

Antigen-independent Effects of Immunoglobulin E

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Immunoglobulin E (IgE) is an important mediator in immediate hypersensitivity, as it facilitates mast cell degranulation and the release of immunomodulatory mediators, such as histamine, prostaglandins, and cytokines. Antigen-specific IgE is a hallmark of allergic diseases. Upon interaction with polyvalent antigen, IgE molecules crosslink and transmit signals that drive this process. Recently, an alternative function of IgE has come to light. Rather than merely priming the mast cell, in the absence of antigen, IgE influences mast cells, including their survival, receptor expression, and mediator release. The mechanisms by which IgE induces these effects and the biological consequences are being discovered and are showing that IgE has an important and active role in facilitating immune responses.

Introduction

The discovery of immunoglobulin E (IgE) in 1966 [1] opened new windows into understanding humoral immunity and was the final piece in the jigsaw of antibody isotypes. We now understand that IgE is synthesized by allergen-specific plasma cells that are driven by signals, such as interleukin 4, to undergo isotype switching and produce epsilon heavy chains [2]. FcεRI, the high affinity receptor for IgE, binds IgE with a high affinity ($K_a = 10^9 \text{ M}^{-1}$) and is found on the surface of rodent mast cells and basophils. Classic type I immediate hypersensitivity reactions arise when allergen-specific IgE, bound via FcεRI, interacts with polyvalent antigen, triggering mast cell activation. This antigen-specific, IgE-driven mast cell activation underlies many forms of acute clinical hypersensitivity responses, including anaphylaxis, food reactions, and allergen-induced bronchoconstriction. The development of anti-IgE therapeutics has been driven by evidence that interfering with antigen-induced mast cell activation might attenuate such reactions.

In recent years, however, it has emerged that IgE can affect cells in the absence of antigen, and the mechanisms and biological consequences are beginning to be eluci-

dated. We now appreciate that the functions of mast cells can be profoundly altered if IgE is present or absent. IgE alone has now been shown to modulate survival, cytokine production, adhesion, and intracellular signaling of mast cells in the absence of antigen. In this review, we describe the published evidence concerning antigen-independent effects of IgE.

History

The first described antigen-independent effects of IgE were in regulating the expression of its own receptors. The high-affinity receptor for IgE, FcεRI, is a heterotetrameric receptor that consists of an IgE-binding α-chain, a β-chain that functions as an amplifier [3], and two signal-transducing γ-chains. It was reported in the 1970s that the expression of FcεRI on human mast cells correlated with the levels of serum IgE [4,5], but it was not until 1997 that researchers described the direct effects of IgE on FcεRI expression [6•,7]. IgE causes a dose- and time-dependent increase in the expression of FcεRI on murine peritoneal mast cells, bone marrow-derived mast cells (BMDMCs), and basophils. Additionally, cells cultured in vitro without IgE actually show a downregulation of receptor expression over time, whereas cells from IgE-deficient mice have reduced expression. Importantly, prior exposure to IgE facilitates an enhanced release of serotonin (5-HT), interleukin (IL)-6, and IL-4 in response to antigen, suggesting that IgE “primes” mast cells for activation. IgE-driven enhancement of FcεRI occurs primarily via stabilization and accumulation of surface FcεRI rather than increased production [8], although a cyclohexamide-sensitive phase of expression has also been described [6•].

IgE also regulates its low affinity IgE receptor, CD23, which is predominantly expressed on B cells in mice [9,10]. IgE-deficient mice possess similar numbers of CD23⁺ B cells as wild-type mice, but have significantly reduced levels of CD23 surface expression. The levels of CD23 can be enhanced by intravenous administration of IgE alone, mirroring findings in parasitized mice (which display high levels of serum IgE) that CD23 expression is elevated and can be reduced by anti-IgE treatment [11]. CD23 may provide a negative feedback loop in the regulation of IgE synthesis because CD23-deficient mice have higher levels of IgE production whereas CD23 transgenic mice produce less than wild-type mice [12,13].

