

REGULATION OF MAST-CELL AND BASOPHIL FUNCTION AND SURVIVAL BY IgE

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Mast cells and basophils are important effector cells in T helper 2 (T_H2)-cell-dependent, immunoglobulin-E-associated allergic disorders and immune responses to parasites. The crosslinking of IgE that is bound to the high-affinity receptor FcεRI with multivalent antigen results in the aggregation of FcεRI and the secretion of products that can have effector, immunoregulatory or autocrine effects. This response can be enhanced markedly in cells that have been exposed to high levels of IgE, which results in the increased surface expression of FcεRI. Moreover, recent work indicates that monomeric IgE (in the absence of crosslinking) can render mast cells resistant to apoptosis induced by growth-factor deprivation *in vitro* and, under certain circumstances, can induce the release of cytokines. So, the binding of IgE to FcεRI might influence mast-cell and basophil survival directly or indirectly, and can also regulate cellular function.

ATOPY

A word meaning 'strange disease' that was coined to describe the familial syndrome of asthma and hay fever. Atopic subjects are predisposed to develop strong IgE responses to common environmental antigens.

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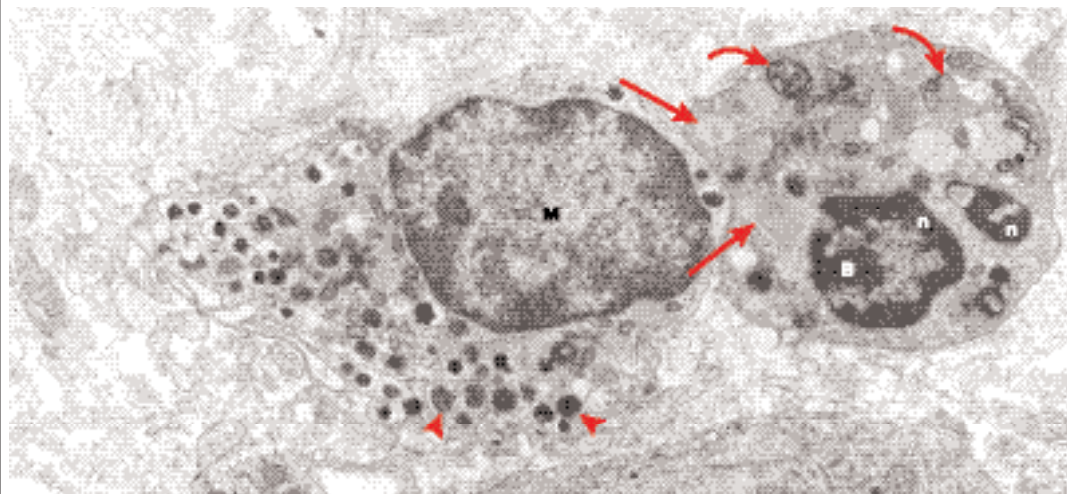
Mast cells are derived from haematopoietic progenitor cells that enter nearly all vascularized tissues, where they complete their maturation and, under some circumstances, can then migrate into epithelia^{1–4}. By contrast, basophils are a type of circulating granulocyte that typically mature in the bone marrow, circulate in the blood and can be recruited to tissues at sites of immunological or inflammatory responses^{5,6}. After appropriate activation (for example, through aggregation of the high-affinity receptor for immunoglobulin E, FcεRI), mast cells and basophils can produce a similar, but not identical, spectrum of pro-inflammatory mediators, including certain cytokines and chemokines^{5–8} (BOX 1; TABLE 1). Crosslinking of FcεRI-bound IgE with multivalent antigen initiates the activation of mast cells and basophils by promoting the aggregation of FcεRI^{9,10}. This FcεRI-dependent cell-activation process has three outcomes: degranulation, with the secretion of preformed mediators that are stored in the cytoplasmic granules of the cell (for example, vasoactive amines, neutral proteases, proteoglycans and some cytokines and growth factors); the *de novo* synthesis of pro-inflammatory lipid

mediators; and the synthesis and secretion of cytokines and chemokines^{2,7–10}.

Mast cells and basophils are regarded as key effector cells in IgE-associated immediate hypersensitivity reactions and allergic disorders, as well as in certain protective immune responses to parasites¹¹. Because the IgE-dependent release of mediators can begin within minutes of antigen challenge, the crucial role of mast cells and basophils in acute allergic reactions (such as anaphylaxis and acute attacks of ATOPIC asthma) is now widely accepted^{2,6,7,9,10}. By contrast, the importance of these cells (compared with other potential effector cells) for the chronic inflammation and other long-term tissue changes that are observed in some IgE-associated disorders, including asthma, has been debated for some time¹². However, two recent studies have shown that mast cells can markedly enhance the antigen-dependent airway hyperreactivity^{13,14}, airway eosinophil infiltration¹³ and increased number of proliferating cells in the airway epithelium¹³ that are induced in mouse models of asthma that either omit artificial adjuvants at the time of antigen sensitization¹³ or use low doses of antigen

Box 1 | Morphology of human mast cells and basophils

Shown below is a transmission electron micrograph (osmium collidine uranyl *en bloc* processing) showing a mast cell (M) and basophil (B) in the ileal submucosa of a patient with Crohn's disease. The mast cell is the larger, mononuclear cell; it has a more complex plasma-membrane surface and cytoplasmic granules (arrowheads) that are smaller and more numerous than those (arrows) of the basophil. In this section plane, the basophil has two nuclear lobes (n). Several basophil cytoplasmic granules are visible that contain whorls of membrane (curved arrows). Reproduced, with permission, from REF. 131.



challenge^{13,14} This work, and other lines of evidence, indicate that a key role of mast cells in IgE-associated immune responses is to amplify both acute and long-term local tissue responses to relatively weak biological signals^{7,11,13,14}. Moreover, through their ability to release immunoregulatory cytokines, such as interleukin-4 (IL-4) and IL-13, and perhaps through other mechanisms^{7,15–17}, mast cells and basophils might also influence the development, strength and/or persistence of T_H2 (T_H2)-cell-associated immune responses. But, such immunoregulatory functions of mast cells or basophils have not been shown yet *in vivo*.

Mast cells and basophils can be activated to express effector functions by many mechanisms that are independent of FcεRI, and these cells are now thought to participate in a wide variety of physiological and pathological processes independently of IgE^{2,3,6,11}. For example, mast cells have been shown to be essential for certain innate immune responses, and they have been implicated as potentially crucial effectors in many other settings (such as angiogenesis, host responses to neoplasia and certain autoimmune disorders)^{2,3,6,7,11}. However, in this review, we concentrate on the proven and potential mechanisms by which IgE, through interactions with FcεRI, can influence the function and survival of mast cells and basophils directly or indirectly.

IgE-dependent activation and effector function

Activation by FcεRI aggregation. The identification of IgE as the long-sought 'reagin' — that is, the activity in plasma that can sensitize tissues in naive subjects to express immediate hypersensitivity reactions within minutes of challenge with specific antigen — was

an important advance in our understanding of the pathogenesis of allergic diseases^{18,19}. This understanding was enhanced by the identification and molecular characterization of FcεRI, the high-affinity receptor for IgE that confers on tissues — specifically, the mast cells that are present in these tissues — the ability to bind IgE and retain it for long periods of time (reviewed in REFS 9,10). Although IgE can also bind to the IgG receptors FcγRII and FcγRIII, and to galectin-3, which are expressed on some mast-cell populations (and to CD23 (FcεRII), which is not expressed on mast cells or basophils), IgE is thought to influence mast-cell and basophil function mainly through its interaction with FcεRI^{9,10}. In mice, mast cells can also be activated to degranulate and release mediators in response to antigen- and IgG₁-dependent signalling through FcγRIII, and this is an additional mechanism to elicit mast-cell function during T_H2-cell-associated responses^{3,10,12,20}.

The mouse FcεRI is a heterotetrameric receptor composed of an IgE-binding α-subunit, a four-transmembrane-spanning β-subunit and two disulphide-bonded γ-subunits. The γ-subunits are signal-transducing molecules, whereas the β-subunits can amplify the signal that is generated by the γ-subunits^{9,10}. FcεRI binds IgE with an affinity of $1 \times 10^{10} \text{ M}^{-1}$, and this interaction has a slow rate of dissociation^{9,10}. In mice, only mast cells and basophils normally express FcεRI (αβγ₂)¹⁰. Similarly, human mast cells and basophils express FcεRI that consists of all three subunits (αβγ₂), whereas an αγ₂ form of FcεRI is expressed on the surface of human monocytes and other cells¹⁰ (BOX 2). The structural basis for the 1:1 interaction between IgE and FcεRI^{21,22} and the regulation of mast-cell activation through FcεRI (FIG. 1 and see below) is understood in some detail^{9,10}.

