New Approaches to Allergen Immunotherapy

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Abstract:

Allergic diseases are common problems affecting 20% to 30% of the US population. Mast cells and basophils are the primary effector cells mediating allergic inflammation through the triggering of membrane immunoglobulin E receptors (FcεRI) with antigen. Allergen immunotherapy is used as one treatment for allergic disease and results in the inhibition of mast cell and basophil responses through unknown mechanisms. In this review, we examine potential mechanisms that could result in blunted human mast cell/basophil functional responses, strategies aimed at using these mechanisms to develop new immunologically based therapies, and recent findings that have broad implications toward our understanding of how mast cells/basophils become desensitized.

Keywords: allergies | immunology | immunotherapy

Article:

Introduction

Allergic reactions are the result of B cell–produced, specific immunoglobulin (Ig)E antibody to common, normally innocuous antigens. These antigens trigger a TH2 response in which naive T cells are induced to develop into TH2 cells in the presence of interleukin (IL)-4, which appears to be derived from a specialized subset of T cells, mast cells, and basophils. These allergen-specific TH2 cells drive allergen-specific B cells to produce IgE. In simplistic terms, mast cell/basophil, natural killer (NK) cells, T cells, and even B cells are responsible for driving the initial, allergen-inducing hypersensitivity reaction through the production of IL-4, and other TH2-specific cytokines, which results in sensitization to allergen with IgE. Re-exposure to the allergen triggers an allergic response through the release of inflammatory mediators from mast cell and basophils.

The IgE produced binds to the high-affinity receptor for IgE (FcεRI) on mast cells and basophils, and the release of allergic mediators is induced when two or more IgE molecules are cross-linked with allergen. Indeed, most allergy medications are aimed at neutralizing (anti-histamines, H1-receptor blockers) or preventing (anti-IgE; “omalizumab”) mast cell/basophil FcεRI responses. Allergen immunotherapy (IT) has also been established as an effective treatment for patients and results in diminished mast cell and basophil responses. In this

review, we discuss various ways that mast cell/basophil IgE responses are blunted, potential mechanisms that could lead to this inhibition following IT, and recent strategies aimed at preventing mast cell and basophil activation.

In Vivo Mast Cell and Basophil FcεRI Nonresponsiveness

Mast cell and basophil nonresponsiveness in patients with heavy parasite burdens

It has been suggested that one important factor in the observed increase in atopy relates to a steady decline in infectious diseases in the developed world. Strong epidemiologic evidence demonstrating an inverse relationship between atopy and infection with various agents appear to support the "hygiene hypothesis" [1], but the hypothesis is very controversial, and certainly not the sole factor responsible. A notable exception to the hypothesis is found in many US inner cities where suboptimal hygienic conditions do not protect against allergies and asthma.

Nonetheless, mast cell and basophil nonresponsiveness to FcεRI stimulus is commonly observed in people from less industrialized areas with high rates of infectious disease where the degranulation and inflammatory responses of mast cells (measured by, eg, skin testing, airway hyperresponsiveness, wheeze) are blunted [1]. Most studies find similar results in spite of high IgE levels to environmental allergens, suggesting mast cell/basophil activation is not inhibited simply due to low IgE levels. A recent study examining infection with Schistosoma mansoni and protection against allergic sensitization and asthma is typical. S. mansoni infection is clearly associated with decreased positive skin test frequency to several indoor allergens (suggesting nonspecific FcεRI downregulation) and significantly lower asthma symptoms [2]. Conversely, treatment of children with anti-helminth medications results in an increase in skin mast cell–driven, skin prick test responses [3]. Therefore, although there is a clear association between increased infection rates and reduced FcεRI-induced responses, the mechanism is not known.

"Natural" basophil/mast cell nonresponsiveness

Ishizaka et al. [4] provided the first detailed description of naturally occurring human basophils that fail to release histamine in response to FcεRI cross-linking (nonreleaser). The nonreleaser basophil FcεRI binds IgE normally, has normal amounts of histamine, is morphologically similar to releaser basophils, and degranulates in response to non–IgE-mediated stimuli, such as f Met peptide, C5a, and Ca2+ ionophores [5–9]. We [10] recently reported that there may also be a human lung mast cell nonreleaser phenotype. However, studies aimed at identifying the defect in nonreleaser cells have not yet identified an obvious mechanism.

