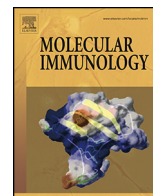




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Review

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

Michael Huber^{a,*}, Bernhard F. Gibbs^b

^a Institute of Biochemistry and Molecular Immunology, RWTH Aachen University, University Clinic, Aachen, Germany

^b Medway School of Pharmacy, University of Kent, Central Avenue, Chatham Maritime, Kent, United Kingdom

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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.

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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 pre-formed 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 LTC₄) 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 PGD₂ 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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* Corresponding author at: Institute of Biochemistry and Molecular Immunology, Medical Faculty, RWTH Aachen University, Pauwelsstr. 30, 52074 Aachen, Germany. Tel.: +49 241 8088717; fax: +49 241 8082428.

E-mail address: mhuber@ukaachen.de (M. Huber).

and are unresponsive to substance P and to stimulation with polybasic 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 (Fcγ₂) (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 Fcγ₂, 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 FcεRI 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

of PI3K-mediated signaling for Ag-triggered MC activation came from the analysis of bone marrow-derived MCs (BMMCs) from mice deficient for the SH2-containing inositol-5'-phosphatase, SHIP1, which hydrolyses PIP3 to yield PI-3,4-P2 (Kalesnikoff et al., 2003). Contrary to p110 δ -deficient BMMCs, SHIP1-deficient BMMCs were found to be more prone to Ag-mediated degranulation than wild-type BMMCs and even degranulated under conditions where wild-type MCs did not i.e. following stimulation with stem cell factor (SCF; also known as mast cell growth factor, steel factor and Kit ligand) or IgE alone (Huber et al., 1998a,b, 2002). These studies established SHIP1 as an important gatekeeper of MC degranulation. Additional signaling pathways important for MC activation are initiated by phospholipase C- γ (PLC- γ), which hydrolyses PI-4,5-P2 concomitantly yielding the 2nd messengers inositol-1,4,5-trisphosphate (IP3) and diacylglycerol (DAG). IP3 induces release of calcium ions from intracellular stores, a mandatory step for subsequent influx of extracellular calcium ions via store-operated calcium channels and secretion of preformed mediators from secretory lysosomes (Baba et al., 2008). DAG is involved in the activation of various PKC isotypes of which PKC- β has been shown to be crucial for induction of Fc ϵ R1-mediated effector functions (Fehrenbach et al., 2009; Nechushtan et al., 2000).

In regard to the bell-shaped dose-response curve of Ag-triggered MC degranulation, the above reports indicate that this curve may not reflect the magnitude of signaling occurring within these cells. Indeed, early overall protein tyrosine phosphorylation was slightly enhanced in supra-optimally vs. optimally stimulated MCs (Gimborn et al., 2005; Kepley et al., 1998). In particular, tyrosine phosphorylation of a 145 kDa protein was increased in parallel to the Ag concentrations used for stimulation and this protein was identified as SHIP1 (Gimborn et al., 2005). This suggested that SHIP1 was involved in regulating the descending part of the bell-shaped dose-response curve, which was further supported by observations showing that SHIP1-deficient BMMCs display almost no reduction of degranulation in response to supra-optimal antigen concentrations (Gimborn et al., 2005; Fig. 1A).

Interestingly, the SFK Lyn has been demonstrated to phosphorylate and activate SHIP1 in MCs and degranulation studies using Lyn^{-/-} BMMCs have revealed that these cells do not display the descending part of the bell-shaped dose-response curve either (Hernandez-Hansen et al., 2004). Lyn also phosphorylates protein kinase C- δ (PKC- δ) and complexes of Lyn with SHIP1 and PKC- δ have been reported (Leitges et al., 2002; Song et al., 1998). Relevant to this, Leitges et al. showed enhanced Ag-triggered degranulation in PKC- δ -deficient BMMCs, especially in response to supra-optimal Ag concentrations (Leitges et al., 2002). These data strongly suggest the existence of an inhibitory signalosome, which seems to be particularly active when MCs are triggered by supra-optimal Ag concentrations.

The Fc ϵ R1 β -subunit seems to play a particularly important role in initiating this inhibitory signalosome. This subunit acts as an amplifier of Fc ϵ R1-induced signals (Dombrowicz et al., 1998; Donnadieu et al., 2000), and has been shown to be highly tyrosine-phosphorylated following supra-optimal stimulation with high Ag concentrations (Dráberová et al., 2004; Gimborn et al., 2005; Xiao et al., 2005). However, supra-optimal triggering not only causes pronounced β -subunit ITAM phosphorylation but was demonstrated to also lead to high levels of Lyn activation, SHIP1 phosphorylation and marked suppression of degranulation (Xiao et al., 2005). The β -subunit contains a unique ITAM which consists of a shorter spacer between its two YXXL sequences as well as an additional tyrosine residue compared to the two γ -subunits of Fc ϵ R1. This unique β -subunit structure allows the recruitment of Lyn to respond to supra-optimal Fc ϵ R1 stimulation and negatively regulate downstream signaling events (Xiao et al., 2005). Thus, the Fc ϵ R1 β -chain and Lyn play a pivotal role in instigating inhibitory