T HELPER 2 CELL
(T_H2 cell). A type of T cell that, through the production of IL-4, IL-13 and other cytokines, can help B cells to produce IgE and other antibodies and, through the secretion of IL-5, IL-3 and others, can promote increased numbers of eosinophilic granulocytes (eosinophils), basophils and mast cells.

Table 1 | Selected products of human mast cells and basophils

Feature	Basophils*	Mast cells*
Main mediators stored preformed in cytoplasmic granules	Histamine, chondroitin sulphates, neutral protease with bradykinin-generating activity, β -glucuronidase, elastase, cathepsin-G-like enzyme, major basic protein, Charcot–Leyden crystal protein, peroxidase, carboxypeptidase A [†]	Histamine, heparin and/or chondroitin sulphates, neutral proteases (chymase and/or tryptase [‡]), major basic protein, many acid hydrolases, cathepsin, carboxypeptidases, peroxidase
Main lipid mediators produced after appropriate activation	Leukotriene C ₄	Prostaglandin D ₂ , leukotriene C ₄ , platelet-activating factor
Cytokines released after appropriate activation [¶]	IL-4, IL-13	TNF, MIP1 α , VPF/VEGF, IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, IL-16, GM-CSF, MCP1 (and probably many more)

*Adapted from REF. 132. [†]Under certain conditions, tryptase-, chymase-, carboxypeptidase-A- and c-Kit-positive granulated cells that seem to be basophils by morphology and react with an antibody specific for BSP1 (which stains basophils, but not mast cells) can be observed in the peripheral blood. Adapted from REF. 133. [‡]Several lines of evidence indicate that certain cytokines produced by mast cells, such as tumour-necrosis factor (TNF) and vascular permeability factor (VPF)/vascular endothelial growth factor (VEGF), are released in part from pre-formed stores, some of which might be associated physically with the cytoplasmic granules of the cell. GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin; MCP1, monocyte chemotactic protein 1; MIP1 α , macrophage inflammatory protein 1 α (CCL3).

ITAM
(Immunoreceptor tyrosine-based activation motif). A sequence — YxxL/I(x)_{6–8}YxxL/I — that is present in the cytoplasmic domains of various activating receptors on immune cells, including Fc ϵ RI, that has a crucial role in recruiting SRC- and SYK-family protein-tyrosine kinases.

LIPID RAFTS
Areas of the plasma membrane that are rich in cholesterol, glyco-sphingolipids, several signalling proteins (such as SRC kinases, RAS, LAT, CBP/PAG) and glycosylphosphatidylinositol-anchored proteins. Also known as glycolipid-enriched microdomains (GEMs) and detergent-insoluble glycolipid-enriched membranes (DIGs).

OSMIOPHILIC PATCHES
A form of plasma-membrane heterogeneity that is detected as 'electron-dense' (osmiophilic) areas, which, in mast cells, can be rich in Fc ϵ RI and some signalling molecules.

ITIM
(Immunoreceptor tyrosine-based inhibitory motif). The cytoplasmic sequence — I/VxYxxL — that is present in various inhibitory immunoreceptors, such as Fc γ RIIB, gp49B1, PIRB and MAFA, is required and sufficient for their inhibitory action by recruiting SHIPs and SHPs through SH2-phosphotyrosine interactions.

Despite the lack of intrinsic enzymatic activity of the receptor, crosslinking of Fc ϵ RI with IgE and antigen (or other reagents) induces the activation of protein-tyrosine kinases (PTKs), including those of the **SRC**, **SYK** and **TEC** families^{23–25}. In the current model of the sequence of activation events, which is based on many studies²⁶, **LYN** — a SRC-family PTK that is expressed predominantly in mast cells and is associated constitutively with the β -subunit of Fc ϵ RI — is activated by Fc ϵ RI aggregation and then phosphorylates tyrosine residues in the immunoreceptor tyrosine-based activation motifs (ITAMs)²⁷ of the β - and γ -subunits of the receptor. Phosphorylated **ITAMs** of the β - and γ -subunits recruit additional **LYN** molecules and SYK, respectively, through interactions with the Src-homology 2 (**SH2**) domains of the PTKs. SYK bound to Fc ϵ RI γ is activated through conformational change and tyrosine phosphorylation by **LYN** or newly activated SYK^{28,29}. Active SYK then phosphorylates many substrates, including **LAT** (linker for activation of T cells), **SLP76** (SH2-domain-containing leukocyte protein of 76 kDa) and **VAV**, which leads to the activation of several signalling pathways, such as those through phosphatidylinositol 3-kinase (**PI3K**), phospholipase C γ (**PLC γ**), RAS/ERK (extracellular signal-regulated kinase), c-Jun N-terminal kinase (JNK), p38 and **AKT** (FIG. 1). These pathways lead eventually to degranulation, the synthesis and release of lipid mediators, and the production and secretion of cytokines, chemokines and growth factors by mast cells and basophils.

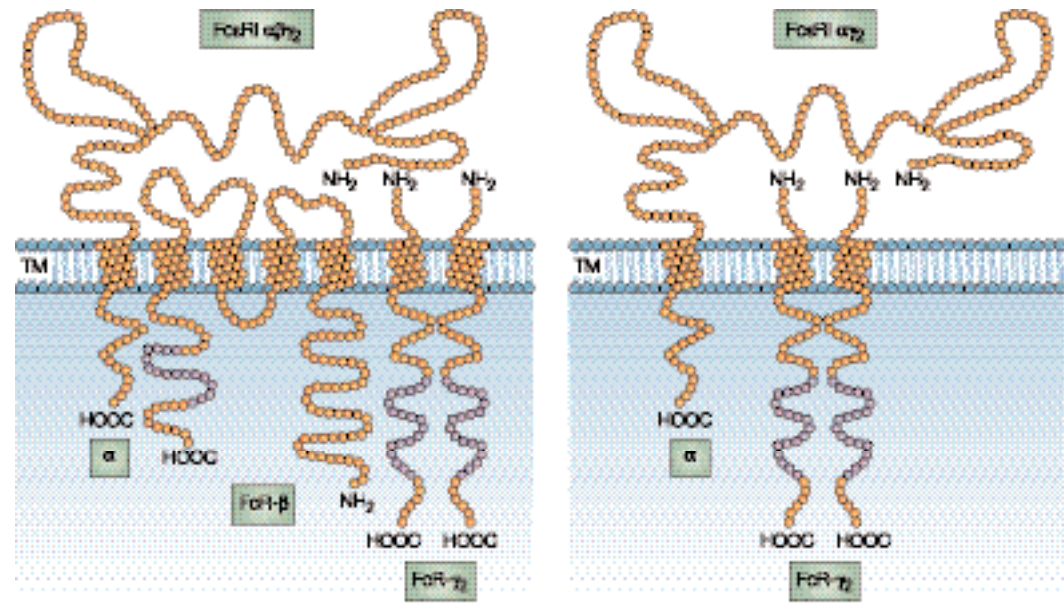
The questions of where the initial activation events take place and the role of membrane heterogeneity (particularly, **LIPID RAFTS**) in this process have been studied intensely^{30–35}. Fc ϵ RI aggregation induces the recruitment of Fc ϵ RI to lipid rafts, which are rich in cholesterol, glyco-sphingolipids and several signalling proteins, such as SRC-family PTKs, RAS and glycosylphosphatidylinositol (GPI)-anchored proteins. These observations indicate that aggregated Fc ϵ RI might move into **LYN**-containing ordered lipid microdomains and thereby initiate Fc ϵ RI signalling. Recent immunoelectron-microscopy

studies^{36,37} indicate the presence of two signalling domains: primary domains, which are formed after Fc ϵ RI aggregation and are localized to the **OSMIOPHILIC PATCHES**, include Fc ϵ RI, SYK, PLC γ 2, PI3K and **GAB2** (GRB2-associated binding protein 2), but exclude **LYN** and **THY1**; whereas secondary signalling domains include **LAT**, PLC γ 1 and PI3K. However, the precise relationship between such osmiophilic patches and lipid rafts remains to be determined.

