

CHAPTER 3

MONOMERIC IgE AND MAST CELL DEVELOPMENT, SURVIVAL AND FUNCTION

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Abstract: Mast cells play a major role in allergy and anaphylaxis, as well as a protective role in immunity against bacteria and venoms (innate immunity) and T-cell activation (acquired immunity).^{1,2} It was long thought that two steps are essential to mast cell activation. The first step (sensitization) occurs when antigen-specific IgE binds to its high-affinity IgE receptor (FcεRI) expressed on the surface of mast cells. The second step occurs when antigen (Ag) or anti-IgE binds antigen-specific IgE antibodies bound to FcεRI present on the mast cell surface (this mode of stimulation hereafter referred to as IgE+Ag or IgE+anti-IgE stimulation, respectively).

Conventional wisdom has been that monomeric IgE plays only an initial, passive role in mast cell activation. However, recent findings have shown that IgE binding to its receptor FcεRI can mediate mast cell activation events even in the absence of antigen (this mode of stimulation hereafter referred to as IgE(-Ag) stimulation). Different subtypes of monomeric IgEs act via IgE(-Ag) stimulation to elicit varied effects on mast cells function, survival and differentiation. This chapter will describe the role of monomeric IgE molecules in allergic reaction, the various effects and mechanisms of action of IgE(-Ag) stimulation on mast cells and what possible developments may arise from this knowledge in the future. Since mast cells are involved in a variety of pathologic and protective responses, understanding the role that monomeric IgE plays in mast cell function, survival and differentiation will hopefully lead to better understanding and treatment of asthma and other allergic diseases, as well as improved understanding of host response to infections.

INTRODUCTION

Over the past few decades, the number of patients with allergic diseases (e.g., asthma, atopic dermatitis) has risen markedly in industrialized nations. Thirty-nine percent of

children and 30% of adults have been diagnosed with one or more of asthma, eczema and hay fever.³ Allergic diseases are manifested by an immune system over-reaction to a substance that the body should not normally identify as dangerous and that reaction can be something as basic as a mild irritation or it can be so extreme as to cause fatal results.

The mast cell is a type of myeloid lineage hematopoietic cell and its development and function suggest that it is involved heavily in the pathogenesis of these allergic diseases (e.g., when a mast cell is “activated” it releases chemicals into the body that cause what we call an allergic reaction). Mast cells originate from precursor cells in the bone marrow, enter the circulation and then localize to mucosal and epithelial sites under the influence of the local microenvironment.⁴ At these sites, facing the outer environment and surrounding blood vessels, mast cells can produce and release a vast array of biogenic amines (e.g., histamine and serotonin), lipids (e.g., leukotrienes and prostaglandins), nucleotides (e.g., adenosine) and polypeptides (e.g., proteases, cytokines and chemokines) that act as effector molecules in allergic or inflammatory reactions as well as innate and adaptive immune responses.^{5,6} Indeed, mast cells from atopic individuals that are sensitized by allergen-specific immunoglobulin E (IgE) respond to allergens by releasing mast cell-derived mediators. Subsequent allergen exposure in susceptible individuals produces a cascade of events orchestrated by immune effector cells such as Type 2 helper T (Th2) cells and eosinophils.⁷ B cells stimulated by IL-4 or IL-13 produce IgE.⁸

Recent studies have shown that monomeric IgE can activate mast cells in the absence of antigen. This suggests that allergic symptoms may be due in part to the effects of monomeric IgE and that these symptoms may occur even in patients who are not exposed to allergens. Different subtypes of IgE have been shown to have different effects on mast cells. It is conceivable that different types of monomeric IgE may give rise to the range of symptoms and symptom severity observed amongst allergic patients. Anti-IgE therapy has been efficacious in treating patients with allergic diseases, pointing to the significant role IgE must have in asthma and other allergic diseases.⁹ It is still unknown how anti-IgE therapy relieves allergic symptoms, but it is highly possible that preventing the effects of monomeric IgE on mast cells is a large part of how anti-IgE therapy mediates its effects. Elucidating the true role and mechanism of action of monomeric IgE's effects on mast cells will further our understanding of allergic diseases, symptoms and treatment.

This chapter will review what is known about the interaction between monomeric IgE and mast cells in the absence of antigen.

IgE+Ag STIMULATION OF MAST CELLS

The initial step in IgE+Ag mast cell activation occurs when monomeric, antigen-specific IgE binds FcεRI, stabilizing and increasing the number of FcεRI receptors on the mast cell surface.¹⁰ This sensitizes the mast cell to a specific antigen so that the mast cell can be activated immediately upon subsequent antigen exposure. Antigen binding to antigen-specific, FcεRI-bound, IgE cross-links FcεRI (FcεRI aggregation/cross-linking), thereby inducing various mast cell activation events (e.g., degranulation, de novo synthesis and secretion of inflammatory mediators, survival promotion, migration, etc) via multiple signal transduction pathways within the mast cell.

In response to FcεRI cross-linking, various phosphorylation cascades are triggered, leading to calcium influx, nuclear importation of transcription factors, mast cell degranulation, eicosanoid generation and chemokine/cytokine production. The FcεRI

receptor consists of four subunits ($\alpha\beta\gamma_2$)—one α subunit that binds IgE, one β subunit involved in signal amplification and Fc ϵ RI stabilization and two disulfide-bonded γ subunits acting as the main signal transducer.¹⁰ Fc ϵ RI aggregation leads to the activation of Lyn, a Src family protein tyrosine kinase (PTK) associated with the β subunit of Fc ϵ RI. The intracellular parts of Fc ϵ RI β and γ contain immunoreceptor tyrosine-based activation motifs, or ITAMs, that consist of six conserved amino acid residues spread out over an ~26 amino acid sequence, (D/E)X₇(D/E)X₂YX₂(L/I)X₆₋₈YX₂(L/I).¹¹ Activated Lyn phosphorylates tyrosine residues in these intracellular Fc ϵ RI β and γ subunit ITAMs. β phosphorylated ITAM recruits Lyn PTK and γ phosphorylated ITAM recruits Syk PTK. Two other Src family PTKs, Fyn and Hck, also work with Lyn and Syk to activate several signaling pathways, especially the PI3K signaling pathway.¹² Signaling events activated by these PTKs (PI3K, phospholipase C/Ca²⁺ and several MAPK pathways) lead to cytokine/chemokine production and degranulation of the effector molecules mentioned in the introduction (chemicals, lipids, nucleotides and polypeptides).^{13,14}

