

BRIEF REVIEWS

Mast Cell Survival and Activation by IgE in the Absence of Antigen: A Consideration of the Biologic Mechanisms and Relevance¹Toshiaki Kawakami³ and Jiro Kitaura²

Mast cells are not only major effector cells in allergy and host defense against parasites and bacteria but also important cellular components in other immune responses. Recent studies on the effects of monomeric IgE on mast cell survival and activation have made an impact on our view of the IgE binding to its high-affinity receptors, FcεRI. Traditionally, IgE binding to FcεRI has been considered as a passive action of "sensitization" before receptor aggregation by Ag. However, recent studies indicate that at high concentrations some monoclonal IgEs have effects on mast cells similar to or identical to those induced by IgE+Ag stimulation. These effects may be due to induction of FcεRI aggregation by these IgEs in the absence of Ag. This review will synthesize recent findings of the heterogeneity of IgEs in their ability to induce survival and activation events, their mechanisms, the potential in vivo significance of IgE-FcεRI interactions, and the implications of the mouse studies to human diseases. The Journal of Immunology, 2005, 175: 4167–4173.

Mast cells reside close to the mucosal and epithelial interfaces with the environment and around blood vessels. A vast majority of mast cell studies have dealt with their predominant role in acute allergic reactions (immediate hypersensitivity) and more recently their roles in late-phase allergic reactions (1) and in the host defense against certain bacteria (2, 3) and parasites (4, 5). Beyond this traditional understanding, our knowledge of mast cell functions has recently been substantially expanded (6, 7) and now includes those in autoimmunity such as experimental allergic encephalomyelopathy and rheumatoid arthritis (8, 9), delayed-type hypersensitivity (10), angiogenesis (11), and congestive heart failure (12).

FcεRI expressed on murine mast cells consists of four subunits ($\alpha\beta\gamma_2$): an IgE-binding α subunit, a signal-amplifying, receptor-stabilizing β subunit, and two disulfide-bonded γ subunits that are the main signal transducer (13). Stimulation of

IgE-sensitized mast cells with Ag (this mode of stimulation hereafter referred to as IgE+Ag) or anti-IgE Ab (referred to as IgE+anti-IgE) induces receptor aggregation or cross-linking. Aggregation of FcεRI leads to the activation of β subunit-associated Lyn, a Src family protein tyrosine kinase (PTK).⁴ Activated Lyn phosphorylates tyrosine residues in the ITAMs in the cytoplasmic regions of β and γ subunits. Phosphorylated β and γ ITAMs recruit Lyn and Syk, respectively. Another Src family PTK, Fyn, was also shown to associate with FcεRI and to play a complementary role, particularly by activating PI3K (14). These PTKs phosphorylate numerous targets and activate several signaling pathways, including the PI3K, phospholipase C/ Ca^{2+} , and several MAPK pathways (15, 16). These signaling events lead to degranulation and cytokine and chemokine production. Chemicals (e.g., histamine and serotonin), lipids (leukotrienes and PGs), nucleotides (e.g., adenosine), and polypeptides (e.g., proteases, cytokines, and chemokines) released from activated mast cells are effector molecules that induce allergic or inflammatory reactions or modulate innate and adaptive immune responses (7, 17).

IgE-induced mast cell survival and activation in the absence of Ag

One of the traditional views that have been widely accepted is on effects of IgE binding to the FcεRI. IgE binding was once thought of as a passive step of sensitization, which would keep mast cells at a "resting" state, before receptor aggregation with multivalent Ag (allergen) or anti-IgE. In stark contrast to this traditional view, it was shown that IgE binding to FcεRI in the absence of specific Ag (the stimulation mode referred to as IgE(-Ag)) engenders several biological outcomes in mast cells: IgE(-Ag) can induce up-regulation of cell surface expression of the receptor (i.e., FcεRI) (18–20), survival (21, 22), increase in histamine content (23), histamine release, leukotriene release, receptor internalization, DNA synthesis (24), increased responses to compound 48/80 and substance P (25), increase in F-actin content (26), membrane ruffling (27), mast cell adhesion to fibronectin (28), and migration (29). These events are

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Received for publication May 10, 2005. Accepted for publication August 4, 2005.