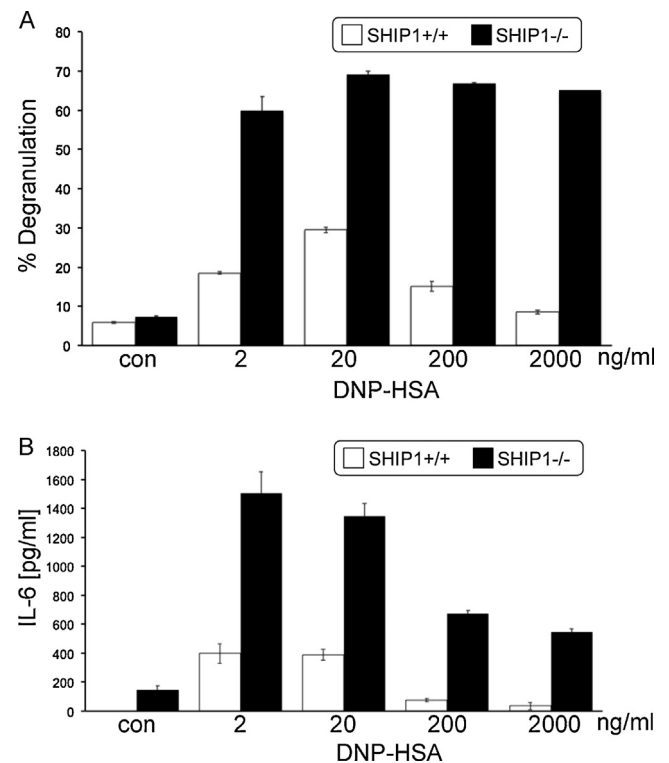


Fig. 1. Augmented antigen-triggered effector responses in SHIP1-deficient mast cells. (A) IgE-loaded SHIP1^{+/+} (white bars) and SHIP1^{-/-} (black bars) BMMCs were left unstimulated (con) or stimulated with the indicated concentrations of Ag (DNP-HSA) for 20 min and degranulation was analyzed performing beta-hexosaminidase assays. (B) SHIP1^{+/+} (white bars) and SHIP1^{-/-} (black bars) BMMCs were treated as under (A) with the exception that stimulation was for 3 h. Production/secretion of IL-6 was measured in the supernatants by ELISA. Data are mean $\pm$ SD of triplicate experiments. Comparable results were obtained in independent experiments using different BMMC cultures.

Fc ϵ R1-dependent signaling which involves the activation of SHIP1 and subsequent control of the PI3K pathway, which plays a prominent role in Fc ϵ R1-mediated activation of MCs and basophils.

In addition to SHIP1, other PIP3 phosphatases that are involved in the control of PI3K signaling in MCs include the 3'-phosphatase PTEN and the 5'-phosphatase SHIP2. Target gene expression silencing approaches using shRNA in BMMCs showed that SHIP2 knock-down gave rise to enhanced Fc ϵ R1-mediated degranulation independent of the Ag concentration employed and was not substantial following supra-optimal triggering (Leung and Bolland, 2007). Similarly, reducing the expression of PTEN, which is a major tumor suppressor expressed in most tissues, was shown to increase IgE-dependent Ca²⁺ mobilization and human MC degranulation (Furumoto et al., 2006), although its role in affecting supra-optimal crosslinking has not been investigated. Interestingly, PTEN does appear to play a role in regulating the constitutive levels of PIP3 in resting MCs, unlike SHIP1 and SHIP2 (Furumoto et al., 2006). Thus, other phosphatases such as PTEN and SHIP2 clearly play a role in governing MC and basophil releasability to IgE-mediated triggering using sub-optimal Ag concentrations whereas SHIP1 appears to be particularly involved in suppressing mediator release from these cells by high Ag concentrations.

