

How IgE upregulates the allergic response

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For several decades it has been known that IgE is central to the allergic response. In recent years, however, IgE has been described as having several actions in addition to passively arming the mast cell or basophil by binding to their high affinity IgE receptors. Recent studies have revealed roles for IgE in the regulation of its own receptor expression and in contributing to cell survival. Furthermore, the presence of trimeric high-affinity IgE receptors on leukocytes other than mast cells and basophils, together with the discovery of previously unknown functions of the β subunit of the complex, has added to the complexity of IgE modulation of the allergic response.

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Introduction

It has been known that IgE is central to type I immediate hypersensitivity reactions since the Ishizakas demonstrated its causal role in arming mast cells to respond to antigenic stimulation by mediator secretion [1]. This central paradigm continues to be the focus of many efforts to treat allergic diseases. However, in recent years it has been discovered that IgE does more than simply arm the mast cell or basophil by binding to their high-affinity IgE receptors (FcεRI). There are four new general areas of IgE/FcεRI biology that significantly influence our understanding of this arm of the immune system. These include 1) the ability of IgE to regulate FcεRI expression, 2) the ability of monomeric IgE to induce survival, 3) the presence of high affinity receptors on other cell types and the role IgE may play on these other cell types, and 4) the role that FcεRI subunit composition plays in determining how IgE signals in cells where it is expressed.

IgE and FcεRI expression

As has been noted in other reviews of this topic, the IgE story has antecedents that go back to 1978. Studies of human basophils showed a good correlation between the level of FcεRI expression and serum IgE concentration [2]. In 1985, Furuichi *et al.* [3] demonstrated that IgE influenced the expression of FcεRI on rat basophilic leukemia cells. After a hiatus of 10 years, a variety of studies in mouse and man more clearly defined the relationship between IgE and FcεRI expression both *in vitro* and *in vivo* [4–6]. The ability of IgE to upregulate the expression of FcεRI, and its absence to lead to downregulation, has marked implications for both the biology of cells expressing FcεRI and the therapeutic approaches seeking to eliminate

IgE from circulation. Indeed, it can be reasonably argued that the recent success of passive immunization with anti-IgE antibodies results not only from the decrease in circulating free IgE observed but also in large part from the marked decrease in FcεRI expression on basophils and presumably mast cells (although the latter has not been directly examined in the published clinical studies). If IgE receptor expression is a critical component of the therapeutic effectiveness of anti-IgE therapies, or any other therapy that reduces circulating IgE, then it is important that the mechanism of regulation of FcεRI expression by IgE is well understood. One of the first general conclusions that can be drawn from the ability of IgE to regulate receptor expression is that monomeric IgE itself provides some sort of signal. The nature of this signal is not yet known, but two recent reports indicate that the signal comes in the form of ‘don’t do something’ rather than ‘do something’. In this case, the ‘don’t do something’ signal directs the cell to not remove an occupied IgE receptor from the cell surface. Data from studies of human basophils indicate that bound IgE protects the receptor from being lost and that IgE itself does not induce mRNA for receptor, nor does it induce protein synthesis or enhance post-translational packaging and movement to the cell surface of preformed receptor [7•]. Studies in which FcεRI was placed into either another hematopoietic cell line or a non-hematopoietic fibroblast cell line demonstrated similar behavior [8••]. This latter study suggested that whatever the mechanism of receptor loss, it operates without regard to the cellular context; that is, the process works in other cell types as well as in mast cells or basophils. The implication of these studies is that there is a general mechanism that recognizes the unoccupied state of FcεRIα and then marks the receptor for removal. An interesting question is whether recognition and/or ‘marking’ for removal of unoccupied receptors takes place on the extracellular side of the membrane, or whether the conformation of the receptor is altered so that it is recognized inside the cell. Whatever the process it is not particularly fast; it can take many hours to remove a few hundred receptors when the starting density of unoccupied receptors is as low as that typically found on circulating basophils.