IgE(-Ag) STIMULATION OF MAST CELLS: MONOMERIC IgE AFFECTS MAST CELL FUNCTION IN THE ABSENCE OF ANTIGEN

As mentioned above, monomeric IgE appears to be involved in more than just mast cell sensitization. IgE(-Ag) induces up-regulation of Fc ϵ RI expression on the cell surface,¹⁵⁻¹⁷ Fc ϵ RI stabilization,^{18,19} increased histamine content,²⁰ histamine and leukotriene release, receptor internalization, cytokine/chemokine expression,²¹ increased response to compound 48/80 and substance P,²² F-actin content release,²³ membrane ruffling,²⁴ fibronectin adhesion,²⁵ and migration.²⁶ Almost all of these effects are seen with IgE+Ag and IgE+anti-IgE stimulation as well (Fig. 1). However, in IgE(-Ag) stimulation, these effects are seen at IgE concentrations 2-3 logs higher than what are required for Ag-dependent IgE+Ag mast cell activation. Since IgE(-Ag), IgE+Ag and IgE+anti-IgE can mediate similar effects at the subcellular level, it follows that IgE(-Ag) would be able to elicit mast cell responses similar to those seen with IgE+Ag and IgE+anti-IgE. Indeed, IgE(-Ag) has been shown to (i) promote mast cell survival, (ii) amplify mast cell accumulation at allergic tissue sites and (iii) mediate mast cell activation events.

(i) IgE(-Ag) stimulation seems to play a role in preventing mast cell apoptosis. Mast cells undergo apoptosis at low levels of growth factor both in vitro and in vivo. Two groups have investigated whether monomeric IgE can inhibit the mast cell apoptosis that is induced by the removal of growth factor; their results showed that increasing the concentration of monomeric IgE can indeed prevent mast cell apoptosis.^{18,27} IgE(-Ag), IgE+anti-IgE and IgE+Ag (at low antigen concentrations) stimuli of Fc ϵ RI all promote mast cell survival.²⁸ The duration and strength of this Fc ϵ RI stimulation determines mast cell survival (further discussed under *Signal Transduction induced by Monomeric IgE*).

(ii) IgE(-Ag) and IgE+Ag are also implicated in amplification of the inflammatory response. Epicutaneous application of IgE(-Ag) and IgE+Ag at allergic tissue sites with local high IgE levels induces mast cell accumulation.²⁹ Mast cell accumulation can amplify inflammatory reactions via molecules such as histamine, leukotriene B₄ (LTB₄) and chemokines/cytokines released during mast cell degranulation. These effector molecules recruit other immune cells such as T cells, eosinophils, monocytes and neutrophils and induce synthesis of other pro-inflammatory effector molecules. Histamine enhances the secretion of Th2 cytokines (involved in mediating allergic responses and host defense

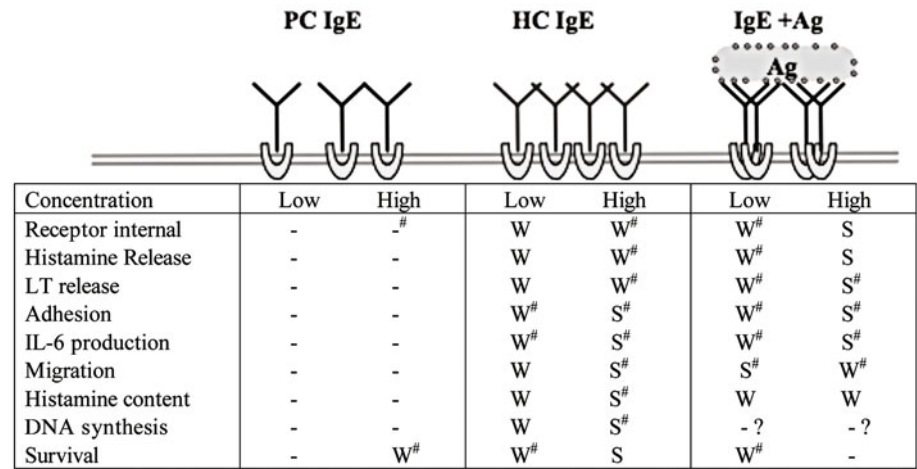


Figure 1. Biological effects by various modes of stimulation via the FcεRI. Experiments using mouse BMMCs are summarized. Concentrations of stimuli are as follows: PC IgE (H1 DNP-ε-206) and HC IgE (SPE-7), 0.5 μg/ml (low) and 5 μg/ml (high); and IgE+Ag (DNP₂₁-BSA), 1 ng/ml (low) and 100 ng/ml (high). Notice that biological events are listed in a rough order of occurrences and receptor aggregation presumably occurs in all modes of stimulation, except for low PC IgE concentrations. -, not detected, -[#], very weak; W, weak; W[#] weak-moderate; S[#], moderate-strong; and S, strong. Reproduced with permission from: Kawakami T, Kitaura J. *J Immunol* 2005; 175:4167-4173;²⁸ ©2005 The American Association of Immunologists, Inc.

against parasitic infections)³⁰ and inhibits the production of Th1 cytokines.³¹ LTB₄ recruits T cells and myeloid cells.³²⁻³⁴ Transcripts coding for the CC chemokines (chemokines in which the first two cysteine residues are adjacent)³⁵ I-309/CCL1, MIP-1α/CCL3, MIP-1β/CCL4 and MCP-3/CCL7 are dramatically enhanced in IgE+Ag-stimulated mast cells.³⁶ These cytokines and chemokines recruit T cells, eosinophils, monocytes and neutrophils.