Interest in antigen-independent effects of IgE was fueled when back-to-back reports by Asai *et al.* [14••] and Kalesnikoff *et al.* [15••] in 2001 described, for the first time, antigen-independent effects of IgE on mast cell survival. BMDMCs deprived of growth factors such as IL-3 die through apoptotic processes. Both studies show that monomeric IgE can antagonize this death, whereas antigen cross-linking of the bound IgE has no effect. The protective effects of IgE cannot occur if the BMDMCs lack FcεRI. The mechanism by which IgE regulated apoptosis is unclear, however, because Asai *et al.* showed no induction of DNA synthesis and ruled out the Fas/Fas ligand and Bcl-2 apoptotic pathways whereas Kalesnikoff *et al.* showed tyrosine phosphorylation of FcεRI β-chain and signaling cascades through extracellular signal-regulated kinase (ERK)1, ERK2, p38, and c-Jun N-terminal kinase (JNK). Importantly, Kalesnikoff *et al.* also demonstrate that IgE prevents DNA fragmentation (an indicator of cell death) and the loss of Bcl-XL, an anti-apoptotic surface protein, whereas, conversely, Asai *et al.* show no effect on Bcl-XL expression. An additional discrepancy between these papers relates to production of cytokines from BMDMCs. Kalesnikoff *et al.* found that IgE enhances the messenger RNA (mRNA) levels of IL-6, tumor necrosis factor (TNF)-α, IL-4, and IL-13, as well as the secretion of IL-6 protein, whereas Asai *et al.* were unable to detect differences in cytokines. In their next paper, Kitauro *et al.* [16], this group unearthed an explanation for the differences in cytokine responses between their study and that of Kalesnikoff *et al.* They studied a range of different IgE clones for their ability to induce cytokine secretion from mast cells. Interestingly, some IgE species are unable to promote cytokine release, including the H1 DNP-ε-206 clone used in their previous study, whereas others are capable of driving cytokine release, including the SPE-7 clone used by Kalesnikoff *et al.* [15••]. The term "cytokinergic" was proposed to define the ability of an IgE species to drive cytokine production in the absence of antigen.

Signaling Pathways

How does IgE mediate cellular changes in the absence of antigen cross-linking? Initial studies [15••] imply activation of intracellular signaling pathways by FcεRI. Aggregation of FcεRI is known to induce the activation of Lyn, an Src family protein tyrosine kinase, and the phosphorylation of immunoreceptor tyrosine-based activation motifs (ITAMs) within the β and γ chains (Fig. 1). ITAM phosphorylation also recruits Lyn and Syk, via a WIP-dependent mechanism [17], which in turn phosphorylate intracellular proteins, such as phospholipase C and Vav, and promote changes in intracellular calcium levels [18]. It is now becoming clear that different pathways regulate the degranulation, survival, and cytokine effects of IgE. IgE alone does not stimulate degranulation of mast cells; this is regulated by Src homology 2 (SH2)-containing inositol-

polyphosphate 5'-phosphatase (SHIP). SHIP-deficient BMDMCs undergo profound degranulation in response to IgE alone [19], suggesting that SHIP is activated by IgE alone and restricts on degranulation response. Additionally, a novel 28 kDa-sized protein has been described to associate with the α-chain of FcεRI in the absence of IgE and to rapidly dissociate upon IgE engagement [20]. This p28-FcεRI complex is present in at least 50% of the surface-expressed FcεRI and dissociates within 2 minutes of monomeric IgE binding by a process that does not require additional proteins. Interestingly, when cells were stimulated to degranulate with low doses of IgE (that did not induce the complex dissociation) and specific antigen, there was no disruption of the p28-FcεRI complex, suggesting that p28 may be a specific sensor of IgE occupancy rather than IgE-driven cell activation. The precise structure of p28 remains unknown, although it was detected owing to cross-reactivity with an antibody against phospholipid scramblase and may be a splice isoform of this enzyme. Phospholipid scramblase enzymes are important for the movement of phospholipids between lipid compartments in cells, and some members of this family have been shown to regulate apoptosis [21,22].