"Induced" FcεRI nonresponsiveness

Allergen IT reduces mast cell and basophil responses. It is aimed at alleviating allergic symptoms by intermittently injecting the patient with increasing doses of allergen over time. The goal of this immunologically based therapy is to induce mast cell nonresponsiveness (determined by the skin prick test) to FcεRI stimulus. Many studies suggest that reduced mast cell responses are a strong predictor for clinical efficacy [11–16]. Basophils also become less responsive to IgE-mediated challenge after allergen IT [12,13,15,17–22]. A recent study in which basophils were obtained before and after IT and challenged with antigen, anti-IgE, or non–IgE-mediated

stimuli typifies this observation. They showed that FcεRI-mediated basophil histamine release is reduced following allergen IT, and no decrease in antigen-specific IgE levels was noted [22]. In general, blunted basophil responses predict the clinical success of IT better than do other parameters [22,23].

A subset of drug hypersensitivity appears to be mediated through immunologic mechanisms [24]. For example, penicillin is a very common drug that induces life-threatening systemic mast cell/basophil responses in susceptible persons. When patients who are allergic to penicillin develop life-endangering infections that require treatment with β-lactam antibiotics, they face the choice between a fatal infection or the possibility of a fatal allergic reaction. A well-known approach to this problem has been the use of desensitization protocols in which patients are given increasing amounts of the drug before full-dose antibiotic therapy. The hallmark for successful drug desensitization is a reduced wheal and flare response in response to drug challenge—a mast cell FcεRI-mediated reaction. However, the mechanisms to explain how drug desensitization protocols induce mast cell nonresponsiveness to FcεRI are not known.

Why Do Mast Cells and Basophils Become Less Responsive to Allergen Following Immunotherapy?

Reduced antigen-specific IgE

One hypothesis suggests that allergen IT simply reduces the amount of allergen-specific IgE available for mast cell/basophil binding. Thus, when patients are subsequently exposed to the allergen, there are not sufficient amounts of specific IgE to crosslink mast cell/basophil–bound IgE. However, a major anomaly exists, as most studies find that IT does not reduce allergen-specific IgE levels but instead tends to increase up to 1 year after allergen injection [25], although mast cell/basophil–induced reactions are diminished. Therefore, although mast cell/basophil nonresponsiveness to FcεRI is induced during IT, there can be a paradoxical rise in allergen-specific IgE in the serum, suggesting that a simple reduction in allergen-specific IgE cannot explain its efficacy.

Blocking-antibody competition

A second hypothesis attempts to explain IT-induced mast cell/basophil nonresponsiveness to FcεRI stimuli through the rise in “blocking antibodies,” but the term is ambiguous. In theory, the increase in serum IgG molecules simply binds up the allergen, preventing it from inducing FcεRI crosslinking, as has been demonstrated in mice [26]. Lichtenstein et al. [27] demonstrated that IT induced blocking antibodies in which pre-incubation of the ragweed allergen with the IgG fraction from patient serum inhibited histamine release from the basophils of ragweed-allergic patients. However, there is little evidence of a correlation between improved symptom scores and serum IgG levels, suggesting that this mechanism does not inhibit mast cell and basophil responses in humans.

Blocking-antibody Fcε/Fcγ receptor coaggregation

A third hypothesis is related to the second “blocking antibodies” theory and has also not been proven. Unlike the competition theory, this is based on the coaggregation of the inhibitory motif-

containing (ITIM) FcγRIIb receptors with the activating motif-containing (ITAM) FcεRI. As antigen-specific IgG levels increase following IT, the serum concentrations increase sufficiently enough that monomeric IgGs (or Ig–antigen complexes) are now able to bind low-affinity FcγRIIb on mast cell and basophils. Upon subsequent exposure, the FcγRIIb-bound IgG and the FcεRI-bound IgE bind the antigen, initiating an inhibitory, rather than activating, signal.