Activated mast cells may also release mediators to further induce activation of themselves and their neighboring mast cells. Adenosine and sphingosine 1-phosphate acutely released by IgE+Ag play an important role in Ca²⁺ influx, degranulation and chemotaxis to antigen.^{37,38} PTK-based signaling pathways and G-protein coupled receptor (GPCR)-based signaling pathways dependent on phosphoinositide 3-kinase γ (PI3Kγ) cooperate to enhance degranulation and migration. It is likely that they induce other activation events as well. Factors that bind to GPCRs (sphingosine 1-phosphate, adenosine, LTB₄ and chemokines MIP-1α/CCL3, MIP-1β/CCL4, MIP-2/CXCL1-3, MCP-1/CCL2 and RANTES/CCL5) contribute to IgE(-Ag) and IgE+Ag mast cell migration using both directional (chemotaxis) and nondirectional (chemokinesis) mechanisms.²⁶

The ability of monomeric IgEs to attract inflammation-inducing mast cells may make it possible for amplification of inflammatory reactions to continue even after the removal of antigen as long as local IgE synthesis continues. A positive feed-back-loop hypothesis was proposed to explain inflammation seen in atopic diseases with high serum IgE levels.¹⁴ ↑ IgE → ↑ FcεRI, ↑ mast cells → ↑ Ag-IgE and FcεRI-dependent release of IL-4, IL-13, MIP-1α/CCL3 and so on → ↑ IgE.

(iii) IgE(-Ag) stimulation exerts a vast number of effects on mast cells that all play a role in the activation of human as well as murine mast cells. For instance, IgE(-Ag) induces the production of various effector molecules. Monomeric IgE induces production

of pro-inflammatory IL-8 and monocyte chemotactic protein-1 (MCP-1) in mast cells derived from umbilical cord blood.³⁹ Steroids known to decrease the inflammatory response inhibit production of these chemokines. In human lung mast cells, monomeric IgE not only induces IL-8 production, but also induces mast cell degranulation, LTB₄ production and intracellular calcium influx.⁴⁰ Mast cells derived from human umbilical cord blood synthesize and release I-309/CCL1 (a ligand for CCR8 that chemoattracts Th2 cells), GM-CSF and MIP-1 α /CCL3 in response to IgE(-Ag) stimulation.⁴¹

IgE(-Ag) also activates mast cells by increasing the number of Fc ϵ RI on the mast cell surface. Although increased numbers of Fc ϵ RI are also seen after IgE+Ag stimulation, IgE(-Ag) increases Fc ϵ RI numbers via receptor stabilization while IgE+Ag does this via actual upregulation of Fc ϵ RI expression.^{42,43} Furthermore, in the case of IgE+Ag, the number of Fc ϵ RI on the mast cell surface may actually decrease at very high levels of Ag due to receptor internalization.⁴⁴

Microarray analysis of 760 human genes showed changes in mast cell gene expression indicative of mast cell activation by IgE(-Ag) stimulation. Fifty-eight genes are upregulated after IgE(-Ag) stimulation and a total of 115 genes are upregulated during IgE+anti-IgE stimulation. The transcripts upregulated by IgE(-Ag) include many that are expected for immune cell activation: genes coding for cytokines (IL-1 β , IL-6 and CSF1), chemokines (IL-8/CXCL8, MIP-1 β /CCL4, MCP3/CCL7, GRO α /CXCL1 and GRO γ /CXCL3), cytokine and chemokine receptors, regulators of signal transduction and transcription factors, as well as other genes involved in immune regulation, cell proliferation and survival, adhesion and cytoskeletal remodeling.⁴⁵

Finally, incubation of human cultured mast cells with IgE significantly increased the expression of two nitric oxide (NO) generating enzyme isoforms, nitric oxide synthase 2 and 3 (NOS2 and NOS3, respectively). Interestingly, IgE+anti-IgE stimulation leads to further upregulation of NOS2 expression but decreased NOS3 expression.⁴⁶ This is consistent with findings in animal asthma models and patients with mild asthma of reduced NOS3 expression 30 minutes after antigen exposure followed by a subsequent increase in NOS2 expression concomitant with increased airway reactivity.^{47,48}

IgE-mediated activation phenomena have also been observed in Fc ϵ RI-expressing cells other than mast cells. For instance, monomeric IgE stimulates basophils (which are Fc ϵ RI-expressing cells) to produce CCL22 (macrophage-derived chemokine, or MDC), a chemoattractant for Th2 cells.⁴⁹ Monomeric IgE also inhibits apoptosis of neutrophils, which express Fc ϵ RI in atopic asthmatics and are thought to play a role in the development of asthma.⁵⁰ Activation of extracellular signal-related kinase (ERK), a required step for IgE(-Ag)-mediated mast cell survival, is required for differentiation of immature B cells into transitional and mature B cells.⁵¹

From these findings, it can be thought that in both mice and humans, monomeric IgE exerts various effects upon cells that express Fc ϵ RI, even in the absence of antigen.

MONOMERIC IgE SUBTYPES (HIGHLY CYTOKINERGIC IgE VS POORLY CYTOKINERGIC IgE)

Monomeric IgEs vary in their ability to induce different IgE(-Ag) mast cell activation events. This was brought to light by the inconsistencies found between the characteristics of monomeric IgE observed in initial studies done by Krystal et al and our research group. For instance, we found that monomeric IgE prolonged mast cell survival, but did

not affect mast cell proliferation, phosphorylation of signaling molecules, production of various cytokines and expression of apoptosis inhibitors. However, Krystal et al were able to induce almost all these mast cell activation phenomena. Another group found that monomeric IgE can induce actin polymerization.²³ It was thought that these discrepancies might have been due to a difference in the monomeric IgEs used in our experiments and various IgE molecules were examined with regards to their effect on mast cell activation and survival. As a result, it was shown that certain types of IgE molecules (highly cytokinergic IgE: HC IgE) not only prolong mast cell survival but also strongly influence mast cell degranulation and proliferation, mast cell migration²⁶ and adhesion to fibronectin,²⁹ leukotriene release, production of chemokines/cytokines such as IL-6 and TNF- α , phosphorylation of signaling molecules, as well as Fc ϵ RI aggregation²¹ and internalization.⁴⁴ On the other hand, there exist types of IgE molecules (poorly cytokinergic IgE: PC IgE) that do not affect mast cell activation processes other than prolongation of mast cell survival.⁵² In this manner, it was shown that individual IgE molecules have different properties (Fig. 1).