Highly cytokinergic IgEs trigger tyrosine phosphorylation of several intracellular proteins, including ERK1, ERK2, p38, and Akt [15••,16], but an essential molecule in IgE-driven cytokine release and mast cell survival appears to be Syk. Syk-deficient BMDMCs are resistant to the effects of IgE on survival and cytokine production, whereas Lyn-deficient cells respond like wild-type cells [16]. One downstream consequence of IgE binding to FcεRI is a rise in cytosolic calcium and the activation of nuclear factor of activated T cells (NFAT) [23], which is a regulator of cytokine transcription, including the mast cell survival factor IL-3 [24].

Additionally, IgE-induced activation of phosphatidylinositol 3-kinase (PI3K), phospholipase C γ, and increased cytosolic calcium regulate another function of IgE, namely mast cell adhesion [25]. Lam *et al.* [25] investigated mast cell adhesion to fibronectin, an important connective tissue protein, and showed that IgE promotes adhesion of mast cells to fibronectin-coated plates, possibly by regulating the levels of very late antigen 5 (VLA-5). Mast cells have been shown to detach from connective tissue and migrate from skin to draining lymph nodes during an immune challenge [26], and it may be that IgE, in the absence of antigen, maintains mast cell location within tissues.

Although studies using BMDMCs have proved extremely useful in characterizing the antigen-independent effects of IgE, they are unable to adequately address questions regarding the biological significance of these findings. This has been addressed using an IgE-deficient mouse.

Physiologic Consequences

It has long been appreciated that IgE is associated with protection against parasites [27]. During a host response to parasite

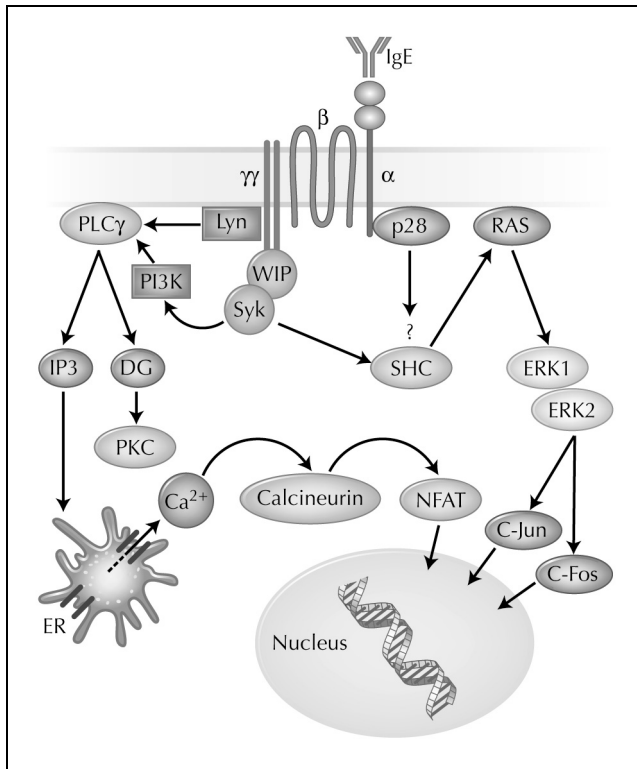


Figure 1. Intracellular signaling by IgE. IgE binds to the extracellular domain of the α -chain of Fc ϵ RI. This triggers the dissociation of p28 and promotes signaling through the β and γ chains. The activation of phospholipase C γ (PLC γ) through Lyn and Syk leads to increases in diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP $_3$), which activate protein kinase C (PKC) and drive release of intracellular stores of calcium from the endoreticulum, as well as promoting calcium influx from the extracellular space (not shown), respectively. Increased calcium and PKC activity can dephosphorylate nuclear factor of activated T cells (NFAT), resulting in its translocation to the nucleus and gene activation. Meanwhile, Syk also drives activation of the MAPK pathway through SHC and Ras proteins, leading to activation of extracellular signal-regulated kinase (ERK1 and ERK2). This pathway stimulates translocation of factors such as C-Jun and C-Fos into the nucleus and facilitates gene transcription. IgE—immunoglobulin E.

infection, serum IgE increases dramatically; however, most of the IgE produced is not specific for the parasite. Previous studies have suggested a correlation between nonspecific IgE titers and protection against infection [28,29]. In a model of *Trichinella spiralis* infection, IgE-deficient mice (which have a disrupted C ϵ exon and cannot form epsilon heavy chains) show a delayed ability to expel the infecting parasite [30]. Additionally, the increase in spleen mast cells that is characteristic of *T. spiralis* infection is absent in IgE-deficient mice, suggesting mast cells fail to expand if IgE is missing. Mouse mast cell protease-1 levels in the serum were also found to increase in wild-type animals but not in IgE-deficient mice. IgE was also suggested to directly affect the survival of the infecting larvae. Larvae in wild-type animals show higher numbers of necrotic larvae and were found by immunohistochemistry to be coated with IgE on their surface.