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¹ This study was supported by Public Health Service Grants AI50209 from the National Institutes of Health (to T.K.).

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⁴ Abbreviations used in this paper: PTK, protein tyrosine kinase; BMDC, bone marrow-derived mast cell; HC, highly cytokinergic; PC, poorly cytokinergic.

almost all inducible by IgE+Ag or IgE+anti-IgE (Fig. 1). Importantly, these IgE(-Ag) effects are induced at high concentrations of IgE 2–3 logs more than required to sensitize a mast cell for Ag-dependent activation. Parenthetically, IgE was shown to augment IL-3-induced mast cell proliferation (30), but unfortunately, this finding in 1986 received little attention.

Earlier studies established that receptor aggregation induced by IgE+Ag or IgE+anti-IgE is required for mast cell activation (31), which is in line with receptor dimerization or oligomerization as the common mechanism for receptor-mediated cell activation, e.g., that by peptide growth factors (32, 33). A biophysical method termed time-resolved phosphorescence anisotropy measurement (that quantifies motions of phosphorescent dye-labeled membrane proteins in the nano- to microsecond range, thus assessing the size and rigidity of the membrane structures containing the labeled proteins) suggests that IgE(-Ag) also induces receptor aggregation (24). The enhancement in the affinity of binding an Ag-combining site of an IgE molecule to an epitope on another IgE was estimated by calculating the probability of finding as nearest neighbor a distinct receptor at distances between r_0 and $r_0 + \Delta r$ on a cell surface (34). This simulation ($r_0 = 75\text{\AA}$ and $\Delta r = 30\text{\AA}$) predicted a significant probability (>0.4 or ~ 0.1 , respectively, for cells with 3×10^5 (RBL-2H3 rat mast cells) or 5×10^4 (mouse bone marrow-derived mast cell (BMMC)) receptors per cell), with which IgE-IgE interactions (presumed FcεRI aggregations) take place on the cell surface at IgE concentrations $> 1\text{ }\mu\text{g/ml}$ in the absence of Ag. These calculations match the experimental data obtained in different laboratories in that IgE effects on survival and activation are seen at $\geq 0.5\text{ }\mu\text{g/ml}$ IgE concentrations, and they are consistent with IgE(Ag)-mediated FcεRI aggregation suggested by the phosphorescence anisotropy data. By contrast, the same simulation predicts a $\sim 10^4$ -fold lower probability of nearest neighbor (IgE-IgE) interactions in solution. Several studies suggested that survival and other activation effects were due to binding to FcεRI by monomeric, but not aggregate, IgE molecules in the IgE preparations. However, IgE(-Ag)-induced FcεRI aggregation will need to be confirmed by other methodologies.

Despite the rapid progress in this area, there are many issues to be resolved (Table I). It has never been absolutely shown if the very high amounts of IgE added under the culture conditions used do not self-aggregate. IgE effects, interpreted as due to monomeric IgE, would be in fact due to aggregated IgE within the culture or IgE sticking to plastic plates. Also, the cultured mast cells exposed to these high levels of IgE have not seen IgE during maturation. This is not what happens in vivo, where an individual IgE receptor expressed on the developing mast cell is immediately engaged by IgE. Furthermore, how would such a system with no Ag specificity mechanistically function in tissues?

Heterogeneity of IgE molecules

Not all monoclonal IgE molecules can induce all the activation events listed above in the absence of Ag: IgEs display a wide spectrum in their ability to induce the production and secretion of IL-6 and TNF-α with highly cytokinergic (HC) IgEs at one extreme end and poorly cytokinergic (PC) IgEs at the other (24). Anisotropy data suggest that more extensive receptor aggregations take place with HC IgEs than with PC IgEs. Consistent with such differences in the extent of receptor aggregation, a strong HC IgE could induce all the tested activation events, whereas PC IgEs cause only a limited set of activation events and even for those to a lesser extent. Importantly, a mixture of seven different monoclonal IgEs exhibited survival effects (24), suggesting that polyclonal IgE should also have survival effects.