There is some knowledge about the differences underlying the divergent effects of HC and PC IgE on mast cell degranulation at a molecular level. Mast cell degranulation involves fusion of the secretory granule membrane with the plasma membrane. This membrane fusion reaction is mediated by a group of integral membrane proteins called SNAREs.⁵³ SNAREs localize to detergent-insoluble lipid raft microdomains in Fc ϵ RI-stimulated mast cells. There are two main categories of SNAREs, vesicle SNAREs (or v-SNAREs) present on vesicle membranes in the vesicle-associated membrane protein (VAMP)/synaptobrevin family and target SNAREs (or t-SNAREs) present on target membranes in the syntaxin and SNAP-23 family. The majority of SNAP-23 is lipid raft-associated, while only small amounts of syntaxin-4 or VAMP-2 are raft-associated. One copy each of SNAP-23/syntaxin/VAMP must form a ternary complex in lipid raft membranes for membrane fusion to occur.⁵⁴ HC IgE seems to regulate VAMP-2 expression at the level of mRNA (transcriptional and/or posttranscriptional). Monomeric IgE also seems to play a role in determining the subcellular location of SNAREs. SNAP-23 and VAMP-8 protein levels were preferentially localized to lipid rafts in mast cells cultured with HC IgE and high concentrations of PC IgE when compared to mast cells cultured with low concentrations of PC IgE.⁵⁵

Still, a clear explanation for the marked heterogeneity among IgE molecules has yet to be uncovered, as no information is truly available about the molecular differences amongst different types of IgEs. Affinity to antigen does not seem to affect IgE function—a typical HC IgE, SPE-7 and a much weaker HC IgE, H1 DNP- ϵ -26, have similar reported affinities (K_d = $\sim$ 15 nM).^{19,56} Anisotropy data has suggested that there is a difference in the extent of receptor aggregation between different IgE types. The more extensive receptor aggregation seen with HC IgE over PC IgE may help explain how a strong HC IgE can induce all activation events, whereas PC IgE can induce only a limited set of activation events to a lesser degree.²¹

The dichotomy between HC and PC IgEs is reminiscent of the variability between different human IgE molecules' ability to prime basophils for stimulation with histamine-releasing factor (HRF). HRF is a cytokine produced by macrophages and platelets. Basophils bound by IgE derived from atopic patients release histamine and cytokines such as IL-4 and IL-13 in response to HRF.^{57,58} Basophils bound by IgE from normal individuals do not show this same response. IgE that has the ability to respond to HRF was termed IgE⁺ and IgE that does not have the ability to respond to HRF was

termed IgE⁻. There is no information about if or how structural differences may underlie the functional differences between IgE⁺ and IgE⁻. However, altered usages of heavy-chain variable (V_H) genes have been reported in IgE of allergic patients. There is overrepresentation of V_H5 (one of the smallest gene families containing two members out of the total of 52 functional V_H genes) in IgE derived from allergic patients.⁵⁹ This indicates that there is indeed structural heterogeneity between human IgE molecules. Further study of human IgE heterogeneity may reveal relevance to human atopic and allergic diseases.

IgE(-Ag) STIMULATION: HYPOTHESIZED MECHANISMS OF ACTION

Questions remain about how IgE(-Ag) mediates mast cell responses similar to those seen after IgE+Ag and IgE+anti-IgE stimuli. It is established that IgE+Ag or IgE+anti-IgE requires FcεRI aggregation to mediate mast cell activation.⁶⁰ This FcεRI aggregation mechanism is consistent with the common mechanism (receptor dimerization/oligomerization) seen with receptor-mediated cell activation.^{61,62} Biophysical methods, including time-resolved phosphorescence anisotropy (which can assess the size and rigidity of membrane structures by quantifying, in the nano- to microsecond range, the motion of phosphorescent dye-labeled membrane proteins that are contained within the membrane), have suggested that IgE(-Ag) stimulation also results in FcεRI aggregation.⁵² Calculations have shown that IgE-IgE interactions on the cell surface (assumed to be FcεRI aggregation) took place at IgE concentrations >1 μg/mL in the absence of Ag. This is consistent with data found at several laboratories that IgE effects on survival and activation are seen at ≥ 0.5 μg/mL IgE concentrations.²⁸

IgE+Ag stimulation requires localization of aggregated receptors in cholesterol/sphingolipid-rich plasma membrane domains (lipid rafts).^{63,64} Lipid rafts are membrane microdomains rich in cholesterol and glycosphingolipids that have been implicated in the regulation of intracellular protein trafficking.⁵⁴ Disruption of lipid rafts prevents IgE(-Ag) induced ERK activation, suggesting that localization of aggregated receptors in lipid rafts is also necessary for IgE(-Ag) stimulation.¹⁹

Although these findings suggest that FcεRI aggregation is required for IgE(-Ag) stimulation, it is still unclear how IgE could cross-link FcεRI in the absence of antigen. There are several models that can be formulated regarding the mechanism of cross-linking that occurs in the absence of a specific antigen, although these proposed models do not escape the realm of conjecture. Since monovalent hapten binds to the variable (V) region and inhibits monomeric IgE effects, the V-region of monomeric IgE is likely involved somehow in mediating those IgE(-Ag) effects (such as receptor aggregation). For instance, a monomeric IgE molecule may bind to FcεRI and then use itself or another molecule to mediate FcεRI cross-linking in an Ig-Ig or Ig-X-Ig (X = unknown bridging molecule) type interaction. One type of HC IgE in particular, SPE-7 IgE, can change the initial structure of its variable region and then bind different antigens, inducing isomerization of the binding site and leading to high-affinity complexes with a deep or narrow binding site.⁶⁵ It is possible that the fragment antigen-binding (Fab) portion of FcεRI-bound IgE directly or indirectly interacts with a neighboring FcεRI-bound IgE via a third molecule that is recognized by the neighboring IgEs. Furthermore, based on findings that IPSE/alpha-1 expressed by *Schistosoma mansoni* binds to IgE,⁶⁶ it is thought that proteins derived from parasites and other organisms cross-link monomeric IgE. Studies have also shown that a 28 kDa protein, p28, may be involved in the FcεRI aggregation mediated by monomeric