This hypothesis importantly depends on the assumption that mast cells express the inhibitory motif-containing FcγRIIb. This assumption has been propagated by the observation that rodent mast cells express only the FcγRIIb isoform (in the mouse there is only a single FcγRIIb gene), which appears to be important in blocking mast cell–induced responses in mouse models [28]. However, in humans, the FcγRII group consists of at least six different proteins encoded by three distinct genes (A, B, and C) [29]. As a rule, these receptors mediate opposing signals. FcγRIIa initiates "activation" functions whereas FcγRIIb displays "inhibitory" signals that downregulate several immune functions. Inhibitory signaling through FcεRI/FcγRII coaggregation has been demonstrated on human basophils [30–32] and cord blood–derived mast cells [32,33].

It is still unclear if this mechanism occurs in humans, owing to the inherent difficulty of performing experiments to address the coaggregation hypothesis and the lack of studies examining the expression of FcγRII isoforms on primary mast cells. For this mechanism to work, the target organ (ie, skin, nasal mucosa) would have to have high amounts of antigen-specific IgG in the microenvironment so that when antigen is introduced (skin testing) there is sufficient enough IgG to immediately bind to the antigen via its antigen-binding fragment (F(ab)2) region and to the mast cell FcγRIIb via its fragment (Fc) region. Coaggregation is induced when mast cell–bound IgE and IgG encounter antigen. Because the wheal response is immediate (< 10–20 seconds), it seems unlikely that these events could occur rapidly enough to prevent mast cell activation.

A great void exists in our understanding of the link between allergen stimulation given during IT, reduced mast cell/basophil FcεRI responses, and clinical efficacy. Although it is clear that certain scenarios invoke mast cell and basophil nonresponsiveness, providing for potential new therapeutic interventions as discussed later, it is not known what the intrinsic defect in FcεRI-positive cells is in these patient populations, which, in turn, could account for the lack of allergic symptoms.

Chimeric Proteins That Inhibit Mast Cell/Basophil Responses Through Fcε-Fcγ Coaggregation

Realizing the therapeutic potential for regulating FcεRI signaling through co-aggregation with ITIM-containing FcγRIIb, based on these previous studies, a molecule was developed in the Saxon laboratory [34] that uses FcεRI coaggregation to FcγRII receptors. This fusion protein (GE2) consists of the human IgG1 γHinge-CHγ2-CHγ3 region linked to the human IgE CHε2-CHε3-CHε4 region. GE2 blocks FcεRI-mediated functions of human basophils and cord blood–derived mast cells, and inhibits passive cutaneous anaphylaxis in FcεRI transgenic mice and skin test reactivity in dust mite allergic rhesus monkeys. GE2 also has the ability to inhibit human Langerhans-like cell function via FcγRIIb crosslinking [35] and interferes in isotype switch and IgE production by B cells via FcγRII (CD23)–FcγRII crosslinking [34]. Similar molecules that coaggregate FcγRI and FcγRI have been developed and block cord blood–derived mast cell and blood basophil IgE responses [32]

The mechanisms of inhibition using the GE2 molecule highlight the differences in the rodent and human systems. In results that are not observed in rodent systems, coaggregation on cord blood mast cells increased the tyrosine phosphorylation and association of the adapter protein downstream of kinase (Dok) with growth factor receptor–bound protein 2 (Grb2) and the SH2 domain-containing inositol 5-phosphatase (SHIP). Surprisingly, the complexes of phosphorylated SHIP-Grb2-Dok were lost upon IgE-receptor activation but retained under conditions of Fcε-Fcγ coaggregation and in nonstimulated cells. These results implicate Dok, SHIP, and Grb2 as key intermediates in regulating IgE-mediated degranulation and cytokine [33]. They further implicate these signaling intermediates as "gatekeepers" of human mast cell degranulation.

Allergen-specific inhibition

Based on the above findings using GE2, Zhu et al. [36] developed an antigen-specific ITIM-binding molecule, GFD, composed of a truncated human IgG Fcγ1 fused to the major cat allergen Fel d1. This molecule blocked cat induced allergic mediator release in vivo and in vitro.