Further evidence for a physiologic role of antigen-independent IgE was shown in a model of contact sensitivity

[31••]. IgE-deficient mice were found to be unable to mount immune responses to the contact-sensitizing haptens oxazolone and 2, 4- dinitrofluorobenzene. By transferring sensitized cells or by administration of IgE intravenously before the sensitization or challenge exposure, IgE was shown to be required for the sensitization arm of the immune response to these chemicals. Interestingly, the amounts of IgE required to fully restore responses are similar to those found in wild-type mice, implying normal mice have sufficient IgE levels to ensure maximal immune sensitization. The skin of IgE mice was shown to have reduced expression of several genes involved in contact sensitivity, mast cell function, or both. Despite this, mast cell numbers in the skin are similar to those in wild-type animals, and, therefore, IgE deficiency does not cause reductions in mast cell frequency, as might be expected if there was reduced survival of mast cells without IgE. The mechanism through which IgE influences immune sensitization was found to involve dendritic cell migration (Fig. 2). In the skin of IgE-deficient mice, dendritic cells do not migrate from the epidermis in response to chemical exposure, whereas reconstitution of IgE recovers this effect. In corroboration, mast cell-deficient WBB6F1/J-Kit^W/Kit^{W-v} mice also lack contact sensitivity to oxazolone and dendritic cell movement from the epidermis. These data put together a pathway whereby IgE, bound to mast cells, in the absence of antigen and/or cross-linking, facilitates release of cytokines and other mediators into the local environment. In contact sensitivity, these mediators then provide stimulation for dendritic cells to mature into efficient antigen-presenting cells and to migrate to regional lymph nodes where antigens can be presented to naïve T cells, driving their expansion and differentiation into specific effector cells. Because mast cells are abundant in many tissues, not just skin, this process hypothetically could regulate immunity to antigen exposure in other systems, such as lung and gut.

Monomeric or antigen-independent

An important question on this topic of IgE biology is whether these effects of IgE are “monomeric” or “antigen-independent.” Both terms have been used, but have important differences in their implications. “Monomeric” suggests that IgE acts to signal through Fc ϵ RI by a mechanism that does not require cross-linking of Fc ϵ RI molecules. “Monomeric” leaves open the possibility of an antigen binding to a single IgE molecule and driving responses. Conversely, “antigen-independent” eliminates this possibility and is suggestive of an interaction or aggregation between adjacent IgE molecules, bound to Fc ϵ RI, which mimic the cross-linking seen if antigen is present. Could “antigen-independent” be simply due to aggregation of IgE molecules within the IgE preparations used? Although care has been taken in all these studies to eliminate aggregates by ultracentrifugation and/or chromatographic purification of monomeric fractions, the possibility of aggregation remains. However, studies performed in vivo (which are unaffected by external influences, such as altered