IgE. p28 is associated with ~50% of Fc ϵ RI in RBL-2H3 rat mast cells in the absence of IgE, but dissociates from Fc ϵ RI at a high concentration (20 μ M) of IgEs.⁶⁷ The identity of this p28 sensor protein is still unknown and further studies must be done to reveal the true involvement of p28 in IgE(-Ag) receptor aggregation. Despite the building evidence that IgE(-Ag) effects are due to binding by monomeric, not aggregate, IgE molecules, it still needs to be conclusively proven that IgE does not simply aggregate before binding Fc ϵ RI at concentrations that result in IgE(-Ag) Fc ϵ RI aggregation.²⁸

The exact events that take place after IgE(-Ag)-induced Fc ϵ RI aggregation also remains to be understood. Currently, it is thought that cytokines such as IL-3 and IL-6 act in an autocrine fashion on mast cells following IgE(-Ag) stimulation. Pro-/anti-apoptotic Bcl-2 family member proteins and mitogen-activated protein kinases (MAPKs) seem to play a role in mediating the effects of IgE(-Ag). Signaling via toll-like receptors (TLRs) synergistically augment the effects of IgE(-Ag) stimulation, likely by modulating the expression of Bcl-2 family member proteins and phosphorylating MAPKs.⁶⁸

Several findings support these proposed mechanisms. HC IgEs do induce secretion of cytokine(s) that support mast cell survival in an autocrine/paracrine manner. Fc ϵ RI aggregation appears to induce autocrine secretion of IL-3, activating IL-3R signaling pathways that promote survival.⁶⁹ Stat5 was found to be an important part of this activated IL-3R signaling pathway.⁷⁰ Neutralization of IL-3 or IL-3 deficiency drastically reduced, but did not completely prevent, HC IgE-mediated survival effects.⁶⁹ Furthermore, Krystal et al reported that monomeric IgE requires phosphorylation of ERK and intracellular calcium influx, whose signal transduction pathways result in production of reactive oxygen species (ROS) that induce IL-3 production.⁷¹

The cytokine IL-6 also seems involved in mast cell survival. Adding IgE to human lung mast cells (HLMC) leads to increased HLMC IL-6 mRNA expression and protein release. Anti-IL-6 antibody, but not anti-SCF antibody, prevents IgE(-Ag) mast cell survival effects. This suggests that IL-6 plays an important role in mediating IgE(-Ag) mast cell survival effects. This is consistent with the knowledge that IL-6 prevents apoptosis in HLMC and cord blood-derived mast cells (HCBMC).^{72,73}

Bcl-2 family members play a key role in mediating IgE(-Ag)-induced mast cell survival. Expression of anti-apoptotic Bcl-2 family members, Bcl-xL and Bcl-2, is dependent on IL-3 and it has been reported that HC IgE prolongs mast cell survival by autocrine IL-3 production to increase the expression of Bcl-xL.⁶⁹ In line with these findings, Bcl-xL can support mast cell survival in IL-3^{-/-} mast cells.⁶⁹ Also, lipopolysaccharide (LPS), a ligand for TLR4, has been shown to accentuate IgE(-Ag)-mediated mast cell survival in the absence of growth factors by working synergistically with monomeric IgE to enhance expression of Bcl-xL. LPS also works with monomeric IgE to reduce expression of pro-apoptotic Bcl-2 family members, Puma and Bim. In the absence of monomeric IgE, LPS does not have these effects.⁶⁸ It seems that multiple mast cell survival pathways converge upon the common mechanism of regulating Bcl-2 family member expression.

Phosphorylation of several MAPKs is also part of the IgE(-Ag)-induced signaling pathway leading to mast cell activation events. LPS and another TLR ligand, peptidoglycan (PGN), augment the IgE(-Ag) induction of inflammatory cytokine production by mast cells via phosphorylation of several MAPKs. Interestingly, the synergistic effects of LPS and PGN (that bind TLR4 and TLR2, respectively) are not mediated by the same set of MAPKs utilized in IgE(-Ag) signaling. The p38 MAPK inhibitor (SB203580) prevents mast cell response to HC IgE, LPS, PGN and to

combinations of HC IgE with LPS or PGN. However, while JNK inhibitor (SP600125) and ERK inhibitor (U0126) inhibit mast cell response to HC IgE, they have little effect on mast cell response to TLR ligands (LPS, PGN) and only partially inhibit response to combinations of HC IgE with LPS or PGN.⁷⁴ Thus, MAPKs are involved in IgE(-Ag)-mediated mast cell activation, but much more must be elucidated about their exact role in IgE(-Ag) activation pathways.

Interestingly, PC IgEs do not support survival of $Fc\epsilon RI\alpha^{-/-}$ mast cells cocultured with wild-type cells.²¹ This indicates that PC IgEs do not induce secretion of survival-mediating cytokines in the way that HC IgEs do and that there are at least 2 different survival mechanisms evoked by IgE(-Ag) at the cellular level.²⁸ There may also be an IL-3-independent survival pathway used by IgE(-Ag) that requires Syk (as no survival effects of monomeric IgEs were seen in $Syk^{-/-}$ cells),²¹ but the exact mechanism remains to be characterized.

IgE+Ag also seems to prevent mast cell apoptosis via an autocrine mechanism, producing IL-3, IL-4 and GM-CSF.⁷⁵ IL-4 renders mast cells reactive to the other 2 cytokines, IL-3 and GM-CSF. IgE+Ag up-regulates expression of FLIP, a caspase-8 inhibitor, to resist Fas-induced apoptosis.⁷⁶ IgE+Ag also up-regulates expression of anti-apoptotic proteins such as A1 and Bcl-xL as well as pro-apoptotic proteins such as Bim-EL and Bim-L.⁷⁷ A1 has been shown to be important for IgE+Ag-induced mast cell survival.⁷⁶ It remains to be seen if A1, Bim-EL and Bim-L play a role in IgE(-Ag)-induced mast cell survival.