Negative regulation of Fc ϵ RI signalling. Activation through ITAM-containing receptors is counter-regulated by the co-aggregation of immunoreceptor tyrosine-based inhibitory motif (ITIM)-containing receptors^{38–40}. Mast cells express the ITIM-containing receptors **Fc γ RIIB** (a low-affinity IgG receptor), gp49B1 (a member of the C2 family of the immunoglobulin superfamily), **PIRB** (a paired immunoglobulin-like receptor) and **MAFA** (mast-cell function-associated antigen). ITIMs, which contain the consensus sequence I/VxYxxL, are required and sufficient for the inhibitory action of these receptors⁴¹. Fc γ RIIB contains two extracellular immunoglobulin-like domains and a single ITIM sequence in its cytoplasmic domain. When co-aggregated with Fc ϵ RI (FIG. 2), Fc γ RIIB is phosphorylated on the tyrosine residue in the ITIM by Fc ϵ RI β -associated **LYN**, and phosphorylated ITIMs recruit SH2-domain-containing inositol polyphosphate 5' phosphatase (**SHIP**)⁴². SHIP catalyses the hydrolysis of phosphatidylinositol-3,4,5-trisphosphate (PtdInsP₃), which inhibits the Fc ϵ RI-induced membrane recruitment and activation of **BTK** and PLC γ . This model can explain how Fc γ RIIB inhibits Fc ϵ RI-induced Ca²⁺ mobilization. The co-aggregation of Fc γ RIIB with the B-cell receptor (BCR) induces the tyrosine phosphorylation of SHIP and its subsequent binding to **SHC**⁴³ and the rasGAP-binding protein **DOK1** (REF. 44). These findings indicate that SHIP inhibits BCR-induced activation of RAS by sequestering SHC away from the growth-factor-receptor-bound protein 2 (**GRB2**)–**SOS** complex, or that SHIP-bound, tyrosine-phosphorylated DOK1 binds rasGAP, which inhibits

Box 2 | Subunit structure of human FcεRI in different cell types

Subunits of human FcεRI (the high-affinity receptor for immunoglobulin E) can be expressed as $\alpha\beta\gamma_2$ and $\alpha\gamma_2$ complexes on transfectants. The $\alpha\gamma_2$ complexes are less stable in detergent than the $\alpha\beta\gamma_2$ complexes. The β -subunit confers stability on the receptor and amplifies the signal that is generated by the γ -subunit. Human mast cells and basophils express the β -subunit, whereas monocytes, Langerhans cells and dendritic cells do not. Messenger RNA encoding the β -subunit can be detected by PCR in human eosinophils and platelets. It has not been determined whether mast cells and other β -subunit-positive cells express only $\alpha\beta\gamma_2$ complexes or both types of complex ($\alpha\gamma_2$ and $\alpha\beta\gamma_2$) in a variable proportion. The purple regions indicate immunoreceptor tyrosine-based activation motifs (ITAMs). TM, transmembrane. Adapted, with permission, from the *Annual Review of Immunology*, Volume 17 ©1999 by Annual Reviews www.annualreviews.org (REF. 134).



RAS by enhancing its intrinsic GTPase activity. Consistent with this model, FcγRIIB-knockout mice have markedly enhanced IgE-associated anaphylaxis⁴⁰, and bone-marrow-derived cultured mast cells (BMCs) from *Ship*^{-/-} mice are extremely sensitive to FcεRI stimulation⁴⁵.

gp49B1 is also a type I transmembrane protein with two extracellular immunoglobulin-like domains, but it has a cytoplasmic tail that contains two ITIMs. The co-aggregation of gp49B1 with FcεRI induces tyrosine phosphorylation of the ITIMs and recruitment of SH2-domain-containing protein tyrosine phosphatase 1 (SHP1)^{46,47}. Both ITIM phosphorylation and SHP1 recruitment are required for gp49B1-dependent inhibition of mast-cell activation⁴⁷. The ligand for gp49B1 has been identified recently as the $\alpha\beta_3$ integrin⁴⁸. Importantly, gp49B1-deficient mice have significantly increased expression of IgE-dependent ACTIVE OR PASSIVE CUTANEOUS ANAPHYLAXIS and active systemic anaphylaxis⁴⁹.

PIRB has four extracellular immunoglobulin-like domains and a long cytoplasmic tail that contains four ITIMs. No ligand has been identified for this receptor yet. When the cytoplasmic tail of PIRB is expressed as a chimaeric receptor with the extracellular domain of FcγRIIB, PIRB inhibits FcεRI- and BCR-induced mast-cell and B-cell activation, respectively^{50–52}. The two most carboxy-terminal ITIMs can bind SHP1 and SHP2. Unlike FcγRIIB, PIRB inhibits BCR-induced tyrosine phosphorylation of Igα/Igβ, SYK, BTK and PLCγ2,

and Ca²⁺ mobilization⁵³. These findings indicate that SHP1 dephosphorylates and deactivates SYK and BTK, which leads to decreased activation of PLCγ2. However, the inhibitory activity of PIRB is not affected in SHP1-deficient mast cells⁵⁴, which indicates that PIRB also uses SHP1-independent mechanisms of inhibition.

MAFA, a type II transmembrane protein of the C-type lectin family, the ligand for which remains to be determined^{55,56}, can interact physically with FcεRI^{57,58}. The amino-terminal cytoplasmic tail has an ITIM-like sequence as the signalling motif, and phosphorylated peptides corresponding to the ITIM-like sequence can bind SHP2 and SHIP directly, but not SHP1 (REF. 59). After stimulation of MAFA, SHP2 can dephosphorylate SYK and LAT, thereby reducing the magnitude of downstream coupling processes, such as Ca²⁺ release from intracellular stores⁶⁰. The recruitment of SHIP might initiate an even more important inhibitory pathway (apparently involving the hydrolysis of PtdInsP₃) that results in reduced membrane mobilization of BTK and PLCγ, as well as the inhibition of RAS by sequestration of SHC and SHIP–DOK1–rasGAP complexes⁵⁹.

Roles of monomeric IgE. Several lines of evidence now indicate that the binding of IgE to FcεRI can have biologically important consequences beyond merely ‘sensitizing’ the cells to undergo activation in response to antigen-mediated aggregation of IgE-bound FcεRI. About 25 years ago, two groups showed that the saturating level of

ACTIVE CUTANEOUS ANAPHYLAXIS

A skin inflammatory response induced by the injection of antigen in subjects who have undergone active immunization with antigen, instead of passive priming with IgE.

PASSIVE CUTANEOUS ANAPHYLAXIS

An inflammatory response that can be elicited within minutes of antigen challenge in IgE-primed (and, in the mouse, IgG₁-primed) skin.

cell-bound IgE (and, therefore, the expression level of FcεRI) on human basophils shows a positive correlation with the serum concentration of IgE^{61–63}. Although these studies did not prove that IgE can influence the expression of FcεRI directly, Furuichi *et al.*⁶⁴ and Quarto *et al.*⁶⁵ have shown that RBL-2H3 rat mast cells have a moderate, and cycloheximide-insensitive (therefore, independent of *de novo* protein synthesis)⁶⁴, increase in the surface expression of FcεRI after incubation with IgE, which is mainly owing to the reduced elimination of FcεRI from the cell surface^{64,65}. More recently, two groups^{66–70} have shown that incubation with monomeric IgE can markedly increase FcεRI surface expression *in vitro* in mouse and human mast cells and basophils (in mouse mast cells, through both cycloheximide-insensitive and -sensitive mechanisms⁶⁷), and that such cells have enhanced IgE-dependent function^{66–70}. Moreover, studies in *IgE*^{−/−} mice indicate that IgE can markedly enhance mast-cell⁶⁷ and basophil⁶⁹ surface expression of FcεRI *in vivo*.

Mouse mast cells that have undergone IgE-induced enhancement of FcεRI surface expression have increased sensitivity to antigen and an increased release of serotonin, IL-4 and other cytokines in response to antigen challenge⁶⁷. Human mast cells also have a marked increase in IgE binding in response to exposure to IgE and have an enhanced ability to secrete histamine, macrophage inflammatory protein 1α (MIP1α; also known as CCL3)⁶⁸ and lipid mediators⁷⁰ after FcεRI aggregation. As CCL3 (similarly to IL-4 and IL-13, another product of human and mouse mast cells⁷¹) has been reported to increase the production of IgE by human B cells, these observations point to a potential positive-feedback mechanism ($\uparrow\text{IgE} \rightarrow \uparrow\text{Fc}\epsilon\text{RI} \rightarrow \uparrow\text{antigen-}, \text{IgE- and Fc}\epsilon\text{RI-dependent release of IL-4, IL-13, CCL3 and so on} \rightarrow \uparrow\text{IgE}$) by which mast cells might enhance the further development and persistence of T_H2-biased, IgE-associated immune responses (see FIG. 3 for an updated version of this model)^{12,67,68}.

Binding of IgE to FcεRI can enhance the surface expression of FcεRI as a result of its ability to promote the stabilization/accumulation of FcεRI on the mast-cell surface in the presence of continued basal levels of protein synthesis^{72,73}. Indeed, it is possible that most (if not all) of the enhanced IgE-dependent function that is observed after antigen- or anti-IgE-induced FcεRI aggregation in cells that have undergone IgE-dependent increases in FcεRI surface expression is a consequence of the higher level of FcεRI expression *per se*.

But, can monomeric IgE also have more direct effects on mast-cell function? Many studies over the years, including our own recent paper⁷⁴, have shown no evidence that the binding of monomeric IgE can induce detectable signalling or production of mediators by mast cells. However, Kalesnikoff *et al.*⁷⁵ reported that the response of mouse BMCMCs to the monoclonal IgE SPE-7 has some similarities to the response that is induced by FcεRI aggregation, in that biochemical activation events (for example, FcεRIβ phosphorylation, activation of mitogen-activated protein kinases (MAPKs) and AKT, and increased BCL-X_L expression),

are induced and some cytokines are secreted⁷⁵. However, the same monomeric IgE did not induce degranulation or the release of leukotriene C₄ (LTC₄).