Models for IgE-induced receptor aggregation

Despite this remarkable heterogeneity among IgE molecules, no information is available about differences among IgEs at molecular levels. Affinity of IgEs to FcεRI does not appear to be a determining factor of the differences between HC and PC IgEs, as a typical HC IgE, SPE-7, and a much weaker HC IgE, H1 DNP-ε-26, have similar reported affinities ($K_D = \sim 15\text{ nM}$) (35, 36). The variable (V) regions of IgE molecules seem important for receptor aggregation and consequent survival and cytokine production, as monovalent hapten (for which the used

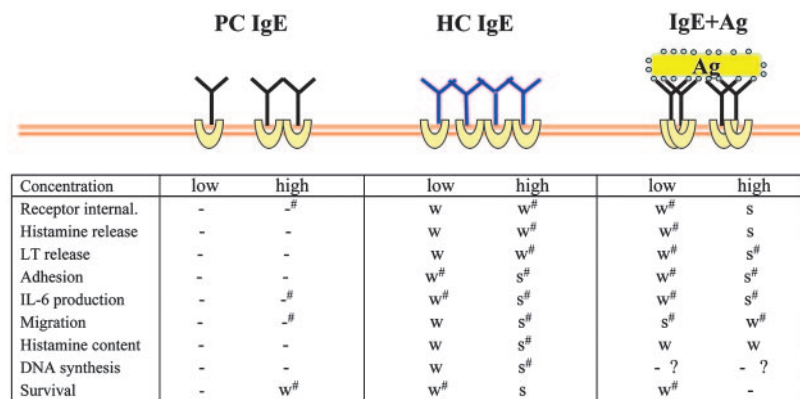


FIGURE 1. Biological effects by various modes of stimulation via the FcεRI. Experiments using mouse BMMCs are summarized. Concentrations of stimuli are as follows: PC IgE (H1 DNP-ε-206) and HC IgE (SPE-7), 0.5 μg/ml (low) and 5 μg/ml (high); and IgE+Ag (DNP₂₁-BSA), 1 ng/ml (low) and 100 ng/ml (high). Notice that biological events are listed in a rough order of occurrences and that 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. References: receptor internalization (24, 44, 50); histamine release (14, 21, 22, 24, 26, 27, 43, 44, 50); leukotriene release (21, 22, 24); adhesion (28, 56); IL-6 production (21, 22, 24, 43, 44, 50, 51); migration (29); histamine content (23, 24, 57); DNA synthesis (21, 22, 24, 86); and survival (21, 22, 24, 43, 44, 50, 51, 58–62). In Ref. 60, MC9 mouse mast cells were used. In Ref. 62, BMMCs were stimulated with anti-TNP IgE (hybridoma supernatants) and TNP_{4,8}-BSA (1 μg/ml). However, it is not clear whether the used Ag concentration corresponds to weak or strong stimulus.

Table 1. Outstanding questions to be addressed in future

1. Why high concentrations of IgE are required for mast cell survival and activation?
2. More definitive evidence for receptor aggregation by monomeric IgE should be obtained.
3. What is the mechanism for receptor aggregation? Is the same mechanism used by monomeric IgE and IgE+Ag to induce receptor aggregation? Does p28 play any role?
4. What receptor components and parts thereof are required for monomeric IgE-induced receptor up-regulation, survival, cytokine production, and other activation events? Are coreceptor or costimulatory receptors present?
5. Are the same sets of signaling pathways used by monomeric IgE and IgE+Ag to cause survival, cytokine production, and other activation events?
6. What makes the difference between HC and PC IgEs? Is there a wide spectrum in the ability of human IgEs similar to that of mouse monoclonal IgEs from HC to PC IgEs. If so, do such differences in human IgEs have pathophysiological significance? Do allergic patients tend to have HC IgEs than nonallergic individuals?