SIGNAL TRANSDUCTION INDUCED BY MONOMERIC IgE

In mast cells, $Fc\epsilon RI$ aggregation induces phosphorylation of ITAMs in β and γ subunits of $Fc\epsilon RI$, initiating specific signaling events through PTKs (Lyn, Fyn, Hck and Syk), LAT (linker for activation of T cells) and SLP-76. Activation of these signaling pathways induces mast cell degranulation and cytokine production.^{78,79} At all levels of the $Fc\epsilon RI$ signaling cascade, both subtle and major similarities and differences are being revealed about how IgE(-Ag) and IgE+Ag are unique. For instance, HC IgE(-Ag) signaling induces phosphorylation of the $Fc\epsilon RI$ β subunit in a manner similar to IgE+Ag.⁸⁰ However, the phosphorylation kinetics of signaling molecules differ between the two types of stimulation.^{26,27} Also, phosphorylation of unique sets of signaling molecules are differentially required for the induction of various mast cell activation events. Differences have been found between the roles of PKC β I and II in IgE(-Ag) and IgE+Ag stimulation of bone marrow mast cells.^{52,81,82} IgE(-Ag)-induced mast cell migration relies on the Lyn, Syk and Fyn PTKs and protein kinase C (PKC) β , ϵ and θ to a lesser degree.²⁶ IgE(-Ag)-mediated survival does not seem to require Fyn, Gab2, PI3K p85, or Akt.⁶⁹ Mast cell adhesion to fibronectin utilizes Lyn and Syk but not Fyn PTK, as well as other Src family kinases and PKC θ .²⁹

The $Fc\epsilon RI$ γ subunit ($FcR\gamma$) ITAM is required in different ways for various mast cell functions. Also, the $FcR\gamma$ -ITAM is utilized differently by IgE(-Ag) and IgE+Ag to mediate the same mast cell function.⁸³ IgE(-Ag) requires the α and γ subunits of $Fc\epsilon RI$ to mediate mast cell survival.^{18,84} IgE(-Ag)-induced cytokine production is $FcR\gamma$ -ITAM-dependent, but IgE(-Ag)-induced upregulation of $Fc\epsilon RI$ is $FcR\gamma$ -ITAM-independent. IgE(-Ag)-induced receptor up-regulation also does not require Lyn, Syk, or other PTKs that are required by degranulation and other activation events.⁴⁴ The differences in $Fc\epsilon RI$ subunits and

signaling molecules utilized by IgE(-Ag)-induced FcεRI up-regulation is consistent with the observation (mentioned earlier in *IgE(-Ag) Stimulation of Mast Cells (iii)*) that IgE(-Ag)-induced FcεRI up-regulation is due more to the increased lifetime of surface-resident receptors rather than to its increased synthesis and/or transport to the plasma membrane.^{15,42,43}

It seems that the duration and strength of FcεRI signaling determines the type of mast cell response. Activation of ERK by activated Lyn and Syk is part of the initial FcεRI signaling pathway.²¹ It is known that the duration of ERK activation alters the quality of biological responses (sustained ERK activation is required for mast cell survival but not degranulation).^{85,86} The prolonged activation of ERK seen with slow on-rate IgE interaction with FcεRI is in line with the pattern of ERK activation required for survival of other cell types such as T cells.⁸⁷ The FcεRI γ subunit signaling required for survival of CD8/γ-expressing γ^{-/-} mast cells is weak relative to the γ signaling required for degranulation.⁸⁵ These findings suggest that FcεRI signaling required for mast cell survival is prolonged but weaker than that required for mast cell degranulation.

In many receptor systems, ERK activation is regulated by Ras, which is activated by guanine nucleotide exchange factors (GEFs) such as Sos. Sos has been found to be preferentially required for FcεRI signaling by IgE(-Ag) more than IgE+Ag.⁸⁸ ERK activation in Syk^{-/-} mast cells is totally abolished, showing that Syk is indispensable to IgE(-Ag)-induced mast cell survival and degranulation, as well as cytokine production.^{69,71} Substrates for Syk PTK, non-T-cell activation linker (NTAL/LAB/LAT2) and LAT (both transmembrane adaptor molecules that localize to lipid rafts and possess multiple tyrosine-based activation motifs) have been shown to play a role in IgE+Ag stimulation of mast cells. These two transmembrane adaptor molecules have also been shown to play a role in IgE(-Ag)-induced mast cell activation and survival via retention of Grb2 and Sos within the plasma membrane.⁸⁸ Interestingly, NTAL/LAB/LAT2 and LAT are phosphorylated with slow kinetics upon IgE(-Ag) stimulation, but are only transiently phosphorylated upon IgE+Ag stimulation. The importance of NTAL and LAT in stabilizing Grb2-Sos within the plasma membrane is supported by the finding that Gads, a Grb2-like adaptor protein, is required for mast cell Ca²⁺ influx, degranulation and cytokine production after FcεRI aggregation.⁸⁹

There have been quantitative differences found in the signaling pathways between IgE(-Ag) and IgE+Ag stimuli. For instance, Lyn is absolutely required for IgE(-Ag) adhesion to fibronectin and essential to FcεRI internalization. However, although Lyn is needed at low levels of antigenic stimulus, at a certain threshold and higher, antigenic stimulus can induce adhesion without relying on Lyn.²⁹ There are also qualitative discrepancies between the required mechanisms of action leading up to intracellular calcium influx resulting from IgE(-Ag) versus IgE+Ag stimulus.^{23-25,90} HC IgE leads to a transient increase in intracellular Ca²⁺. SK and F96365, a specific inhibitor of store-operated Ca²⁺ channels, suppresses SPE-7 IgE(-Ag) induced intracellular Ca²⁺ increase. Broad-spectrum Ca²⁺ channel inhibitors, La³⁺ or Gd³⁺, do not. IgE+Ag induced Ca²⁺ responses are suppressed by all three inhibitors.⁹⁰ This suggests that IgE(-Ag) and IgE+Ag induce Ca²⁺ influx via different mechanisms (Fig. 2).

While IgE(-Ag) and IgE+Ag both elicit mast cell responses via FcεRI signaling pathways that utilize similar sets of signaling molecules, key differences between the signaling pathways are being uncovered. Elucidating the similarities and differences between IgE(-Ag) and IgE+Ag signaling will further our understanding of allergic disease at a cellular level.