5. Structure, function and interactome of SHIP1

SHIP1 expression is restricted to hematopoietic cells where it negatively regulates myeloid cell proliferation and survival as well as suppressing supra-optimal Ag triggering of allergic effector cells. It is thought that these actions are caused by the hydrolyzing

activity of SHIP1 on PIP3, a major stimulatory product of the PI3K pathway, leading to the generation of PI-3,4-P2 and a marked reduction in important PIP3-dependent pathways. These include Ca^{2+} mobilization, Btk activation and, ultimately, cessation of mediator synthesis and release (Bolland et al., 1998; Hata et al., 1994). However, SHIP1 has both catalytic and adaptor functions within its structure (Rohrschneider et al., 2000) and PI-3,4-P2 itself may have important effects due to interacting with the PH-domains of several adaptor proteins such as Bam32/DAPP1, TAPP1, and TAPP2 (Marshall et al., 2002). Interestingly, Bam32-deficient mouse MCs display enhanced Ca^{2+} mobilization, PLC- γ 1 phosphorylation and degranulation to supra-optimal IgE-mediated activation. This clearly suggests that this adaptor protein may mediate a portion of the negative signaling functions of SHIP1 (Hou et al., 2010). However, the extent of adaptor as opposed to catalytic function input needs to be verified in primary MCs and basophils, especially of human origin.

The adaptor/scaffolding function of SHIP1, has revealed a number of potential interaction partners. The phosphatase expresses an N-terminal SH2-domain, a centrally located 5'-phosphatase domain, as well as a C-terminus which contains several proline-rich sequences and two NPXY motifs (Rohrschneider et al., 2000). Phosphorylated ITAM sequences on the Fc ϵ RI β -chain could interact with the SH2-domain (Kimura et al., 1997; Osborne et al., 1996). Moreover, three of the proline-rich motifs on the C-terminus of SHIP1 have been shown to associate with SH3-domains of various proteins including Grb2, CIN85, and Src kinase (Büchse et al., 2011; Kalesnikoff et al., 2003; Wisniewski et al., 1999). Importantly, Lyn can also bind to SHIP1 due to its SH3-domain. Indeed, we have observed that SHIP1 can be pulled-down from BMMC lysates using a GST-SH3(Lyn) fusion protein (data not shown). A pleckstrin homology-related domain has also been discovered to mediate membrane localization of SHIP1 by binding to PIP3 (Ming-Lum et al., 2012).

The adaptor proteins Shc and p62Dok1, which contain phosphotyrosine-binding domains (PTB), bind to SHIP1's C-terminal NPXY motifs after phosphorylation (Lamkin et al., 1997; Ott et al., 2002; Tamir et al., 2000). Fc ϵ RI-induced Shc tyrosine phosphorylation depends on SHIP1 expression (Huber et al., 1998a) and Shc may therefore limit SHIP1's activity at the Fc ϵ RI by moving the enzyme away from the receptor following tyrosine phosphorylation of Shc by Lyn. However, Shc may also serve as a linker between SHIP1 and PKC- δ through association of its SH2-domain with PKC- δ (Leitges et al., 2002). Shc (ShcA), is expressed as three isoforms (p46shc, p52shc, and p66shc) with p46shc and p52shc being expressed ubiquitously and p66shc showing a more restricted expression pattern. p46shc and p52shc were shown to be involved in Ras-MAPK activation in the context of epidermal growth factor stimulation, however, p66shc appeared to act rather inhibitory in the context of this signaling pathway (Migliaccio et al., 1997). p66shc has been demonstrated to be expressed in MCs and the study of Fc ϵ RI signaling in p66shc-deficient BMMCs has revealed that p66shc is limiting Ag-triggered degranulation and proinflammatory cytokine secretion (Ulvieri et al., 2011). Furthermore, p66shc was demonstrated to promote SHIP1 recruitment to the transmembrane adaptor LAT offering a molecular mechanism for p66shc's negative regulatory function (Roget et al., 2008; Ulvieri et al., 2011). Of importance, however, the suppressive function of p66shc with respect to MC degranulation did not appear to be involved in regulating supra-optimal Fc ϵ RI signaling (Ulvieri et al., 2011).

Another adaptor protein, p62Dok1, is also known to bind to SHIP1 and inhibit p21Ras and thus serves as an important negative regulator of the Erk1/2 MAPK pathway (Ott et al., 2002; Tamir et al., 2000). Erk1/2 itself may also be directly involved in upregulating Fc ϵ RI-induced MC mediator release. Xu showed that Erk1 is an

integral part of a feed-forward loop which upregulates Syk activity following Fc ϵ RI crosslinking and pharmacological blockade of the Erk kinase MEK reduced IgE-dependent MC degranulation (Xu et al., 1999), a result that has since been verified using novel, highly selective MEK inhibitors (Marschall et al., 2012).

Erk1/2 could also positively regulate MC degranulation by an alternative mechanism reported by Pozo-Guisado et al. who showed that this MAPK phosphorylates STIM1, which controls store-operated calcium entry, an essential step in facilitating mediator synthesis and degranulation (Baba et al., 2008; Pozo-Guisado et al., 2010). Because SHIP1 tyrosine phosphorylation is enhanced upon supra-optimal Fc ϵ RI stimulation (Gimborn et al., 2005) which results in greater p62Dok1-SHIP1 binding (Tamir et al., 2000), Erk1/2 input in IgE-mediated signaling could be abrogated resulting in diminished degranulation.