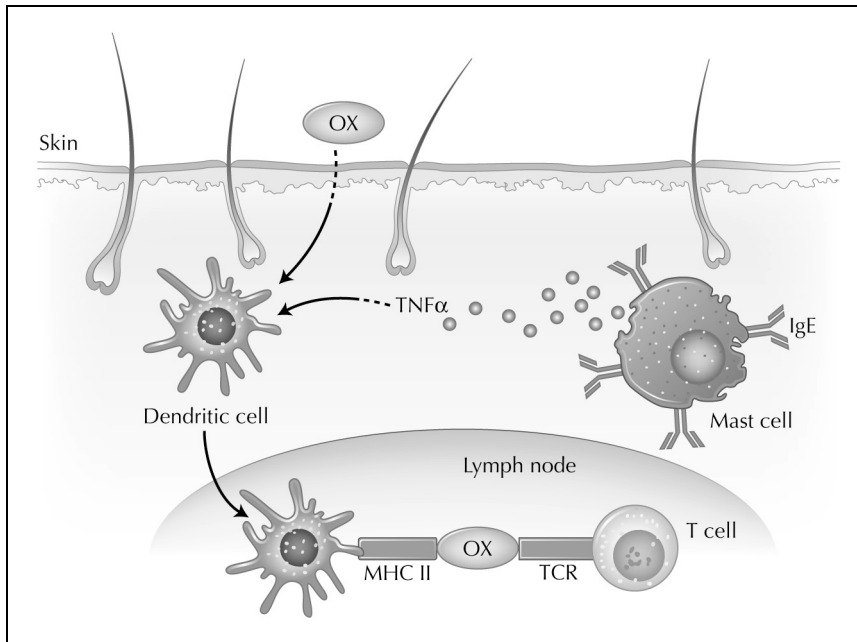


Figure 2. The role of IgE in promoting immune sensitization via the skin. IgE, bound to resident mast cells in the epidermis, promotes the synthesis and release of mediators such as TNF- α and IL-6. These act, via their receptors (eg, TNF receptor I or II), to stimulate immature epidermal dendritic cells. In concert with these signals, exposure to antigen, such as the contact sensitizer oxazolone (OX), drives the dendritic cell to mature and migrate from the epidermis to the draining regional lymph node, where presentation of the antigen via major histocompatibility (MHC) class II molecules triggers the expansion of naïve T cells to become antigen-specific effector T cells. IgE—immunoglobulin E; IL—interleukin; TNF—tumor necrosis factor.

temperature, exposure to surfaces, and handling) using the IgE-deficient mouse [31••] or an IgE-secreting hybridoma transplanted into normal mice [16] support the findings of the studies performed *in vitro*. It, therefore, seems unlikely that this body of work is the consequence of IgE aggregation before interacting with Fc ϵ RI. There is evidence, however, for “antigen-independent” aggregation of IgE molecules once bound to Fc ϵ RI. Using phosphorescence anisotropy, Kitaura *et al.* [16] showed that anisotropy values increased over time after IgE binding, indicating a spatial movement of Fc ϵ RI that would be consistent with receptor aggregation. Additionally, this effect was greater for highly cytokinergic IgE species than for poorly cytokinergic IgEs.

The possibility of “monomeric,” but antigen-driven, effects of IgE was given weight by a study investigating the crystal structure of IgE. Surprisingly, a single IgE species was found to be capable of four alternate conformations [32•]. Serendipitously, the IgE species studied was SPE-7, the same highly cytokinergic IgE used by Kalesnikoff *et al.* [15••]. Two conformations blocked the binding site entirely, making them inactive, whereas another bound the immunizing hapten (DNP). The final conformation possessed an altered, more open, binding domain and, instead of binding DNP, bound to an unrelated protein—thioredoxin, identified by screening a peptide library. This intriguing finding raises the possibility that IgE binding domains may be promiscuous for other antigens, especially protein antigens. Could these effects of IgE molecules be due to a loose binding of an endogenous protein? Recent work by Kitaura *et al.* [33] has also addressed this issue. They used monovalent hapten, which can disengage antigen-driven aggregation of Fc ϵ RI via IgE, to show that the effects of “monomeric” IgE on IL-6 release required the antigen-binding site of IgE. Interestingly, more recently they have gone on to show that the IgE-induced upregulation of Fc ϵ RI does not have such a requirement, implying multiple

pathways may underlie the effects of IgE. Although monovalent hapten is a useful tool in addressing the role of antigen in IgE-driven effects, it could be postulated that its binding *per se* might hold the IgE molecule in the antigen-binding conformation and, thereby, eliminate the ability for that IgE to adopt the alternative, open conformation that allows promiscuity for other antigens. This remains to be determined.

Conclusions

Our understanding of the functions and biology of IgE have rapidly expanded over recent years. IgE is no longer seen as a simple sentinel, waiting for a specific antigen to trigger it and the mast cell it is bound to. We have come to realize that IgE alone is actively driving the survival, mediator release, and functions of the mast cell and has an important regulatory role to play on the pathways leading to immune protection.

Acknowledgments

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