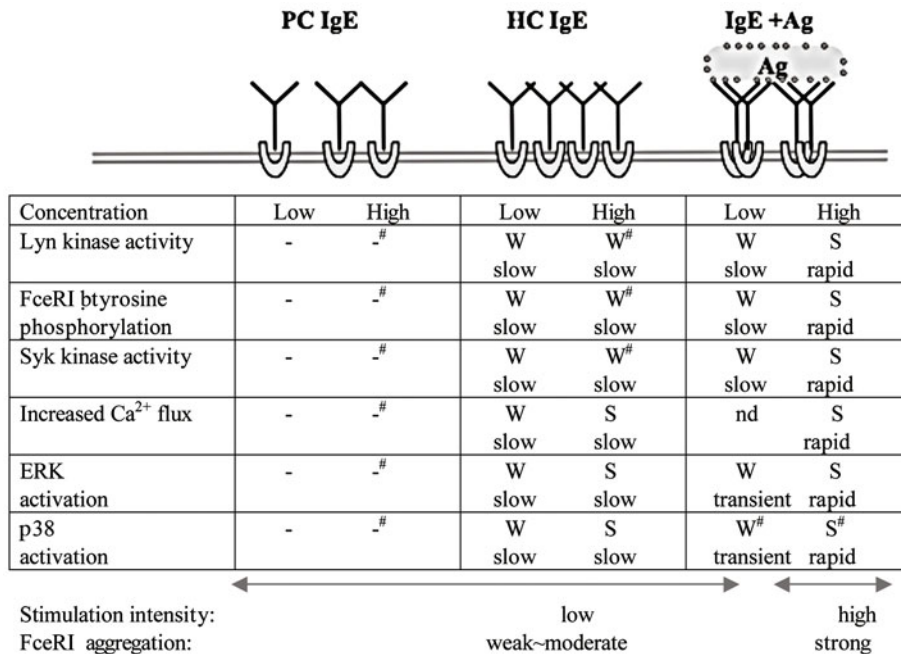


Figure 2. Comparison of signaling events induced by different modes of FcεRI stimulation. Data with mouse BMMCs are summarized. Concentrations of stimuli are as follows: PC IgE (H1 DNP-ε-206) and HC IgE (SPE-7), 0.5 μg/ml (low) and 5 μg/ml (high); and IgE+Ag (DNP₂₁-BSA), 1 ng/ml (low) and 100 ng/ml (high). Notice that biological events are listed in a rough order of occurrences and receptor aggregation presumably occurs in all modes of stimulation, except for low PC IgE concentrations. -, not detected, -[#], very weak; W, weak; W[#], weak-moderate; S[#], moderate-strong; and S, strong. Reproduced with permission from: Kawakami T, Kitaura J. J Immunol 2005; 175:4167-4173;²⁸ ©2005 The American Association of Immunologists, Inc.

IN VIVO EFFECTS OF IgE(-Ag) STIMULATION

Although it is not easy to generate definitive in vivo evidence of IgE(-Ag)-mediated mast cell survival and activation, supporting data have recently accumulated. Increased mast cell numbers were seen in mice transplanted with IgE-producing hybridomas that increased serum IgE levels.²¹ However, mast cell numbers were not increased in IgE-transgenic mice,⁹¹ and the presence of IgE was not found to be a requirement for mast cell development.⁹² These findings may suggest that IgE levels are not the key to maintaining homeostasis of mast cell populations in vivo. However, these findings do not undermine the previously hypothesized positive effects that IgE(-Ag) may have on mast cell survival, since the concentration of IgE must be relatively high for the survival effects of IgE(-Ag) to take place. In fact, several findings do support IgE(-Ag)-mediated mast cell survival and activation. There is in vivo evidence that IgE may be needed to maintain mast cell survival during parasitic infections. In mice infected with *Trichinella spiralis*, monomeric IgE is needed to protect the survival of mast cells.⁹³ In wild-type mice, *Trichinella spiralis* infection elicits an IgE response (pronounced expansion of the mast cell population in the intestine and spleen).⁹³ In IgE^{-/-} mice, mast cells migrate from the gut to the spleen, where they undergo accelerated apoptosis compared to wildtype

mice. This suggests that high-level IgE is essential in promoting mast cell survival in vivo by preventing mast cell apoptosis in the spleen. There is also in vivo support of the proposed IL-3 autocrine mechanism for mast cell survival induced by IgE(-Ag). In IL-3^{-/-} mice, mucosal mastocytosis is impaired during *Strongyloides venezuelensis* infection.⁹⁴

Thus, in certain conditions where high levels of IgE are seen, such as parasite infection (53.3 µg/mL serum IgE in *T. spiralis*-infected mice)⁹³ and atopy (sometimes >100 µg/mL in NC/Nga mice),⁹⁵ monomeric IgE may play a role in regulating mast cell survival. However, it is also possible that in parasite infections, antigen is somehow involved in enhancing mast cell survival in an IgE+Ag manner, although IgE produced in response to nematode infections has not been shown to be specific to the parasite.⁹⁶

With regards to the pathological role of IgE in vivo, Oettgen et al have studied the role of IgE in contact sensitivity using IgE-deficient mice. IgE was required for optimal sensitization but not for elicitation of contact sensitivity.⁹⁷ Mast cells were required for both sensitization and elicitation phases. Contact sensitivity was depressed in IgE-deficient mice as compared to wild-type mice, but was restored once hapten-irrelevant IgE or sensitized cells from wild-type mice were administered. During the sensitization phase, IgE was required for the generation of cytokines. Similarly, IL-6, IL-1β and MCP-1/CCL2 (and weakly TNF-α) can replace IgE in sensitization. Thus, even in the absence of a specific antigen, monomeric IgE plays a role in the contact hypersensitivity reaction. The same group also showed that inhalation of an *Aspergillus fumigatus* extract in mice induces a dramatic rise in IgE accompanied by an increase in airway activated mast cells.⁹⁸ Both mast cell expansion and activation were markedly attenuated in IgE-deficient mice. The recruitment of eosinophils to the airways was also reduced in these mice. IgE Abs were shown to sustain the survival of mature mast cells. From these findings, it can be suggested that monomeric IgE is required not only for mast cell sensitization, but also for maintenance of mast cell survival in vitro and in vivo.