Monomeric IgE signaling

Because IgE regulates its own receptor expression, a role for monomeric IgE in upregulating the allergic response is already clear. As IgE performs this task as a monomer — indeed, oligomers of IgE actually reduce upregulation by monomeric IgE — observations demonstrate that the interaction produces a state that is recognized by the cell. Two recent reports have expanded upon this perspective by suggesting that monomeric IgE increases mast cell survival and might even lead to specific cytokine release. One of these studies was an outgrowth of work on the role

SHIP (SH2-containing 5' inositol phosphatase) in mast cell IgE-mediated signaling [9•]. The authors noted that the particular IgE (SPE-7) that was used to sensitize murine bone marrow-derived mast cells was also inducing substantial signaling of the kind traditionally associated with aggregation of FcεRI. Fractionation of the IgE (a murine monoclonal IgE with specificity for dinitrophenyl [DNP]) to obtain putatively monomeric IgE did not change the outcome of their studies; the monomeric IgE induced syk phosphorylation, followed by the entire cascade of MAPK enzyme activation and induced secretion of a particular profile of cytokines. Curiously, despite activation of the early signaling elements, 'monomeric' IgE did not induce degranulation or arachidonic acid metabolism (and therefore no subsequent leukotriene C₄ [LTC₄] or prostaglandin D₂ [PGD₂] secretion). A second study did not find any of these results, but did find that monomeric IgE promoted mast cell survival in culture [10•]. Although not yet published, but presented at several recent meetings [11], resolution of these discrepancies sheds some light on what is happening. First, it is apparent that the source of IgE used for these studies is critical to their outcome. Indeed, it is possible to define a spectrum of IgE types; those that induce the entire panoply of observations described in the Kalesnikoff study [9•] to those that only induce mast cell survival [10•]. Two telling experiments provide some insight into these discrepancies. In the first study, addition of monovalent dinitrophenyl lysine to mast cells sensitized with the SPE-7 anti-DNP IgE used in the Kalesnikoff study [9•], ablates the responses previously described [9•]. In the second experiment, knocking out the syk kinase gene ablates all the responses, including the ability of monomeric IgE to induce survival [11]. Curiously, mast cells from *lyn*^{-/-} mice (*lyn* being an important *src*-family kinase that regulates signal transduction from aggregated FcεRI) show normal monomeric IgE-dependent survival [10•]. It is possible that, in these mast cells, another *src*-family kinase can substitute for *lyn* kinase, but there is no substitute for syk in the *syk*^{-/-} mouse mast cells. Although an all-encompassing hypothesis to explain the monomeric IgE story is not yet in hand, one possibility is that some IgE antibodies have the ability to aggregate when bound to the receptor and that low level aggregation induces a range of responses not previously appreciated in studies of antigen-induced secretion. The extent of spontaneous aggregation, which in the case of the SPE-7 IgE antibody used in the Kalesnikoff study can be inhibited by blocking the antigen binding site with monovalent ligand, can be extreme enough to induce reasonably strong signaling, or weak enough to only influence survival without measurable signaling. If this viewpoint is correct, then there are two perspectives of interest: low level aggregation induces behavior not formerly recognized and the ability of a particular monomeric IgE to induce a wide range of cell behavior can only be determined by experimental testing at this time. The nature of the attribute of IgE that influences its ability to signal in these ways is not yet known. Thus far, the ability of IgE to promote survival of mast

cells has not been translated to *in vivo* observations and the unusual characteristics of IgE that lead to aggregation and/or signaling may be only an *in vitro* phenomenon. Previously there have been no clear observations that high IgE titers result in significantly greater numbers of mast cells [12].

The role of the β subunit in FcεRI surface receptor expression

Allergen and specific IgE interactions result in crosslinking of surface FcεRI complexes, which activates multiple signaling pathways leading to diverse mediator responses and cytokine transcription in mast cells and basophils. The role of the high affinity IgE receptor β subunit (FcεRIβ) in allergic disease has been the focus of active research since it was shown to amplify FcεRI signaling responses and, most recently, to amplify FcεRI cell surface expression [13,14•]. The latter function for the β subunit was noted in cell lines stably expressing the human trimeric FcεRI (αγ2) that, upon transfection with the β subunit, rapidly enhanced surface FcεRI complexes in the range of threefold to sixfold. The proposed mechanisms are that FcεRIβ presence facilitates intracellular processing of immature FcεRIα chains to the Golgi apparatus for surface expression as well as offering greater stability to the complex as measured by resistance to detergent solubility. In human basophils, levels of FcεRIβ protein have been correlated to surface expression of the FcεRIα chain in support of the newly described FcεRIβ function [15•]. However, levels of FcεRIβ among donors were not strictly proportional to FcεRIα and raise the possibility of variable FcεRI stoichiometry on the basophil surface (αγ2 versus αβγ2). Furthermore, it has also been noted that IL-3 and, to a lesser degree IL-5, can significantly enhance FcεRIβ mRNA and protein expression [16]. However, in IL-3 cultured human basophils, the levels of surface FcεRIα chain were not altered despite dramatic upregulation of FcεRIβ protein expression.