IgE is specific) can inhibit IgE(-Ag)-induced receptor aggregation, survival, and cytokine production (24). Potentially related to the receptor aggregation mechanism and as a likely factor that determines the potency to induce receptor aggregation, a recent study showed that the SPE-7 Ab expressed as a recombinant single-chain Fv (V_H - V_L) molecule can adopt different Ag binding site conformations before Ag binding and that binding of different Ags can induce isomerization of the binding site, leading to high-affinity complexes with a deep or narrow binding site (37). Therefore, it is conceivable that IgEs can induce FcεRI aggregation in the absence of specific Ag when the Fab portion of a FcεRI-bound IgE molecule interacts with a neighboring FcεRI-bound IgE directly or indirectly via a third molecule whose epitopes are recognized by neighboring IgEs. Similar aggregation models via Ig domain-Ig domain interactions or Ig-X-Ig ("X" represents an unknown bridging molecule) interactions were proposed for pre-BCR (38).

Interestingly, a recent study described a molecule termed p28 in RBL-2H3 cells (39). This molecule, which cross-reacts with a mAb against phospholipid scramblase 1, is associated with ~50% of FcεRI in RBL-2H3 cells in the absence of IgE and dissociates from the receptor when the cells are incubated with a high concentration (20 μ M) of IgEs. Therefore, p28 might be involved in sensing IgE binding. However, the identity and function of p28 remain to be revealed. Both IgE and FcεRI α subunit are glycoproteins. However, potential involvement of the carbohydrate moiety vs polypeptide backbone of IgE in receptor aggregation remains to be studied in future.

Another interesting feature of IgE(-Ag) effects is that at least some of them require the continuous, but not transient, presence of IgE, hence prolonged receptor aggregation: survival promotion by both HC and PC IgEs and proliferation by HC IgEs could be seen only in the presence of IgE but not after washing off IgE even after surface expression of FcεRI was dramatically increased by preincubation with IgE (21). Prolonged FcεRI aggregation was also suspected to be required for augmented proliferation of peritoneal mast cells, although this proliferation was induced in a semisolid culture by IgE+Ag in the presence of IL-3 (40).

FcεRI components and membrane microenvironments required for IgE(-Ag) effects

IgE+Ag stimulation induces localization of the aggregated receptors to cholesterol/sphingolipid-rich plasma membrane domains termed lipid rafts (41, 42). Experimental disruption of lipid rafts suggests that localization of the receptor in lipid rafts is required for the activation of ERK induced by IgE(-Ag) stimulation and therefore for survival as well (see *Strength and duration of FcεRI stimulation determine mast cell survival*) (22). Among the obligate subunits (α , β , and γ) of the murine FcεRI, both α and γ subunits were shown to be required for IgE(-Ag)-mediated survival (21, 43). Interestingly, the ITAM of γ subunit, which is critical for IgE+Ag-induced mast cell degranulation and cytokine production and IgE(-Ag)-mediated survival, is not essential for IgE(-Ag)-mediated receptor up-regulation (43). Consistent with this but unlike degranulation or other activation events, receptor up-regulation induced by IgE(-Ag) does not require Lyn, Syk, or other PTKs (44). This phenomenon is due to the increased lifetime of the surface-resident receptors rather than to its increased synthesis and/or transport to the plasma membrane (18, 45, 46). Thus, this effect of IgE binding to the FcεRI is of a nature totally different from the other effects.

Strength and duration of FcεRI stimulation determine mast cell survival

Mast cells die by apoptosis in the paucity of growth factors in vitro and in vivo. In vivo apoptosis was reported in mast cells that had been proliferated by repetitive injections of stem cell factor, following the cessation of the injections (47) and splenic mast cells during parasite infection (48). Survival-enhancing stimuli of FcεRI include IgE(-Ag), IgE+anti-IgE, and IgE+Ag (low Ag concentrations) (44). Importantly, a slow on-rate of IgE interaction with FcεRI together with prolonged activation of ERK matches with the required pattern of ERK activity for survival of T cells and other cell types (49). Consistent with this, relatively weak signals emanated from the γ subunit were required for the survival of mast cells expressing CD8/ γ chimera in the genetic background of $\gamma^{-/-}$, compared with the signals required for degranulation (50). However, the duration of the γ signals seems more essential for survival because sustained ERK activation, even strong enough to induce degranulation, could induce survival.