As described below, both our study⁷⁴ and that by Kalesnikoff *et al.*⁷⁵ found that monomeric IgE enhanced mast-cell survival, and both groups tried to show that the effects that were observed were due to monomeric IgE, rather than IgE aggregates. Kalesnikoff *et al.*⁷⁵ proposed that certain monoclonal IgEs can induce small clusters of FcεRI aggregates in lipid rafts of mast cells by reducing repulsion between the neighbouring FcεRI molecules. A slow on-rate of IgE interaction with FcεRI, but with prolonged activation of MAPKs, might result in a type of incomplete activation that favours cytokine production and secretion. Of the other possible explanations for the apparent aggregation of FcεRI (for example, with SPE-7 IgE) in the absence of specific antigen, it should be considered that unknown factors might result in interactions between IgE molecules that, in turn, influence FcεRI function. For example, FcεRI aggregation could be induced by bi- or multivalent compounds (in tissue-culture media or biological fluids) that are immunochemically crossreactive with the specific antigen that is recognized by the monoclonal IgE, or that bind to IgE by other mechanisms.

In contrast to SPE-7 IgE, the IgE that we used (H1 DNP-ε-206) did not detectably induce any of these activation events⁷⁴. Although this could be due to low-level FcεRI aggregation that is below the detection limit, the survival-enhancing effect of this IgE did not seem to involve autocrine and/or paracrine effects of anti-apoptotic cytokines. Therefore, there is no direct or indirect evidence so far (apart from the ‘survival effect’ itself) that FcεRI bound by this monoclonal IgE undergo aggregation.

Regulation of development and survival

Regulation by cytokines. Before considering how IgE might influence mast-cell or basophil survival and/or proliferation directly or indirectly, we first briefly review the roles of cytokines in the development, survival and proliferation of these cells. Mast cells and basophils are derived from CD34⁺ haematopoietic progenitor cells, but mast-cell maturation typically occurs in peripheral tissues. Differentiation and maturation in the periphery are tightly regulated by various cytokines, which results in the generation of heterogeneous populations of mast cells that vary in patterns or levels of expression of cell-surface proteins and granule constituents, such as histamine, heparin and other proteoglycans, and serine proteases^{1,3,4,76}. Although mouse mast cells have been classified traditionally into ‘mucosal’ and ‘connective tissue’ types, based on their location, morphological features and pattern of expression of granule constituents, it is now understood that the phenotypical features of mast-cell populations can change markedly in response to changes in the cytokine milieu and, perhaps, other environmental cues^{1,3,4,76}. In addition, in T_H2-cell-associated immune responses (in mice and humans) and in other settings, tissue mast cells can increase in number significantly during the course of the response;

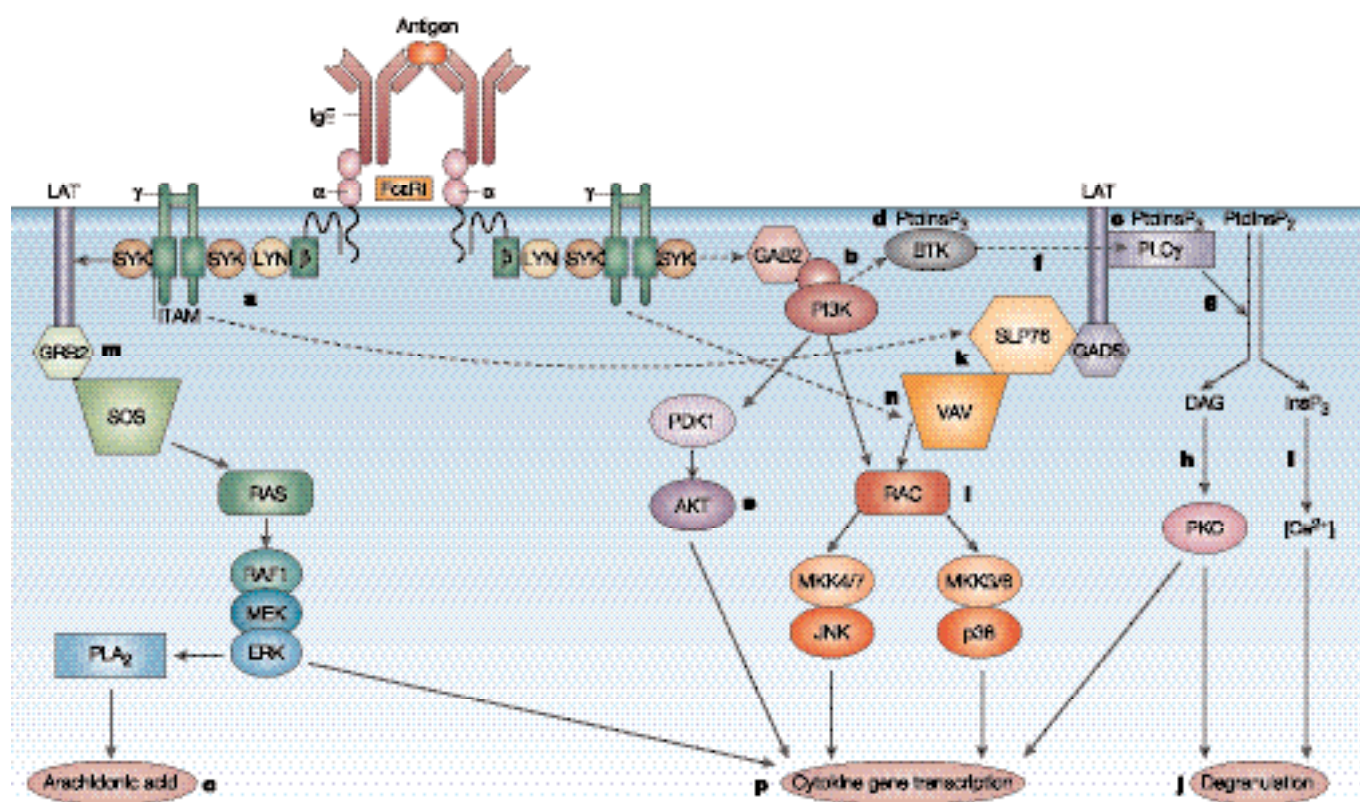


Figure 1 | FcεRI-mediated activation pathways. After FcεRI aggregation by antigen-crosslinked immunoglobulin E, β-subunit-associated LYN is activated and phosphorylates immunoreceptor tyrosine-based activation motifs (ITAMs) in the β and γ subunits of FcεRI; LYN and SYK are recruited to the receptor (a) and activated, and then phosphorylate (dashed arrows) many signalling proteins. After activation, phosphatidylinositol 3-kinase (PI3K) catalyses the synthesis of phosphatidylinositol-3,4,5-trisphosphate (PtdInsP₃) and phosphatidylinositol-3,4,-bisphosphate in a GRB2-associated binding protein 2 (GAB2)-dependent manner¹³⁵ (b). By interacting with pleckstrin-homology (PH) domains¹³⁶, PtdInsP₃ recruits phospholipase Cγ (PLCγ) (c), BTK (d) and AKT (not shown) to the plasma membrane. Membrane-recruited AKT is activated by dual phosphorylation^{137,138} and has a crucial role in cell survival and the transcriptional regulation of cytokine genes (e). PtdInsP₃-bound BTK becomes activated through phosphorylation by LYN and SYK, and subsequent autophosphorylation^{139–141}. Through interactions with the adaptor protein linker for activation of T cells (LAT) and PtdInsP₃, PLCγ becomes a target of BTK^{142–145} (f). Phosphorylated PLCγ generates diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (InsP₃) from phosphatidylinositol-4,5-bisphosphate (PtdInsP₂) (g). DAG activates many isoforms of protein kinase C (PKC) (h), and InsP₃ recruits Ca²⁺ from intracellular stores (i). Cellular Ca²⁺ responses consist of the initial release of Ca²⁺ from the endoplasmic reticulum and a BTK-dependent sustained Ca²⁺ influx, which promotes degranulation and the release of granule-associated products (j). The adaptor protein SLP76 (k) can interact with VAV¹⁴⁶, a guanine nucleotide exchange factor for RAC GTPase¹⁴⁷ (l), through GRB2 or GADS¹⁴⁸. So, active SYK, through the phosphorylation of substrates such as LAT (m), SLP76 (k) and VAV (n), activates pathways that result in degranulation (j), the synthesis and release of lipid mediators derived from arachidonic acid (o) and synthesis and secretion of cytokines (p). ERK, extracellular-signal-regulated kinase; MEK, mitogen-activated protein kinase kinase; PLA₂, phospholipase A₂.

mast-cell numbers often return to 'baseline' levels after the resolution of such processes^{1,3,4,76}.