Such chimeric human gamma-ITIM or gamma-allergen fusion proteins may provide a new approach for immune-based therapy of allergic disease. Given that allergen IT already reduces mast cell and basophil responses, these molecules may provide a way for IT maintenance doses to be achieved more quickly. In addition, they are theorized to be safer based on the hypothesis that human mast cell/basophil IgE responses can be downregulated through Fcε-Fcγ coaggregation in vivo. Whereas GE2 may be used to reduce mast cell/basophil responses to several allergens, the GFD molecule is theorized to specifically block cat-allergic responses. This platform for producing allergy-specific and nonspecific molecules represents a new approach in developing therapeutics aimed at blunting allergic mediator release from mast cells and basophils [37••,38••].

Human Skin Mast Cells Express FcγRIIa Isoforms

The presence and inhibitory capabilities of FcγRIIb on human basophils and cord blood–derived mast cells has been established. However, it is not known what FcγR receptors are expressed on primary human mast cells. Recently, we showed that mast cells derived from human skin express FcγRIIa, but not FcγRIIb, and provide the first evidence that human mast cells can be activated through FcγRIIa [39]. Coaggregating FcγR with FcγRIIa results in a significant increase, rather than a decrease, in FcγRI-dependent mediator release. Thus, unlike rodent mast cells, cord blood-derived mast cells, and peripheral blood basophils, which express the inhibitory receptor FcγRIIb that is capable of dampening FcγRI function, human skin mast cells express the activation receptor FcγRIIa, which augments FcγRI responses.

Several factors regulate FcγRII isoform expression. Cytokines can influence whether monocytes express FcγRIIa (activating) or FcγRIIb (inhibitory) isoforms [40]. Specifically, the expression of FcγRIIb is highly upregulated by IL-4. IL-10, a cytokine upregulated after allergen IT, has been shown to increase FcγIIa expression on human monocytes [41]. Additionally, it appears that cell–cell interactions (how dense the cells are grown in culture) can profoundly influence FcγRII isoform expression on human monocytes [40]. Moreover, recent studies show that a promoter haplotype in FcγRIIb results in an altered expression in some individuals [41,42], suggesting that donor variability may exist in mast cell and basophil FcγRII isotype expression.

Work is under way to determine which FcγRII isoforms are expressed on lung mast cells, what parameters result in the differences observed in the FcγRII expression on cord blood-derived versus skin mast cells, and what effect Fcγfusion proteins have on skin and lung mast cells.

The Tyrosine Kinase Syk Regulates FcγRI Function

The spleen tyrosine kinase (Syk) is a critical molecule that is involved in Fc receptor signaling in many cell types, including mast cells. Studies examining the function of Syk using mutated Syk and tyrosine (Tyr) phosphorylationspecific antibodies (Abs) suggest that subtle changes in Syk phosphorylation sites have profound functional consequences [43]. For example, mutation of the amino acids 519 and 520 in the putative activation loop does not reduce in vitro kinase activity, but the mutated Syk is incapable of transducing FcγRI-signaling. Further data from Lupher et al. [44] suggest that tyrosine 317 may be a possible site for binding c-Cbl. Cbl is a negative regulator of protein tyrosine kinases and has been shown to regulate FcγRI function. Mutation of Tyr-317 to Phe (Y317F) results in an increase in the catalytic activity of Syk. Indeed, phosphorylation of Tyr-317 of Syk negatively regulates signal transduction in mast cell [45]. Taken together, different stimuli invoke varied Syk phosphorylation "profiles" that precisely regulate which downstream event will occur.

There are still unresolved questions in understanding the relationship between Syk tyrosine phosphorylation and its function. For example, in rat basophilic leukemia cells (RBL; a common cell line with mast cell-like characteristics), FcγRI stimulation with antigen or with the anti-ganglioside mAb AA4 induces similar levels of Syk tyrosine phosphorylation. However, the functional response of the cell to these two stimulants is dramatically different. Whereas FcγRI aggregation results in degranulation, mAb AA4 binding does not [46]. Similarly, phosphorylation of Tyr 323 in the linker region between the Src homology 2 and kinase domains of Syk induces Cbl binding and coexpression of Cbl with Syk in COS-7 cells. This led to a dose-dependent decrease in the autophosphorylated pool of Syk and in phosphorylation of an in vivo substrate, CD8-ζ. Interestingly, these effects were largely due to the loss of Syk protein. The decrease in Syk protein levels were blocked by Y323F mutation in Syk [44]. These data suggest that Syk function and regulation are dependent on qualitative and/or quantitative changes in its tyrosine phosphorylation. The importance in this distinction will be explained in more detail later.