In contrast to the above, the MEK-Erk1/2 pathway seems to play a more differential role in human basophils, where pharmacological inhibition leads only to a suppression of LTC₄ production but not degranulation or cytokine synthesis (Gibbs and Grabbe, 1999). Jun kinase, another MAPK family member, plays a prominent role in MC function but not in basophils (Gibbs et al., 2005). Although SHIP1 appears to be a universally important regulator of both MC and basophil activity, the above examples indicate that not all IgE-dependent signal transduction pathways are identical in these cells. Thus, the regulation of MC/basophil activation under supra-optimal Ag conditions potentially use a combination of catalytic as well as adaptor functions of SHIP1 and it is essential to elucidate all these functions in different allergic effector cell types in the future.

6. Putting SHIP1 into context

The releasability of MCs and basophils to IgE-dependent stimulation and the shape of the dose-response curve is unlikely to depend solely on the simple interplay of increasing SHIP1 activity cancelling stimulatory signaling. In human basophils, relative Syk expressions strongly determine basophil releasability *per se* (MacGlashan, 2007), though not in human lung MCs (Havard et al., 2011). Altered stimulatory signaling at high Ag concentrations may itself also play a role in concert with increased inhibitory input. Comparing IgE-induced histamine release in wild-type, Lyn-deficient, and Btk-deficient BMMCs, it has been shown that, although degranulation in Lyn-deficient BMMCs at high Ag concentrations is not reduced compared to wild-type cells, Btk-deficient BMMCs function is more strikingly attenuated than wild-type BMMCs (Kawakami et al., 2000). This supports the notion of a positive regulatory role for Btk in IgE-dependent signaling (Hata et al., 1998). The generation and integration of both positive and negative signals, therefore, ultimately govern the magnitude and type of response.

Although SHIP1 is likely to play a prominent role in the attenuation of MC and basophil responses caused by high, supra-optimal Ag concentration challenge, it is certainly not the only inhibitory signal. The releases of various mediators from these cells follow marked differences in the shape of the bell-shaped curve. Although degranulation (histamine release) and the production and release of *de novo*-generated eicosanoids and cytokines all give rise to bell-shaped curves, the descending portion of these curve is considerably steeper for eicosanoid and cytokine release (Fig. 1B; Fehrenbach et al., 2009; Gibbs et al., 2006; Gimborn et al., 2005; Ra et al., 2012). In the case of human basophils, the releases of IL-4 and IL-13 are not only more bell-shaped but the curves are leftward-shifted compared to histamine release (Gibbs et al., 1996, 2006). Rivera and coworkers have also reported that cytokine and chemokine releases caused by IgE-mediated triggering required different Ag concentrations for their optimal expression (Gonzalez-Espinosa et al., 2003).

The control of MC and basophil mediator releases is therefore not dependent on merely one activating and one inhibitory signal but involve the interplay of differential signaling networks. Despite this complexity, SHIP1 does appear to be majorly involved in negative regulation of both immediate (degranulation) and sustained (cytokine production) FcεRI-mediated MC/basophil activation with a particularly prominent effect at high, supra-optimal Ag concentrations (Fig. 1B; Fehrenbach et al., 2009; Gimborn et al., 2005). With respect to Ag-triggered cytokine secretion, SHIP1 has been demonstrated to control NFκB and 38^{MAPK} activation (Kalesnikoff et al., 2002), both of which are well-known to be crucial for gene transcription as well as mRNA stability (Ronkina et al., 2010; Saccani et al., 2002).

7. Conclusion

The fact that MCs and basophils release mediators that follow a bell-shaped dose–response curve provides us with an interesting shut-off mechanism of FcεRI responses. It is tantalizing to see whether a better understanding of this inhibitory mechanism could in future be exploited in the treatment of allergies. To date, SHIP1 has been identified as a prominent inhibitory signal in these allergic effector cells, although other candidates are likely to be discovered. Shedding more light on these inhibitory mechanisms will also help us understand the concept of releasability of the cells, especially in the context of optimizing allergen immunotherapy and rush desensitization strategies. Moreover, the bell-shaped dose–response phenomenon is not only restricted to FcεRI but also FcγRIII (CD16) and B cell antigen receptors (Campbell et al., 1992; Hazenbos et al., 1996). A greater understanding of FcεRI signaling control would also enhance that of other important receptors involved in other immunological diseases. Finally, identifying cell surface receptors and their ligands that directly enhance the activity of inhibitory signals such as SHIP1 could greatly facilitate utilizing inhibitory signaling as a means of therapy for these diseases. Indeed, a small-molecule SHIP1 activator, AQX-1125, has been developed and was shown to suppress leukocyte accumulation and release of pro-inflammatory mediators in rodent models of allergy and pulmonary inflammation (Stenton et al., 2013).

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