The number of mast cells at baseline and during inflammatory or immune responses seems to be crucially regulated by local concentrations of factors that influence tissue mast-cell survival and proliferation, as well as by factors that regulate the tissue homing, survival and maturation of circulating mast-cell precursors^{1,3,4,76,77}. Stem-cell factor (SCF, also known as KIT ligand) is a crucial mast-cell developmental, survival and proliferation factor in both mice and humans⁴. Several lines of evidence, from many laboratories, indicate that IL-3 is a crucial growth and survival factor for human basophils (reviewed in REFS 3,78). Although IL-3 is an important mouse mast-cell growth factor *in vitro* and can greatly enhance mouse mast-cell development in response to

SCF *in vitro*, IL-3 (unlike SCF) cannot be used as the only exogenous growth factor to generate mast cells from human haematopoietic progenitors *in vitro*. Moreover, IL-3-knockout mice have few or no abnormalities in the number of tissue mast cells (or blood or bone-marrow basophils) under baseline conditions, but have significantly (~50–97%) reduced expansion of some mast-cell populations (and essentially no increase in the number of basophils) during T_H2-cell-associated immune responses to nematodes *in vivo*⁷⁸.

Many other cytokines, including IL-4, IL-6, IL-9, IL-10, tumour-necrosis factor (TNF) and nerve growth factor (NGF), can promote the development of various populations of mouse or human mast cells, at least *in vitro*^{2,5,6,79}. By contrast, various external stimuli — such as growth-factor deprivation, certain cytokines,

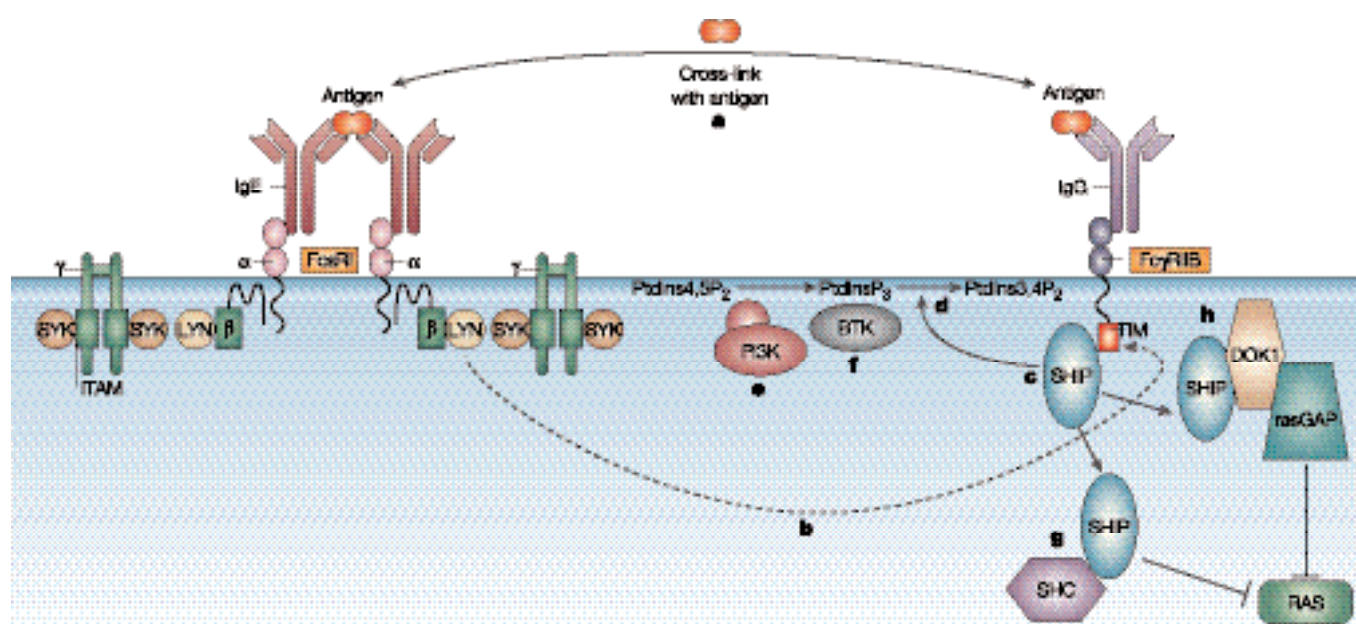


Figure 2 | **A representative inhibitory pathway in mast cells.** The co-aggregation of FcεRI with FcγRIIB — for example, by an antigen that is bound by both immunoglobulin E and immunoglobulin G antibodies (a) — induces the phosphorylation of tyrosine residues in the immunoreceptor tyrosine-based inhibitory motif (ITIM) of FcγRIIB by LYN associated with the FcεRI β-chain (b). Phosphorylated ITIMs recruit Src-homology 2 (SH2)-domain-containing inositol polyphosphate 5' phosphatase (SHIP) (c). SHIP hydrolyses phosphatidylinositol-3,4,5-trisphosphate (PtdInsP₃) (d) generated by phosphatidylinositol 3-kinase (PI3K) (e) and thereby inhibits the activation of BTK (f), phospholipase Cγ (PLCγ) and AKT (not shown). FcγRIIB-recruited SHIP is tyrosine phosphorylated and bound to SHC (g) and DOK1 (h).

drugs (such as glucocorticoids and some cytotoxic agents) and exposure to physical insults — can induce the apoptosis of mast cells⁸⁰. For example, mouse mast cells undergo apoptosis after withdrawal of IL-3 (REFS 81,82) or SCF⁸³, and such apoptosis can be prevented by SCF in a dose- and c-Kit (SCF receptor)-dependent manner.

Evidence such as this, together with data regarding the ability of mast cells to produce cytokines in response to the aggregation of FcεRI, is compatible with a complex model of the regulation of mast-cell number and phenotype during IgE-associated immune responses. Cytokines, including IL-3 and IL-4, that are secreted by allergen-activated T_H2 cells might trigger the local proliferation of mast cells near the site of allergen exposure (and, through systemic effects, contribute to the expansion of basophil populations); once mast cells are activated by IgE and allergen, mast-cell development and/or proliferation might be augmented by autocrine mechanisms due to the production and secretion of various cytokines by the activated mast cells (FIG. 3). However, the specific cytokines and/or growth factors that are involved in such responses might vary according to the species or type of mast-cell population. The resolution of such responses after the disappearance of allergen (and the associated reduction in the size of local populations of mast cells) might be favoured by reductions in the antigen-driven secretion of products that promote mast-cell development, survival and proliferation, as well as by the production of cytostatic and pro-apoptotic cytokines and other mediators by various cell types, including mast cells themselves.

Regulation by IgE. Two recent studies have shown that binding of monomeric IgE to FcεRI can promote mouse mast-cell proliferation induced by IL-3 or SCF *in vitro*^{74,75}. In one, the binding of monomeric IgE to FcεRI did not induce DNA synthesis, but it rendered mast cells resistant to apoptosis induced by growth-factor deprivation⁷⁴. By contrast, Kalesnikoff *et al.*⁷⁵ showed that treatment with IgE induced tyrosine phosphorylation of FcεRIβ, activation of MAPKs — such as ERK1, ERK2, JNK and p38 — and AKT, and upregulation of expression of BCL-X_L, an anti-apoptotic member of the BCL-2 family, as well as increased messenger RNA expression and the production and/or secretion of various cytokines (such as IL-4, IL-6, IL-13 and TNF). A combination of these cytokines mimicked the anti-apoptotic effects of IgE in naive BMCMCs. None of these activation events or cytokine secretion was observed in our study⁷⁴. Our study also indicated that neither the FAS–FAS ligand (FASL) death receptor–ligand system nor several protein kinases that are involved in FcεRI-aggregation-induced activation are required for monomeric-IgE-mediated mast-cell survival.

Opposing results have also been reported regarding the effects of FcεRI aggregation on mast-cell proliferation and survival. In the CFTL mouse mast-cell line, aggregation of FcεRI was shown to enhance mast-cell proliferation, perhaps owing to the autocrine effects of IL-3 and IL-4 (REF. 84). However, Tsai *et al.*⁸⁵ have shown that mouse BMCMCs undergoing antigen- and IgE-dependent activation *in vitro* can have significantly reduced proliferation in response to either SCF or IL-3 compared with control cells or cells that are exposed

only to IgE. In these experiments, IgE alone did not significantly affect mast-cell proliferation in response to SCF or IL-3, either in quiescent or non-quiescent mast cells. Notably, under the conditions tested, mouse mast cells had similar patterns of activation of MAPKs, **p90^{RSK}** and **p70 S6 kinases**⁸⁶, and the early response genes ***c-Fos***, ***c-Jun*** and ***JunB***⁸⁵, either after aggregation of FcεRI by IgE and antigen or after exposure to SCF.