Relationship Between Cellular Syk Levels, Mast Cell/Basophil Nonresponsiveness, and IT—A Hypothesis

The functional nonresponsiveness of post-IT mast cells and basophils is similar to the nonreleaser; the cells demonstrate little or no mediator release when allergen challenged. No hypothesis has been able to explain how graded increases in allergen dosing given during IT results in reduced functional responses without inducing systemic mast cell activation and anaphylaxis. It has been assumed that as the dose of allergen is slowly titrated up, some degree of "tolerance" is induced that renders the next dose safe. It is plausible that the mechanisms underlying chronic allergen stimulation and concomitant blunted FcεRI responses may involve decreased kinase levels, resulting in a nonresponsiveness to FcεRI stimuli. How could chronic, low-dose antigen stimulation induce degradation of FcεRI-specific kinases without affecting

protein levels in other cells (as with nonreleaser basophils and possibly mast cells)? The following hypothesis is proposed (Fig. 1).

As discussed, allergen IT initially induces increases in antigen-specific IgE and is given at a dose that does not result in noticeable symptoms. Fatalities have been associated with allergen skin testing and IT injections. Therefore, ensuring that the patient receives a dose of allergen that will result in mast cells and basophil nonresponsiveness, and not activation, is an important safety concern. Similarly, suboptimal FcεRI crosslinking likely occurs in populations in which helminth infections result in high concentrations of IgE, which "saturates" FcεRI so that one particular antigen-IgE crosslinking does not dominate. The specificity for mast cell/basophil–specific kinase reductions is ensured through the high-affinity interaction of antigen with mast cell–bound, antigen-specific IgE. This results in suboptimal, nonsecreting activation by the specific antigen, which, in turn, leads to the downregulation and degradation of FcεRI receptor–associated Syk kinases. The recurrent stimulation with increasing doses of allergen would ensure continued, subacute activation and kinase ubiquitination, whereas the specific, high-affinity interaction between mast cell/basophil surface IgE with antigen would ensure only downregulation of kinase levels in these cells. Evidence for this hypothesis is presented in the following section.

Inhibition of FcεRI Through Syk Deficiency in "Nonreleaser" Basophils

As mentioned earlier, nonreleaser basophils are "naturally" desensitized. We [47] showed that nonreleaser basophils have a deficiency in Syk. Other laboratories confirmed these findings [48]. The observation that an early signal transduction event is affected is reminiscent of the defect detected in mast cell/basophil desensitization described previously. Syk suppression is lineage-specific, fluctuates, and occurs post-transcriptionally [49]. Our studies show a clear relationship between the lineage specific lack of Syk expression and the lack of FcεRI mediated signaling activity in human nonreleaser basophils.