MONOMERIC IgE AND MAST CELL DIFFERENTIATION

Mast cells differentiate from hematopoietic progenitor cells in the bone marrow after progenitor cells migrate to local tissues.⁹⁹⁻¹⁰¹ Stem cell factor (SCF) and its receptor, KIT, are essential for the differentiation, proliferation and survival of mast cells.¹⁰² However, other cytokines, growth factors and other factors are also involved in the development, proliferation, survival and differentiation of mast cells.¹⁰³ Monomeric IgE acts not only upon mature mast cells, but works on mast cell progenitors to induce differentiation into mature mast cells. Bone marrow cells grown for 4 weeks in IL-3 culture supplemented with monomeric IgE, especially HC IgE, resulted in an increased number of mast cells compared with cultures grown without IgE. Also, although bone marrow cells cultured in IL-3-containing medium do eventually consist of >85% mast cells, the presence of IgE (especially HC IgE) reduces the time period required for the mast cell purity to reach ≥80%.⁵⁵

Properties of mast cells exhibit heterogeneity, depending on what tissue and species they are derived from.^{104,105} Mast cells can exhibit differences in lifespan, morphology, development, expression pattern of mouse mast cell proteases (mMCPs) and proteoglycans and sensitivity to immunologic and nonimmunologic stimuli.¹⁰⁶⁻¹¹¹ Monomeric IgE binds the FcεRI receptor expressed on mast cell precursors in the early stages of mast cell differentiation to alter the expression of various genes, especially

those genes that are relevant to differentiation. As a result, different IgE subtypes can modulate the mast cell phenotype in various ways and likely play a role in giving rise to mast cell heterogeneity.

For example, the expression of early growth response factor-1 (Egr-1) is upregulated in cultures containing high and low concentrations of HC IgE and a high concentration of PC IgE. Egr-1 is a transcription factor involved in the production of TNF- α in mast cells. TNF- α helps mediate IL-3-dependent differentiation of mast cells.¹¹² TNF- α expression was increased in bone marrow cells cultured with HC IgE and high concentrations of PC IgE (consistent with the increased Egr-1 expression seen with HC IgE and high concentrations of PC IgE). However, levels of TNF- α protein were barely detectable even in bone marrow cells cultured with HC IgE. Expression of IL-13 and TGF- β is comparable in bone marrow cells cultured with different subtypes of IgE.⁵⁵

These findings suggest that monomeric IgE induces mast cell differentiation and plays a role in determining mast cell phenotype.

POLYCLONAL IgE AND MAST CELL ACTIVATION

Although there is evidence that monoclonal IgE acts in an IgE(-Ag) manner to induce mast cell activation and mast cell differentiation, the effect of polyclonal IgE on mast cells has not been studied until recently. A mixture of equal amounts of several different types of monoclonal monomeric IgE promoted cell survival and induced cytokine production at the level that would be expected based on the average of the different types of monomeric IgE.²¹ This suggested that polyclonal IgE would also be able to affect mast cell survival and function.

Polyclonal monomeric IgE taken from the serum of mice with atopic dermatitis induced by epicutaneous administration with mite antigen and Staphylococcus Enterotoxin B (SEB)^{113,114} resulted in prolonged mast cell survival and cytokine production in comparison to serum from mice without atopic dermatitis. This prolonged mast cell survival and cytokine production was not seen when IgE was removed from the blood or when the serum utilized was from IgE-deficient mice with atopic dermatitis. Also, prolonged mast cell survival and cytokine production was not seen when polyclonal monomeric IgE from the serum of mice with atopic dermatitis was added to mast cells from Fc ϵ RI α -deficient mice. These results suggest that polyclonal IgEs mediate their effects via Fc ϵ RI in a similar manner to monoclonal IgEs. The effect of human polyclonal monomeric IgE, from the serum of patients with atopic dermatitis, on human peripheral blood-derived cultured mast cells was additionally studied. IL-8 production was increased in mast cells cultured with serum taken from patients with atopic dermatitis when compared to mast cells cultured with serum added from healthy subjects. Removing IgE from the serum inhibited IL-8 production.¹¹⁵

Previous studies have dealt with the effects of monoclonal monomeric IgE. However, monomeric IgE in the serum of actual patients is polyclonal. The results of these polyclonal monomeric IgE studies are of great significance, because they provide the first evidence that polyclonal monomeric IgE in the serum of patients does actively stimulate mast cells. Better understanding the effects of polyclonal monomeric IgE is the next step in proving that the effects of monomeric IgE play an active role in the development of allergic diseases.

CONCLUSION

Mast cells play a major role in allergy, anaphylaxis, innate immunity and acquired immunity. The role of monomeric IgE was previously thought to be limited to passive sensitization of the mast cell for IgE+Ag activation. It is now understood that monomeric IgE plays a much larger role in mast cell function and can induce mast cell activation events via IgE(-Ag) stimulation. The mechanisms of action by which IgE mediates its effects on mast cells via IgE+Ag and IgE(-Ag) stimulation are being uncovered. FcεRI aggregation seems to play a large role, as do the signaling molecules Lyn, Fyn, Hck and Syk. Evidence supports the role of autocrine IL-3 secretion in IgE(-Ag) stimulation. Experiments being done studying the differences between HC IgE and PC IgE suggest that different subtypes of monomeric IgEs elicit different sets of mast cell responses and that the different IgE subtypes may work via different mechanisms of action. While there is a lack of definitive evidence of IgE(-Ag) effects in vivo, there have been promising studies, especially those looking at mast cell survival in mice with parasitic infections. The more recent studies have elucidated a role for IgE(-Ag) in mast cell differentiation in addition to its previously acknowledged role in mature mast cell survival and activation. The most recent studies have also found that polyclonal monomeric IgE has effects on mast cell function similar to those seen with monoclonal monomeric IgE stimulation.

The clinical importance of these findings lies in the fact that monomeric IgE plays a larger role in mast cell function, survival and differentiation than previously believed. Since the mast cell is involved in allergy, anaphylaxis and both innate and acquired immunity, better understanding of the effects and mechanisms of action of IgE(-Ag) stimulation will lead to an improved understanding of these pathologic and protective responses. This, in turn, may lead to the uncovering of better treatments for asthma and other allergic diseases, as well as ways to improve host response to infections.

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