Yoshikawa *et al.*⁸⁷ showed that FcεRI aggregation prevents apoptosis induced by growth-factor deprivation in the IL-3-dependent mouse mast-cell line MC9. Of the cytokines that are secreted by FcεRI-stimulated MC9 mast cells, IL-4 was shown to cause cell aggregation, by inducing the expression of lymphocyte function-associated antigen 1 (**LFA1**), and to make the cells more reactive to other autocrine growth factors, such as IL-3 and granulocyte-macrophage colony-stimulating factor (**GM-CSF**). Xiang *et al.*⁸⁸ also showed a survival effect of FcεRI aggregation with mouse BMCMCs and MCP5/L, a mast-cell line that is derived from BMCMCs. These authors found that expression of the anti-apoptotic Bcl-2-family protein **A1** is markedly induced by FcεRI aggregation in a calcium-dependent manner. Mast-cell survival was abrogated in *A1a*^{-/-} mast cells, and the increase in the number of mast cells that is induced by FcεRI aggregation *in vivo* was not observed in *A1a*^{-/-} mice. By contrast, Yeatman *et al.*⁸⁹ reported that long-term (six-day) FcεRI stimulation induces apoptosis in BMCMCs even in the presence of IL-3, and that IL-10 enhances this effect.

The results of the various studies discussed are not directly comparable, because of differences in the cells, culture conditions, IgE preparations, crosslinking procedures and periods of crosslinking that were used. Moreover, mast cells that are activated by aggregation of FcεRI can secrete many products that might influence mast-cell survival or proliferation directly or indirectly (*in vitro* or *in vivo*), and the different conditions that are used to induce FcεRI aggregation might variably affect the type or amount of secreted products that have positive or negative effects on cell viability. Nevertheless, taken together, the reported findings indicate that, depending on the conditions of the experiment, FcεRI aggregation can have neutral effects on mast-cell survival, favour apoptosis or promote survival and/or proliferation. Furthermore, it seems that the binding of monomeric IgE can promote survival by an autocrine mechanism⁷⁵ and/or by an unknown mechanism⁷⁴.

It will be of interest to determine the extent to which the various consequences of binding of monomeric IgE (or aggregation of FcεRI) on survival are determined only by the strength of signalling that is induced, as opposed to other factors (BOX 3). For example, the simplest explanation for the 'survival effect' of monomeric IgE is that both the non-autocrine and autocrine mechanisms are the result of FcεRI aggregation — in the former case, below current limits of detection, and in the latter case, associated with biochemical evidence of aggregation. But, this hypothesis remains to be tested. Similarly, the biological relevance of the effects of monomeric IgE and/or FcεRI aggregation to mast-cell

survival is not clear yet. It is possible that a short-term 'survival effect' associated with FcεRI aggregation might favour mast-cell regranulation, in preparation for further activation. By contrast, a long-term 'apoptotic effect' (for example, owing to excessive FcεRI crosslinking?), together with the pro-apoptotic effects of T_H2 cytokines (for example, IL-4 plus IL-10 (REF 89)), might limit further increases in the number of mast cells during the resolution of the response. Finally, evidence from studies of other haematopoietic cells indicates that different cell types can have different relationships between the mode and strength of receptor stimulation, and the consequences of such stimulation on cell survival. For example, in eosinophils and neutrophils stimulated through FcγRII, receptor aggregation with soluble ligands enhances cell survival, whereas aggregation with immobilized ligands induces apoptosis^{90–92}. By contrast, in natural killer cells, stimulation of FcγRIIIA with soluble ligands induces apoptosis^{93,94}. So, it seems probable that many factors might contribute to the survival consequences of immunoreceptor stimulation with soluble ligands, including the status of other receptor component(s) or ligand-interaction sites in the receptor, as well as cell-type-specific factors.

Regulation by adhesion molecules. Adhesion events (between cells or to extracellular matrix proteins) influence not only mast-cell migration, but also proliferation, differentiation and survival, and adhesion molecules use signalling pathways that have similarities to those that are elicited by FcεRI aggregation. BMCMC adhesion molecules include various integrins, intercellular adhesion molecule 1 (**ICAM1**) and c-Kit, and BMCMCs can adhere to plate-bound **laminin**, **fibronectin** and **vitronectin** when activated through FcεRI or by non-immune stimuli, such as phorbol 12-myristate-13-acetate^{95–97}. BMCMCs bound to vitronectin show enhanced proliferation in response to IL-3, but this adhesion does not sustain BMCMC survival in the absence of IL-3. Interestingly, adhesion to fibronectin enhances monomeric-IgE-mediated survival effects on BMCMCs (J. Kitaura and T. K., unpublished observations).

The differentiation and maturation of mast cells in co-culture with fibroblasts depends on the interaction between c-Kit on mast cells and fibroblast-bound SCF^{98,99}. *Sl/Sl^f* mice — which have mutations in *Scf* that result in a lack of membrane-associated SCF, but continued production of a soluble form of SCF — have only 1% of the normal number of tissue mast cells^{4,100}. Transfection of mast cells that do not express endogenous c-Kit with wild-type or mutated *c-Kit* showed that Tyr567, the Src-docking site, and Tyr719, the PI3K-docking site, of c-Kit are both necessary for SCF-mediated adhesion. However, Tyr719 is not necessary for SCF-induced mast-cell proliferation and survival¹⁰¹. Similar studies indicate that mast-cell adhesion to fibronectin, but not proliferation, through the platelet-derived growth factor (**PDGF**) receptor is also regulated by PI3K and PLCγ1 (REF. 102). c-Kit selectively activates the transcription factor signal transducer and activator of transcription 5 (**Stat5**) in BMCMCs, and studies with