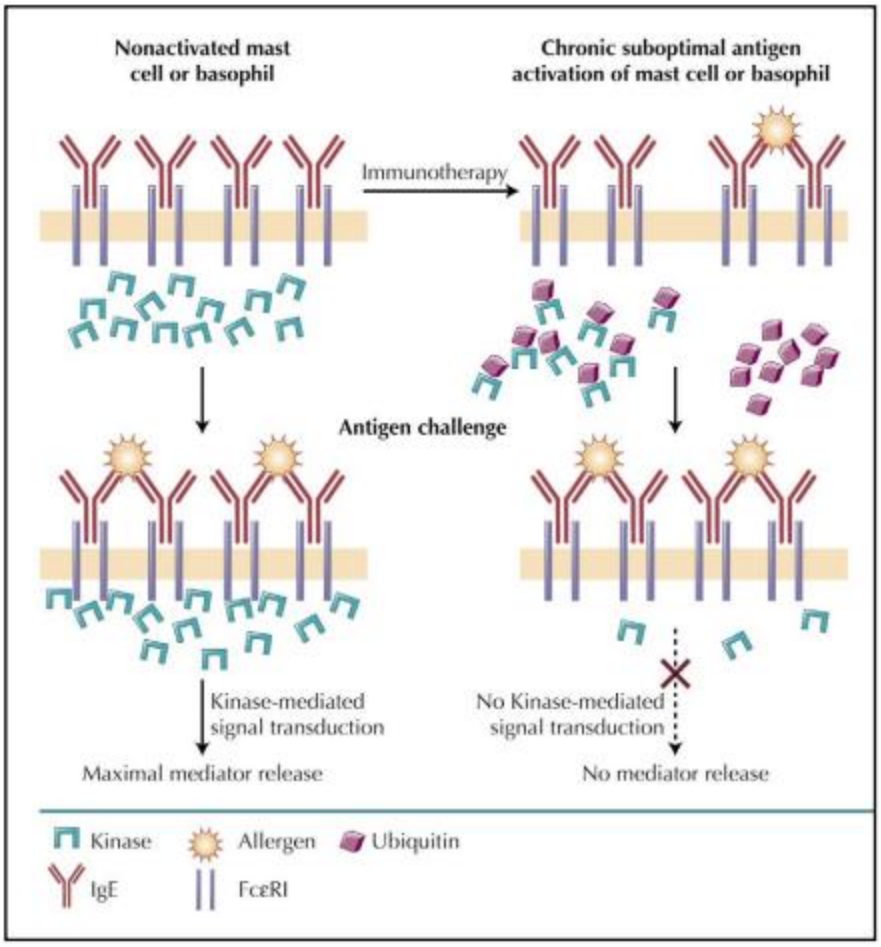

Figure 1. Chronic antigen stimulation results in lower kinase levels in mast cell/basophils. Normally, when people from areas with low FcεRI stimulation (ie, low infectiousdisease rates, pre-IT patients) are challenged with antigen, there is degranulation that is mediated through kinases such as Syk. However, low doses of antigen suboptimally crosslink surface-bound IgE on mast cells and basophils, resulting in the ubiquitination and subsequent proteasome degradation of kinases such as Syk. When cells are then antigen challenged, there is less kinase signaling and reduced mediator release. FcεRI—high-affinity receptor for IgE; IgE—immunoglobulin E.

FcεRi Nonresponsiveness after Challenge with Low Doses of Antigen Is Syk Dependent

We hypothesized that crosslinking FcεRI on mast cells and basophils leads to FcεRI nonresponsiveness through reductions in Syk protein levels. To test this hypothesis, human mast cells and basophils were used to determine if FcεRI hyporesponsiveness correlated with reduced Syk levels. It was shown that suboptimal antigen challenge that did not lead to significant mediator release induced nonresponsiveness and correlated with reduced Syk. The ability of IgE-unresponsive mast cells to regain FcεRI responsiveness was paralleled by increased cellular Syk levels in vitro. The reduction of Syk levels with suboptimal antigen concentrations was calcium-independent and mediated through a proteasome-dependent mechanism [50]. We conclude that these findings extend our knowledge about a novel regulatory mechanism for maintaining FcεRI in a quiescent state. This mechanism may also explain how low concentrations of allergen given

to patients during the initial phases of IT induce FcεRI nonresponsiveness and therapeutic benefit without inducing systemic anaphylaxis.

Conclusions

It remains to be determined if IT strategies aimed at inhibiting mast cell and basophil-mediator release through Fcε-Fcγ coaggregation will provide improved efficacy compared to current IT regimens using allergen extracts. The hallmark for successful IT is the reduced mast cell and basophil functional response, and naturally occurring nonreleaser FcεRI-positive cells demonstrated reduced Syk levels. Recent studies in our laboratory (Kepley, Unpublished data) suggest that reduced Syk levels occur in parallel with reduced FcεRI responses in basophils obtained before and after 2 months of allergen IT. Therefore, finding ways to target Syk, either through Sykspecific inhibitors or through reduced Syk cellular levels in mast cells and basophils, may be a new approach for blunting the allergic response in patients.

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•• Of major importance

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