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Review

SHIP1 and the negative control of mast cell/basophil activation by supra-optimal antigen concentrations☆

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ABSTRACT

IgE-mediated, antigen-triggered activation of mast cells and basophils often results in bell-shaped dose–response curves for the release of various pro-inflammatory mediators. The degree of suppression of mediator release observed following supra-optimal stimulation varies widely for different allergens as well as for different experimental agents that cause crosslinking of high-affinity IgE receptors (FcεRI) on these cells. While the reasons for these differences have not yet been resolved it has become increasingly apparent that supra-optimal stimulation in many cases causes a shift in the balance of stimulatory and inhibitory signal transduction mechanisms arising from FcεRI triggering. In particular, the lipid phosphatase SHIP1 has been shown to be centrally involved in explaining the bell-shaped phenomena in both mast cells and basophils in different species and appears to play a fundamental role in limiting the IgE responsiveness of these allergic effector cells. Elucidating the nature of this inhibitory signaling pathway may provide crucial knowledge in order to optimize desensitization strategies in the treatment of allergic diseases.

1. Origins and basic functions of mast cells and basophils

Mast cells (MCs) are derived from hemopoietic precursor cells and are prominently found in tissues close to the external environment, i.e. the skin and the mucosal membranes of the intestine as well as the airways (Metz and Maurer, 2007). MCs are key effector cells in innate immune responses (Marshall, 2004). In addition, they are widely recognized as detrimental effector cells in allergic disorders and other IgE-associated acquired immune responses (Galli et al., 2005, 2008). Depending on the mode of activation, MCs are capable of releasing preformed mediators (e.g. histamine, proteoglycans, and different proteases (e.g. chymases, tryptases, granzyme B, and even active caspase-3, Garcia-Faroldi et al., 2013; Metz and Maurer, 2007; Pardo et al., 2007; Zorn et al., 2013) from secretory lysosomes by a process called degranulation and/or secreting *de-novo* produced arachidonic acid metabolites, such as leukotrienes and prostaglandins, as well as various cytokines, chemokines, and growth factors (Metz and Maurer, 2007). Whereas stimulation of MCs *via* innate immune receptors, like toll-like receptors, only activates arachidonic acid metabolism and cytokine production, antigen (Ag)-mediated MC activation *via* the IgE-bound high-affinity receptor for IgE (FcεRI) results in both release of preformed mediators and secretion of *de-novo* synthesized factors (Leal-Berumen et al., 1994).

Basophils are often seen as a blood-borne MC counterpart. Indeed, they share many of the main morphological and functional properties of MCs such as FcεRI expression and the ability to rapidly release histamine, certain eicosanoids (such as LTC_4) and various cytokines. Like MCs basophils also arise from hemopoietic precursor cells but fully mature in the bone marrow rather than at other tissue locations. Although basophils are mainly found in the blood they invade various tissues affected by allergic inflammation, parasite infestation and certain autoimmune diseases. While comparatively rare cells, basophils are thought to play an important role in orchestrating pro-allergic Th2-type immunity since, in humans, they are probably more capable than their MC counterparts in rapidly generating IL-4 and IL-13, archetypal cytokines involved in allergy (reviewed in Falcone et al., 2011). Unlike MCs, basophils are polymorphonuclear cells which produce only very low levels of PGD_2 and do not store large quantities of tryptase or chymase-like proteases. They have also been shown to be relatively insensitive to stem cell factor priming (Frenz et al., 1997)

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and are unresponsive to substance P and to stimulation with poly-basic amines, IgE-independent triggers that can activate connective tissue-like MCs (MC_{TC}). In this regard basophils may resemble more certain functional properties of mucosal-like MCs (MC_T) in humans but unlike these cells their responses are crucially not inhibited by cromolyn. Despite some functional similarities, therefore, there are significant differences as well between basophils and MCs but it is also worth emphasizing that MCs themselves differ considerably in function depending on their tissue location and species.

2. The high-affinity IgE receptor

FcεRI on MCs and basophils usually consists of an α-subunit, a β-subunit, and two disulfide-bridged γ-subunits (FcRγ) ($\alpha\beta\gamma_2$) (Blank et al., 1989). The α-subunit comprises of a short 17 amino acid cytoplasmic tail which binds to the constant Cε3 region of IgE by its second extracellular immunoglobulin-like domain. The β-subunit belongs to the tetraspanin family and together with the γ-subunits it contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain (Reth, 1989). FcεRI crosslinking by multivalent Ag causes tyrosine phosphorylation of these subunits and triggers signaling events by binding to cytoplasmic proteins that contain phosphotyrosine-binding SH2-domains (Turner and Kinet, 1999). Whereas FcεRI on murine MCs and basophils always contains the β-chain (Blank et al., 1989), the receptor can be expressed in two forms in human MCs and basophils, one containing and one lacking the β-chain (Miller et al., 1989). The exclusive expression of the β-chain-containing FcεRI in murine cells is because all three chains must be present for cell surface expression. In human cells, expression of the β-chain is expendable for surface expression of FcεRI but does play a role in amplifier functions (Dombrowicz et al., 1998; Donnadieu et al., 2000). Unlike the form of human FcεRI, which only comprises FcεRIα and FcRγ, FcεRIβ-containing FcεRI shows enhanced FcεRI surface expression and stability as well as augmented stimulatory functions.