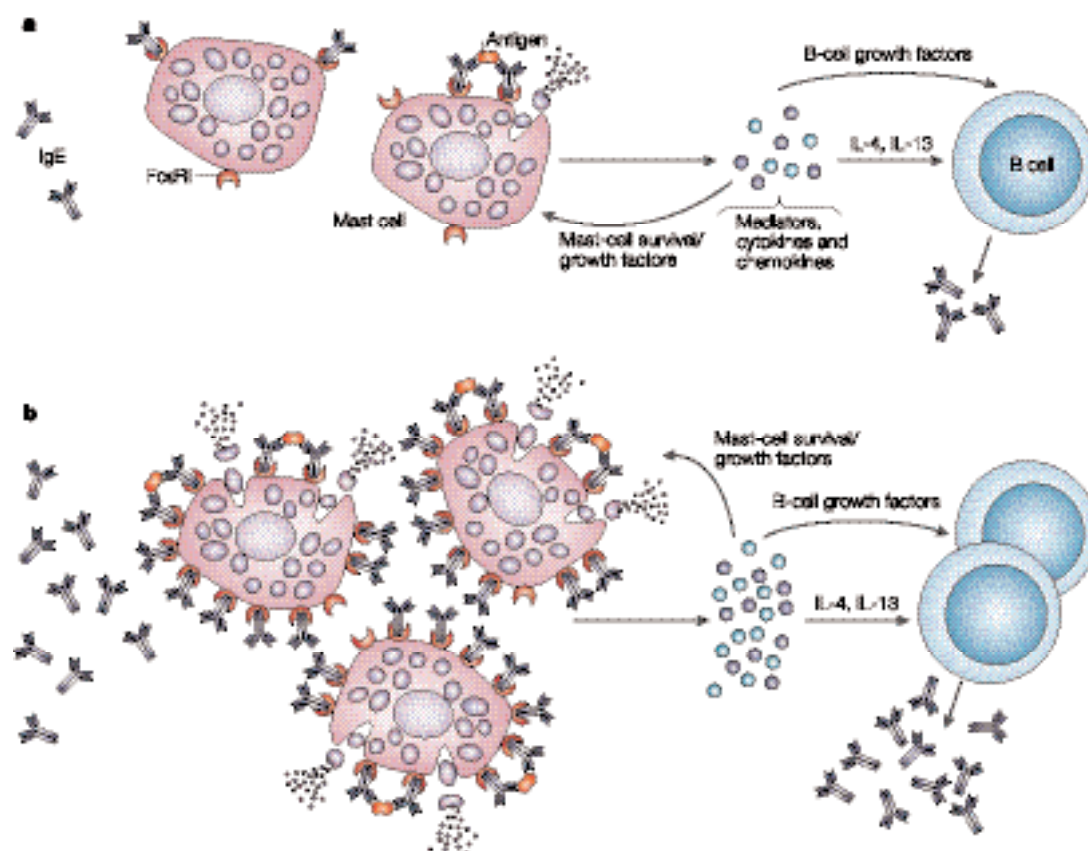


Figure 3 | A positive-feedback model for IgE production and IgE-dependent mast-cell activation, survival and/or proliferation. a | The development of a typical T helper 2 (T_H2) response is shown. **b** | Exposure to progressively higher levels of immunoglobulin E during an ongoing T_H2 response leads to increased surface expression of FcεRI on mast cells and on basophils (not shown). Mast cells that are sensitized by high levels of IgE can respond to lower concentrations of specific antigen and release greater amounts of mediators, cytokines and chemokines after antigen-dependent aggregation of FcεRI. These products include immunoregulatory cytokines (for example, IL-4 and IL-13) and, perhaps, chemokines (such as MIP1α) that can promote the production of IgE by B cells, as well as B-cell growth factors (for example, IL-6 and perhaps others⁸). Activated mast cells can also secrete survival/growth factors for mast cells (for example, IL-3, IL-4 and SCF), basophils (for example, IL-3) and eosinophils (for example, IL-5), as well as cytokines and chemokines that can promote the recruitment and/or influence the function of eosinophils, basophils, neutrophils, T cells, monocytes and dendritic cells^{7,8}. So, mast cells (and/or basophils) that are activated through FcεRI can initiate many potential positive-feedback mechanisms in T_H2 -associated responses. Monomeric IgE itself can also enhance mast-cell survival, at least *in vitro*, which is another potential mechanism for increasing/maintaining the number of mast cells (or basophils) in this setting. Note that in ongoing T_H2 responses, T cells, eosinophils, basophils and other recruited or resident cell types (not shown in the figure) are additional sources of cytokines that have effects on IgE production, B cells, mast cells, basophils and eosinophils. For example, in humans, basophils seem to be a more important potential source of IL-4 than do mast cells, but mast cells can produce substantial amounts of IL-13. This is a revised version of the hypothesis that was proposed originally by Yamaguchi *et al.*⁶⁷ and Yano *et al.*⁶⁸. IL, interleukin; MIP1α, macrophage inflammatory protein 1α (CCL3); SCF, stem-cell factor.

Stat5^{-/-} BMCMCs have shown that Stat5 is required for IL-3- and SCF-mediated mast-cell survival, which correlates with the Stat5-dependent expression of Bcl-X_L and Bcl-2. Consistent with these *in vitro* results, adult *Stat5*^{-/-} mice have been reported to lack mast cells completely in various tissues (J. J. Ryan, personal communication).

Molecules that regulate life and death in mast cells. Mast-cell anti-apoptotic cytokines (for example, SCF, IL-3, IL-6, IL-9, **interferon-γ** and NGF) and pro-apoptotic cytokines (for example, transforming growth factor β1, **TGF-β1**), many of which can be produced by activated mast cells, have been studied extensively (reviewed in REFS 4,103,104). By contrast, fewer studies have been carried out on death receptors in mast cells. Mouse peritoneal mast

cells express the death receptor Fas and undergo apoptosis when stimulated with anti-Fas antibodies^{105,106}. Although both Fas and FasL are expressed on BMCMCs, these molecules are not targeted directly in IgE-mediated survival⁷⁴. Another death-receptor ligand — TNF-related apoptosis-inducing ligand (**TRAIL**) — that is expressed on HMC-1 human mast cells¹⁰⁷ has not been analysed for its involvement in IgE-mediated cell survival.

FcεRI-dependent effects on mast-cell activation, survival and apoptosis probably use overlapping signalling pathways that might be recruited by other stimuli also. For example, stimulation of both FcεRI and c-KIT induces the activation of PI3K in mast cells. In many other cell types, PtdInsP₃ (produced from phosphatidylinositol-4,5-bisphosphate by PI3K) activates AKT.

Box 3 | Questions on the effects of IgE on mast-cell survival

- Can the effects of monomeric immunoglobulin E on mast-cell survival (or cytokine production) be observed *in vivo*?
- What happens to the FcεRI that binds monomeric IgE; does it undergo IgE-induced aggregation?
- What are the roles of individual components of FcεRI in mediating the effects of monomeric IgE? Is the γ-subunit essential for this function, as it is for FcεRI-aggregation-mediated mast-cell activation? Does the β-subunit amplify the survival effect?
- Which part of IgE is required for the survival effect? Is the Fc portion alone sufficient?
- What are the mechanisms that promote mast-cell survival when FcεRI binds monomeric IgE or is aggregated? Interleukin-4 has been shown to be a crucial survival factor that is secreted by mast cells under certain conditions of FcεRI aggregation. However, survival mechanisms that are independent of the autocrine and/or paracrine effects of anti-apoptotic cytokines seem to exist also⁷⁴.
- What are the mechanisms that underlie the differential outcomes of FcεRI stimulation by different crosslinking reagents (that is, positive, neutral or negative effects on cell survival and/or proliferation)? Excessive crosslinking (for example, with secondary antibody or immobilized reagents) could involve the co-crosslinking of FcεRI with other cell-surface receptors that can inhibit FcεRI-dependent signalling. In this regard, it is interesting to note that mast-cell function-associated antigen (MAFA) can interact with FcεRI^{57,58}.

AKT phosphorylates several substrates that are important for the regulation of cell survival, including **BAD** — a pro-apoptotic member of the BCL-2 family^{108,109} — **caspase-9** (REF. 110) and forkhead transcription factors such as **FKHRL1**, **AFX** and **FKHR**^{111–113}. Levels of PtdInsP₃ are negatively regulated by two phosphoinositide phosphatases, SHIP and phosphatase and tensin homologue (**PTEN**). Although the role of PTEN in mast-cell survival has not been analysed yet, the role of SHIP is well characterized. BMCMCs derived from *Ship*^{−/−} mice were less susceptible to apoptosis induced by various stimuli, including growth-factor withdrawal¹¹⁴. Importantly, *Ship*^{−/−} BMCMCs are also extremely sensitive to monomeric IgE binding (without antigen stimulation), which leads to extensive degranulation that correlates with high and sustained intracellular calcium levels and MAPK activation⁴⁵.

As described above, another PtdInsP₃ target, BTK, together with SYK, phosphorylates and activates PLCγ isoforms. It seems that through this pathway, BTK can regulate PKCβI in a diacylglycerol- and calcium-dependent manner¹¹⁵. Downstream of PKCβI, **JNK1** and **JNK2** (stress-activated protein kinases of the MAPK family) are regulated by BTK¹¹⁶, which leads eventually to the transcriptional activation of cytokine genes, such as those encoding **IL-2** and TNF^{115,117}. Interestingly, however, BTK was shown to be pro-apoptotic in the context of growth-factor deprivation in mast cells¹¹⁶. Monomeric IgE-mediated survival was observed in *Btk*^{−/−} BMCMCs⁷⁴, which indicates that the BTK–PKCβI–JNK pathway might not be crucial for IgE-mediated regulation of cell survival.

Alternatively, BTK might have an anti-apoptotic function under certain conditions. Indeed, in B cells, BTK regulates the activation of the nuclear factor-κB (**NF-κB**) family of transcription factors, which are important for survival, through the phosphorylation

and degradation of inhibitor of NF-κB (**IκB**) in a PKCβ-dependent manner^{118–120}. Because this pathway is also activated in mast cells after FcεRI aggregation, its role in mediating the effects of binding of monomeric IgE should be examined. Consistent with this possibility, **RAC2** was shown to be crucially important for the survival of mast cells¹²¹. RAC2 is a haematopoietic-lineage-specific member of the RHO family of GTPases that regulates JNK and, therefore, might be an intermediate signalling molecule between BTK and JNK. *Rac2*^{−/−} mice have reduced numbers of mast cells, and BMCMCs derived from *Rac2*^{−/−} mice have a severe defect in IL-3- and SCF-induced survival, which correlates with a lack of activation of Akt, reduced expression of Bcl-X_L and increased expression of Bad¹²¹.

Although PI3K has a crucial role in mast-cell survival, it is not clear which member(s) of the PI3K family is responsible for this function. Heterodimeric PI3Ks consist of a 110-kDa catalytic subunit (p110α, β or δ) and an 85-kDa regulatory subunit (p85α, β or γ) combined in a random manner. Mast cells derived from mouse fetal livers express all three p110 isoforms, and p85α and p85β isoforms. PI3K activity is indispensable for c-KIT-dependent mast-cell degranulation and adhesion, but it has only contributing roles in mediating cell survival and proliferation¹²². Studies with *p85α*^{−/−} mouse mast cells have shown that p85α is crucial for maximal c-Kit-mediated degranulation, proliferation and Akt phosphorylation, but not for survival¹²². By contrast, p85α was shown to be dispensable for FcεRI-induced degranulation and Akt phosphorylation.

Calcineurin is a calcium/calmodulin-dependent phosphatase that dephosphorylates the transcription factor **NFAT** (nuclear factor of activated T cells) and the pro-apoptotic BCL-2-family protein BAD¹²³. The dephosphorylated NFAT is translocated to the nucleus, where it upregulates the transcription of pro-apoptotic genes, such as *FasL*¹²³. Dephosphorylation of BAD, by counteracting the PI3K–AKT pathway, also favours apoptosis. Inhibitors of calcineurin, such as cyclosporine and FK506, augment SCF-dependent human mast-cell proliferation¹²⁴.