Autocrine and paracrine mechanisms for mast cell survival and activation

Survival mechanisms elicited by IgE(-Ag) and IgE+Ag are not completely understood. At cellular levels, HC IgEs use an autocrine or paracrine mechanism at least in part by secreting a cytokine(s) that supports mast cell survival (22, 24). Kohno et al. (51) recently identified IL-3 as the predominant cytokine induced by HC IgEs that is responsible for the survival effects; survival effects were drastically reduced (but not abrogated) by neutralization of IL-3 or IL-3 deficiency. By contrast, PC IgEs do not support *FcεRI* $\alpha^{-/-}$ mast cells in coculture with wild-type cells (24), indicating that PC IgEs do not induce secretion of survival-mediating cytokines. These data suggest that there are at least two different survival mechanisms evoked by IgE(-Ag) at cellular levels.

In addition to the predominant role of autocrine IL-3 for mast cell survival, recent data indicate that the mediators released from activated mast cells affect not only other cell types

(e.g., neutrophil recruitment by mast cell-derived TNF- α in late-phase allergic responses (52)) but also themselves and neighboring mast cells in terms of scale and duration of several aspects of activation. Thus, recent studies demonstrated the importance of adenosine and sphingosine 1-phosphate acutely released by IgE+Ag in Ca^{2+} influx, degranulation, and chemotaxis to Ag (53, 54). Unlike degranulation that is completed within several minutes after the initiation of IgE+Ag stimulation, migration induced by IgE(-Ag), as well as IgE+Ag, is affected by many factors; in addition to sphingosine 1-phosphate, adenosine, leukotriene B_4 , and several chemokines (MIP-1 α /CCL3, MIP-1 β /CCL4, MIP-2/CXCL1-3, MCP-1/CCL2, and RANTES/CCL5) contribute to the full migratory activity using both directional (chemotaxis) and nondirectional (chemokinesis) mechanisms; as these factors all use G protein-coupled receptors, this form of migration is dependent on PI3K γ (29). Therefore, the PTK-based signaling pathways and G protein-coupled receptor-based signaling pathways seem to converge or cross-talk to enhance degranulation and migratory responses (and probably other activation events as well).

