reduced levels of Shc1 after siRNA treatment.

III. Conclusion

We leveraged a microfluidic platform enabling precisely controlled complex waveform inputs and mathematical modeling to elucidate how signaling processes operating on distinct time scales (quickly induced and rapidly reversed Syk activation, slowly induced and slowly reversed Ship1 activation, and very slow decay of the Ship1 co-factor Shc1) can give rise to short- and long-term molecular memories of antigen exposure.

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