3. The bell-shaped dose–response curve phenomenon

MC and basophil activation, for instance degranulation in response to increasing Ag concentrations, follows a peculiar bell-shaped dose–response curve, characterized by weak responses at both low (sub-optimal) and high Ag (supra-optimal) concentrations (Huber, 2013). While it appears logical that low stimulus concentrations cause weak cellular responses, the reduction of responses when supra-optimal Ag concentrations are applied is less easily explained. It is currently accepted that the reduction of MC/basophil responses after stimulation with supra-optimal Ag concentrations is the result of qualitative changes in the interplay of various signal transduction enzymes compared to lower Ag concentrations. As early as 1973, Becker et al. discovered that histamine secretion from human basophils was strongly attenuated in response to supra-optimal FcεRI crosslinking although FcεRI/IgE redistribution was increased under these conditions (Becker et al., 1973). This interesting finding was re-addressed by Magro and Alexander, who concluded that the descending portion of the bell-shaped dose–response curve might be the result of an active turn-off mechanism caused by excessive bridging of the IgE-loaded FcεRI (Magro and Alexander, 1974).

An important next step in unraveling the mechanism of MC/basophil activation by supra-optimal FcεRI engagement was also made by Baird and coworkers who observed that stimulation of IgE-bound FcεRI by anti-IgE induced a detergent-resistant association of these complexes with the cellular cytoskeleton (Robertson et al., 1986). The extent of the cytoskeletal association followed the extent of FcεRI bridging and, crucially, continued to increase beyond the point where anti-IgE concentrations caused maximal degranulation (Robertson et al., 1986). This showed that MC/basophil triggering with supra-optimal Ag concentrations caused intracellular rearrangements despite a lack of degranulation. Oliver et al. confirmed that detergent-insolubility of FcεRI–IgE–Ag complexes did not necessarily follow degranulation events (Seagrave and Oliver, 1990). These authors also showed that blocking actin polymerization increased degranulation following supra-optimal Ag challenge even though detergent-insolubility was diminished (Seagrave and Oliver, 1990). Ag-induced actin polymerization was therefore likely to be part of the inhibitory mechanisms at supra-optimal Ag concentrations (Seagrave and Oliver, 1990). This study also suggested that actin polymerization and associated signaling events were effectively supported by both supra-optimal as well as optimal Ag concentrations.

The above observations have since been verified by our own study in human basophils showing that the kinetics of IgE-dependent histamine release and phosphorylation of various intermediate signal transduction enzymes (e.g. ERK1/2) appear to follow the law of mass action. In other words, the greater the concentration of anti-IgE employed (which was used as an experimental crosslinking agent) the faster the rate of signaling and histamine release despite that maximum responses were strikingly diminished at the supra-optimal range (Gibbs et al., 2006). Interestingly, the phosphorylation of several stimulatory kinases became progressively more transient with increasing supra-optimal concentrations of stimulus suggesting that inhibitory signaling mechanisms increasingly come into play under these settings.

4. IgE-mediated signaling and the role of SHIP1

The regulation of FcεRI signal transduction is increasingly better understood (Gilfillan and Rivera, 2009; Turner and Kinet, 1999) and the blank picture of supra-optimal "attenuating" signaling was filled with several interesting molecules. But first, a short description of general Ag-triggered FcεRI signaling shall be included. The Src family kinase (SFK) Lyn, *via* its unique domain, is constitutively bound to the β-chain of the FcεR1 and, after receptor engagement by multivalent Ag, phosphorylates the ITAMs of the β- and γ-chains (Vonakis et al., 1997; Yamashita et al., 1994). This enables the cytoplasmic tyrosine kinase, Syk, *via* its tandem SH2-domains to interact with the doubly phosphorylated ITAMs of the γ-chains, thus stabilizing Syk in its active conformation, and initiating amplification of several downstream signaling pathways necessary for MC activation (Costello et al., 1996; Jouvin et al., 1994; Kihara and Siraganian, 1994). In basophils Syk also plays a particularly prominent role in determining releasability to IgE-dependent stimulation where Syk deficiency has been shown to be largely responsible for a non-releasing basophil phenotype affecting up to 20% of healthy donors (Laven-Phillips and MacGlashan, 2000; Kepley et al., 2000).

An additional pathway crucial for MC activation is mediated by the SFK Fyn, which phosphorylates the adaptor protein Gab-2, enabling its subsequent interaction with the lipid kinase phosphatidylinositol-3-kinase (PI3K)(Gu et al., 2001; Parravicini et al., 2002). PI3K phosphorylates its substrate, phosphatidylinositol-4,5-bisphosphate (PI-4,5-P2), to produce phosphatidylinositol-3,4,5-trisphosphate (PIP3), an important 2nd messenger for the regulation of different MC activation pathways (Marone et al., 2008). Deficiencies in Fyn, Gab-2 and the p110δ isoform of PI3K have been demonstrated to result in abrogation or severe attenuation of Ag-triggered MC degranulation as well as allergic hypersensitivity responses (Ali et al., 2004; Gu et al., 2001; Parravicini et al., 2002). Additional proof for the importance

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