Signal transduction for IgE(-Ag)- and IgE+Ag-induced survival and activation

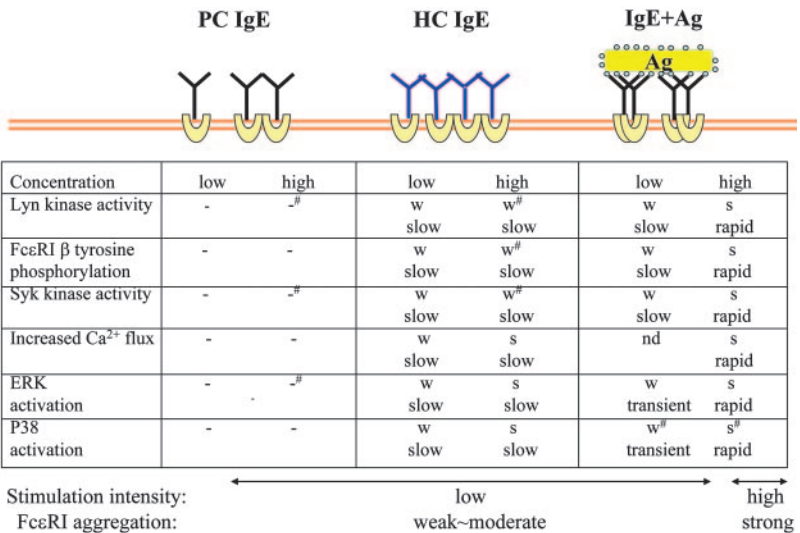
Because both IgE(-Ag) and IgE+Ag engage the Fc ϵ RI, it would not be irrational to assume that similar, if not identical, signaling events are elicited by the two modes of Fc ϵ RI stimulations. Indeed, recent studies using pharmacological agents and mast cells derived from gene-targeted mice showed very similar signaling requirements for IgE(-Ag)- and IgE+Ag-induced biological outcomes (refer to Refs. 15, 16, 55 for detailed reviews of IgE+Ag-induced signaling): degranulation induced by these stimuli depends on Syk; production of IL-6 and TNF- α also requires Syk; Fc ϵ RI internalization requires Lyn but not other Src family PTKs, Syk, or Btk; optimal adhesion via fibronectin requires Lyn and Syk but not Fyn; and migration toward HC IgEs or Ag depends heavily on Lyn and Syk and Fyn, PKC β , PKC ϵ , and PKC θ to a lesser extent (24, 28, 29, 44, 56). However, quantitative differences were observed for the required signaling molecules in the IgE(-Ag)- and IgE+Ag-induced biological consequences: for instance, the Lyn requirement for HC IgE-induced adhesion is almost absolute, but the role of Lyn for IgE+Ag-induced adhesion is at best contributory (56). Quali-

tative differences were also observed between the two stimulation modes: concentrations of intracellular Ca^{2+} are increased transiently by HC IgEs (26–28). A recent study showed that Ca^{2+} responses induced by SPE-7 IgE are suppressed by SK&F 96365, a specific inhibitor of store-operated Ca^{2+} channel but not by broad-spectrum Ca^{2+} channel inhibitors, La^{3+} or Gd^{3+} , whereas Ca^{2+} responses induced by IgE+Ag are suppressed by these three inhibitors (57). These results suggest that IgE(-Ag) and IgE+Ag induce Ca^{2+} influx by different mechanisms (Fig. 2).

A recent study showed that Bcl-2 and Bim, anti- and proapoptotic members of the Bcl-2 family proteins, respectively, antagonizes and promotes mast cell apoptosis induced by growth factor withdrawal (58). The major survival pathway induced by IgE(-Ag) appears to be dissected into two consecutive events: the Fc ϵ RI-activated signaling pathway leading to autocrine secretion of IL-3 and then activation of IL-3R signaling pathways leading to survival (51). Although details still remain to be worked out, the initial signaling events seem to include the activation of Lyn and Syk (24) leading to the activation of ERK. Interestingly, however, Fyn, Gab2, PI3K p85, or Akt are not required for IgE(-Ag)-mediated survival (51). Downstream of IL-3R signaling, Stat5 was shown to be important for mast cell survival (59). Expression of Bcl-x $_L$ and Bcl-2 is induced in an IL-3-dependent manner. Importantly, Bcl-x $_L$ can support mast cell survival in *IL-3*^{-/-} mast cells (51).

IgE+Ag stimulation can also enhance mast cell survival under certain conditions (44): IgE+Ag prevents apoptosis of MC9 mast cells by an autocrine mechanism, producing IL-3, IL-4, and GM-CSF (60). Although secretion of endogenous IL-3 and GM-CSF is not sufficient for MC9 survival, IL-4 renders the cells reactive to these cytokines. IgE+Ag can induce enhanced expression of FLIP, a caspase-8 inhibitor, and consequently a resistance to Fas-induced apoptosis (61). An antiapoptotic member of the Bcl-2 family, A1, was shown to be important for the IgE+Ag-induced survival effects (62). IgE+Ag stimulation induces the expression of not only antiapoptotic proteins such as A1 and Bcl-x $_L$ but also proapoptotic proteins such as Bim $_{EL}$ and Bim $_L$ (58). However, it remains to be examined whether these proteins with opposing functions