Of the Bcl-2-family proteins, Bcl-2 positively regulates the number of mast cells in the mouse stomach mucosa, whereas **Bax** negatively regulates this population¹²⁵. However, a lack of these proteins does not affect the baseline number of mast cells in other tissues, such as the stomach submucosa or muscularis, or the skin¹²⁵. The expression of Bcl-2 is induced by IL-3, and the level of *Bcl-2* mRNA is reduced in mouse BMCMCs after IL-3 deprivation¹²⁶. However, SCF does not induce the expression of *Bcl-2* mRNA, but rather, it increases the expression of *Bax* mRNA⁸², which indicates that survival induced by IL-3 and SCF might be mediated by different mechanisms. As described above, enhanced Bcl-X_L expression was induced by SPE-7 anti-dinitrophenyl IgE, and the expression of A1 was crucial for FcεRI-crosslinking-induced survival. Therefore, many BCL-2-family proteins seem to be involved in regulating cytokine- and/or FcεRI-dependent effects on mast-cell survival and apoptosis.

Clinical implications

The IgE-dependent activation of mast cells and basophils (through aggregation of FcεRI) is widely regarded as a key effector mechanism of acute allergic reactions in humans, such as anaphylaxis and acute attacks of atopic asthma^{2,3,7,9,10}. Studies in genetically mast-cell-deficient *Kit^W/Kit^{W-v}* mice, the congenic normal (+/+) mice and *Kit^W/Kit^{W-v}* mice that have been selectively reconstituted with +/+ mast cells or genetically altered mast cells can define the importance of mast cells (and specific mast-cell activation mechanisms, signalling pathways or products) in IgE-associated and many other biological responses^{7,11}. By contrast, in humans, it is much more difficult to define the relative importance of a single type of effector cell (such as mast cells or basophils), compared with other cells that can secrete similar or identical products, in the expression of specific adaptive or pathological processes. However, the results of *in vivo* studies in mice, taken together with the observations of many groups who have analysed human subjects *in vivo*, or tissue derived from such subjects or human mast cells *in vitro*, indicate that mast cells that are activated through aggregation of FcεRI can contribute not only to acute allergic reactions, but also to many of the long-term consequences of exposure to allergens, including leukocyte recruitment, tissue remodelling and alterations of the function of the affected organs^{2,7}.

There is much less evidence regarding the clinical importance of the effects of monomeric IgE on mast-cell FcεRI surface expression or function. Studies in *IgE^{-/-}* mice^{67,69} indicate that the concentration of circulating IgE (even at baseline levels) is one of the main regulators of the level of mast-cell and basophil FcεRI expression *in vivo*. And, several *in vitro* studies indicate that the ability of IgE to enhance FcεRI surface expression can markedly influence mast-cell and basophil function, in both mouse and human cells^{67–70}. However, so far, there is only a single study that has investigated the possible clinical implications of these findings directly. MacGlashan *et al.*¹²⁷ have shown that the treatment of atopic patients with humanized anti-IgE monoclonal antibody, which reduced free IgE levels to 1% of pre-treatment levels, was associated with a significant reduction in the expression of FcεRI on human basophils and wheal and flare (type I immediate hypersensitivity) responses to challenge with intra-dermal antigen (a measure of the IgE-dependent function of skin mast cells). Moreover, for basophils from anti-IgE-treated subjects who were sensitive to many allergens, the reduction in FcεRI expression was associated with a significantly decreased secretion of mediators in response to allergen challenge *in vitro*¹²⁷.

In contrast to the findings with IgE-mediated regulation of FcεRI surface expression, evidence that monomeric IgE can regulate mast-cell survival (and, in certain circumstances, induce mast-cell cytokine release) in the absence of deliberate crosslinking of IgE with antigen has been restricted so far to studies of mouse mast cells *in vitro*^{74,75} and to a preliminary report indicating that monoclonal human IgE can induce

chemokine production by human umbilical-cord-blood-derived mast cells *in vitro*¹²⁸. Therefore, the *in vivo* relevance of this phenomenon remains to be determined. Notably, *IgE^{-/-}* mice¹²⁹ (H. Oettgen and S.J.G., unpublished observations) and transgenic mice that overexpress antigen-specific IgE¹³⁰ seem to have levels of skin mast cells that are similar to those of the congenic wild-type mice. This result indicates that baseline levels of IgE (that can markedly enhance mast-cell FcεRI surface expression⁶⁷), or even markedly elevated IgE levels¹³⁰, might have little or no effect on the number of mast cells in some anatomical sites (for example, the skin). By contrast, the percentage of bone-marrow basophils was significantly, albeit moderately (~40%), higher in IgE-treated compared with IgG2a-treated *IgE^{-/-}* mice⁶⁹.

Concluding remarks

IgE and its high-affinity receptor FcεRI have long been recognized as key molecules that allow mast cells and basophils to have immunologically specific effector functions. Several lines of evidence indicate that the antigen- and IgE-dependent activation of mast cells and basophils, through aggregation of FcεRI, is crucial for acute reactions to allergen challenge (for example, anaphylaxis or asthma), and that mast cells (and perhaps basophils) that are activated by this mechanism can contribute to the development of certain long-term consequences of allergen challenge, such as leukocyte recruitment, tissue remodelling and functional changes in the affected organs.

Also, it is now clear that exposure to high levels of monomeric IgE, beyond simply 'sensitizing' cells to recognize specific antigen, can also markedly increase the surface expression of FcεRI on mast cells and basophils. Moreover, such cells can both show an IgE-dependent response to lower concentrations of specific antigen and release greater amounts of mediators, cytokines and chemokines after antigen-dependent aggregation of FcεRI. These secreted products include those with potential immunoregulatory actions, such as IL-4 and IL-13 (which can enhance IgE production), as well as cytokines that can stimulate B-cell development or have autocrine effects on mast-cell or basophil development and survival. In addition to influencing the surface expression of FcεRI, IgE can also promote mouse mast-cell survival, at least *in vitro*.

As shown in FIG. 3, these findings indicate that the high levels of IgE that develop during T_H2-cell-associated immune responses not only can increase FcεRI surface expression on mast cells (and, accordingly, enhance the effector and potential immunoregulatory functions of the cell), but might also contribute directly or indirectly to an increase in the number of mast cells (or basophils). In such settings, enhanced IgE- and FcεRI-dependent production of certain cytokines and chemokines can also favour increased IgE production by B cells, which results in additional positive-feedback effects on mast-cell and basophil effector and/or immunoregulatory functions and, possibly, numbers.

It should be emphasized, however, that important aspects of the scheme that is shown in FIG. 3 — including the proposed IgE-dependent immunoregulatory and autocrine/paracrine functions of mast cells and basophils — have not been documented *in vivo* yet. Similarly, the recent demonstration that the binding of monomeric IgE to FcεRI can promote mouse mast-cell survival (and, in some cases, cytokine production) is based solely on *in vitro* evidence. Although this work is provocative in proposing that IgE might be able to enhance mast-cell effector, immunoregulatory or autocrine functions in the absence of aggregation of

FcεRI by specific antigen, it remains to be determined whether, and under what circumstances, this mechanism of mast-cell activation can occur *in vivo*. It will be even more challenging to determine whether the phenomenon has any physiological or pathological relevance. Nevertheless, it is possible that this newly recognized potential activity of IgE could contribute to both the occurrence of increased numbers of mast cells at sites of chronic allergic inflammation and the persistence of the signs and symptoms that are associated with allergic inflammation, even in the absence of the offending allergen.

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ITAM | PH | SH2

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A1 | AFX | AKT | BAD | Bax | Bcl-2 | BCL-X_L | BTK | caspase-9 | CD23 | *c-Fos* | *c-Jun* | c-Kit | DOK1 | ERK1 | ERK2 | FAS | FASL | FcεRI | FcγRII | FcγRIIB | FcγRIII | fibronectin | FKHR | FKHL1 | GAB2 | galectin-3 | GM-CSF | GRB2 | ICAM1 | IκB | IL-2 | IL-3 | IL-4 | IL-5 | IL-6 | IL-9 | IL-10 | IL-13 | interferon-γ | JNK1 | JNK2 | *JunB* | laminin | LAT | LFA1 | LYN | MAFA | MIP1α | NFAT | NF-κB | NGF | PDGF | PI3K | PIRB | PLCγ | PTEN | p70 S6 kinases | p90^{RSK} | RAC2 | SCF | SHC | SHIP | SHP1 | SHP2 | SLP76 | SOS | SRC | Stat5 | SYK | α-subunit | β-subunit | γ-subunit | TEC | TGF-β1 | THY1 | TNF | TRAIL | VAV | vitronectin

OMIM: <http://www.ncbi.nlm.nih.gov/Omim/>

asthma

FURTHER INFORMATION

The Fourth International Workshop on Signal Transduction in the Activation and Development of Mast cells and Basophils, 2001: <http://info.md.huji.ac.il/conf/mast-meeting/>

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