Several genetic studies across populations have shown linkage to the site of the FcεRIβ gene on chromosome 11q13 and several candidate polymorphisms in the FcεRIβ protein have been identified [17]. Among the identified β polymorphisms, I181L–V183L and E237G have not shown alterations in either of the two defined functions of the FcεRIβ subunit [18]. Furthermore, a functional comparison of subjects expressing 181I rather than 181L did not find any significant differences in terms of skin test reactivity and antigen-induced basophil degranulation [19]. At this point, the association of the FcεRIβ gene with atopy and its defined functions in relationship to the atopic state is unclear.

Function of IgE binding or activation on other leukocytes

Given the elevation of allergen specific IgE in atopic diseases, the presence of a functional trimeric FcεRI on other leukocytes might contribute to the initiation and perpetuation of atopic responses. Monocytes of both normal and atopic subjects express a trimeric version of the high affinity IgE

receptor ($\alpha\gamma 2$), which can serve in antigen presentation to T cells [20,21]. In normal donors, monocytes variably express Fc ϵ RI (in the range 2–40%, mean 11%) and expression is rapidly lost *in vitro* [22]. Fc ϵ RI crosslinking prevents apoptosis of monocytes by induction of Bcl-2 and Bcl-xL expression, and also protects from CD95 Fas-mediated apoptosis. Furthermore, Fc ϵ RI crosslinking on monocytes triggers IL-10 production and, in cultures with IL-4 and GM-CSF, alters monocyte differentiation towards a macrophage species away from a dendritic cell phenotype [23]. In terms of mechanism, Fc ϵ RI activation has been shown to involve the downstream activation of the pro-inflammatory transcription factor NF- κ B in monocytes, dendritic cells and Langerhans cells of atopic donors with high Fc ϵ RI expression [24*]. In addition, monomeric IgE binding to Fc ϵ RI rescued monocytes from apoptosis, an effect reminiscent of the reports of monomeric IgE binding to cultured mast cells [22]. Whether this was related to the presence of small numbers of IgE aggregates or the nature of the IgE has not been tested. Thus, the potential of IgE and Fc ϵ RI activation to prolong monocyte survival, promote IL-10 release and alter their maturation paths into either macrophages or dendritic cells might hold significance for the character of allergic responses. Similar to monocytes, other antigen presenting cells, such as dendritic cells and Langerhans cells, have been shown to possess a trimeric Fc ϵ RI [25]. Recently, peripheral blood dendritic cells from normal and asthmatic subjects were noted to possess similar densities of Fc ϵ RI, but differ in the level of IgE occupancy. Only dendritic cells from asthmatic subjects demonstrated the ability to bind IgE both *in vivo* and *ex vivo* after stripping and re-sensitization [26].

Conclusions

Although the basic role of IgE in mediating atopy is clear, it is becoming apparent that there is more to IgE biology than first appreciated. The four areas of IgE/Fc ϵ RI biology discussed in the introduction are relatively new avenues of research, and the implications of the basic observations remain to be explored. The apparent signaling of monomeric IgE needs further study to define the attributes of IgE that lead to its ability to induce aggregation-like signaling. But, even if this characteristic is primarily shown to exist with monoclonal IgE operating under *in vitro* conditions, the observations raise the possibility that some unusual circulating IgE antibodies may cause pathology in rare individuals. Observations on the roles of IgE in antigen presentation and in the functions of macrophages and dendritic cells are now leading to a needed phase of study where this aspect of IgE biology can be shown to influence either normal immune responses or pathological states.

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