FIGURE 2. Comparison of signaling events induced by different modes of Fc ϵ RI 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 $\mu\text{g/ml}$ (low) and 5 $\mu\text{g/ml}$ (high); IgE+Ag (DNP $_{21}$ -BSA), 1 ng/ml (low) and 100 ng/ml (high). -, not detected; -#, very weak; W, weak; W#, weak~moderate; S#, moderate~strong; and S, strong. References: Lyn kinase activity (14, 21–24, 44); Fc ϵ RI β tyrosine phosphorylation (14, 21, 22, 24); Syk kinase activity (14, 21, 22, 24, 43, 44, 50); increased Ca^{2+} flux (14, 21, 23, 26, 27, 57); ERK activity (14, 21, 22, 24, 43, 50, 51); and p38 activity (14, 21, 22, 24).



contribute to IgE(-Ag)-induced mast cell survival. An IL-3-independent pathway used by IgE(-Ag) remains enigmatic, but it requires Syk because no survival effects of mIgEs were seen in *syk*^{-/-} cells (24).

In vivo effects of IgE(-Ag) stimulation and their implications in diseases

IgE(-Ag) effects on survival and activation may not represent mere in vitro artifacts in mouse mast cells, although the definitive in vivo evidence for these phenomena has yet to come. However, there are several in vivo observations that support roles for IgE in mast cell survival and activation. Increased serum IgE levels in mice transplanted with IgE-producing hybridomas were correlated with increased mast cell numbers in some mucosal tissues (24). However, the presence of IgE is not required for the development of mast cells (63), and no increase in mast cell numbers was reported in IgE-transgenic mice (64). These results do not contradict the IgE(-Ag) effects on mast cell survival because relatively high concentrations of IgE are required for survival effects. These observations suggest that IgE levels do not determine the homeostasis of mast cell populations in vivo under physiological conditions. By contrast, IgE might play a critical role in maintaining mastocytosis during parasite infection. Infection with the parasitic helminth, *Trichinella spiralis*, elicits a vigorous IgE response and pronounced intestinal and splenic mastocytosis in wild-type mice. However, splenic mastocytosis was diminished in *IgE*^{-/-} mice while similar levels of eosinophilia and jejunal mastocytosis occurred in wild-type and mutant mice (48). Mast cells in *T. spiralis*-infected mice migrate from the gut to the spleen and die there by apoptosis. Therefore, high-level IgEs seem essential to protect mast cells from apoptosis in the infected spleens. Furthermore, mucosal mastocytosis is impaired in *IL-3*^{-/-} mice during nematode (*Strongyloides venezuelensis*) infection (5). These observations fit well with the IL-3 autocrine mechanism for IgE(-Ag)-induced mast cell survival. Therefore, under conditions where IgE may reach very high levels, e.g., parasite infection (53.3 µg/ml serum IgE in *T. spiralis*-infected mice (48)) and atopy (sometimes >100 µg/ml in NC/Nga mice (65)), tissue density of mast cells could be regulated in part by the survival (and proliferative) effects of IgE. However, the potential involvement of Ag, for which IgEs are specific, in the role for IgEs in enhancing mast cell survival has not been addressed in parasite infections, although much of the IgE that is produced in response to nematode infections is found not to be specific to the parasite (66). Additionally, contributions to mast cell survival by other factors, e.g., cytokines and chemokines, have not been known in the above parasite experiments.

A recent study showed that mast cell accumulation can be induced by epicutaneous application of HC IgEs and IgE+Ag (29). These results suggest that HC IgEs (in the absence of specific Ag) and IgE+Ag can induce accumulation of mast cells without prior inflammation. However, it is conceivable that, in allergic individuals, some inflammatory reactions such as the infiltration of helper T cells have occurred when IgE synthesis in B cells takes place at mucosal sites in response to Ag exposure (67–72). Given the vast variety of proinflammatory mediators secreted from activated mast cells (73), IgE(-Ag)- and IgE+Ag-induced mast cell accumulation would amplify inflammatory reactions by recruiting other immune cells such as T cells, eosinophils, monocytes, and neutrophils: for instance, histamine plays an important role in the pathogenesis of atopic asthma by

enhancing the secretion of Th2 cytokines and inhibiting the production of Th1 cytokines (74). Leukotriene B₄ recruits T cells and myeloid cells (75–77), and mast cell-produced cytokines and chemokines can recruit T cells, eosinophils, monocytes, and neutrophils. CC chemokine transcripts coding for I-309/CCL1, MIP-1α/CCL3, MIP-1β/CCL4, and MCP-3/CCL7 are among the most dramatically enhanced in IgE+Ag-stimulated mast cells (78). The ability of HC IgEs to attract mast cells suggests that this amplification of inflammation can last as long as local IgE synthesis continues even after the elimination of Ag. Therefore, IgEs in the presence as well as absence of allergen are implicated in mast cell accumulation at allergic tissue sites with local high IgE levels.

Importantly, IgE was shown to be essential for optimal sensitization in contact sensitivity but not for the elicitation phase, unlike mast cells, which are required for both the sensitization and elicitation phases (79). Contact sensitivity was markedly impaired in *IgE*^{-/-} mice but was restored by either transfer of sensitized cells from wild-type mice or administration of hapten-irrelevant IgEs (including two HC IgEs) before sensitization. IgE was required for generating an appropriate cytokine milieu for immunization. IL-6, IL-1β, and MCP-1/CCL2 (and weakly TNF-α) can replace IgE in sensitization. However, it is not clear in this study whether or how IgE-mast cell interactions are required for the oxazolone sensitization, whether specific endogenous Ag interacts with IgE, or whether PC IgEs can restore responses in *IgE*^{-/-} mice.

Several studies indicate the ability of IgE to enhance mast cell and basophil function in both mouse and human cells (20, 80, 81). Together with the enhanced cell survival, a positive feedback-loop hypothesis was proposed to explain inflammatory situations often found in atopic diseases associated with high-serum IgE levels: ↑ IgE → ↑ FcεRI, ↑ mast cells → ↑ Ag-, IgE-, and FcεRI-dependent release of IL-4, IL-13, MIP-1α/CCL3, and so on → ↑ IgE. Now, studies have been extended to human monoclonal IgEs: human umbilical-cord-blood-derived mast cells incubated with IgE synthesize and release I-309/CCL1 (a ligand for CCR8 responsible for chemoattraction of Th2 cells), GM-CSF, and MIP-1α/CCL3 (82).

The distinction between HC and PC IgEs is reminiscent of the dichotomy found in human IgE molecules in their ability to prime basophils for the stimulation with histamine-releasing factor, a cytokine produced by macrophages and platelets: basophils bound by IgEs (termed IgE⁺) derived from atopic patients, but not those (termed IgE⁻) from normal subjects, can release histamine and cytokines such as IL-4 and IL-13 in response to histamine-releasing factor (83, 84). Structural basis for the difference between IgE⁺ and IgE⁻ is not known yet. Interestingly, unusual usages of V_H regions have been noted in allergic individuals: V_H5, one of the smallest gene families containing two members out of the total of 52 functional V_H genes, is overrepresented in the IgE from allergic patients (85). These observations together indicate the structural and functional heterogeneity among human IgE molecules. Therefore, it is interesting to study the human IgE heterogeneity and its relevance to human atopic and other diseases.

Conclusions

Although numerous unanswered issues still remain (Table I), IgE(-Ag) effects on mast cell survival and activation have been well established. Several reports summarized in the previous

section also presage future directions. Particularly, translational research together with basic mechanistic studies should be encouraged. Such studies will lead to a better understanding and, hopefully, better treatment of asthma, other allergic diseases, and host responses to parasite infections. Additionally, we hope this review stimulate studies in related fields, e.g., studies on effects of IgG-FcγRs.

Acknowledgments

We thank Ralph Kubo and two anonymous reviewers for critical reading of the manuscript.

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