

IgE-Facilitated Antigen Presentation: Role in Allergy and the Influence of Allergen Immunotherapy

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One mechanism by which antigen-presenting cells (APCs) sample antigens from the local microenvironment is a crude process involving direct phagocytosis or pinocytosis and is independent of surface receptors. This “scavenging” is inefficient and triggers T-cell activation only in the presence of high antigen concentrations [1]. In addition, B cells are able to focus very low concentrations of antigen via specific membrane immunoglobulin into endosomal compartments, a process that results in greatly enhanced cell activation. This receptor-mediated “antigen focusing” may occur at antigen concentrations 10^3 to 10^4 fold lower than by nonspecific uptake [2]. Antigen focusing by B cells amplifies capture of antigen without the requirement for receptor cross-linking. Capture, per se, may not result in activation of the B cell, which also requires subsequent T-cell recognition. After internalization by either mechanism, antigen is degraded into antigenic peptides within endosomal compartments. Peptides subsequently bind to major histocompatibility complex (MHC) class II molecules, and the complexes are loaded onto the cell surface before presentation to antigen-specific T cells [1]. Activation of immune responses in healthy individuals depends on very high antigen concentrations or the presence of B cells with antigen-specific membrane immunoglobulin.

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In allergic individuals, exposure to common inhalant allergens involves minute allergen concentrations at respiratory mucosal surfaces. In patients who have seasonal pollinosis, the quantity of allergen that reaches the nasal mucosa, even at the peak of the pollen season, is estimated to be a few micrograms per year [3]. In normal nonatopic individuals, such small quantities of allergen fail to activate an immune response. In atopic individuals, allergen-specific IgE produced at least in part by plasma cells activated locally within mucosal tissue [4–6] binds to allergen at the mucosal surface in a process referred to as IgE-facilitated allergen presentation (FAP). This process is fundamentally different from antigen focusing mediated by antigen-specific B cells in normal subjects. In contrast to the lack of effect in normal nonatopic subjects, the additional presence of high local IgE allows APCs that express IgE receptors to process extremely low concentrations of antigen. FAP is dependent on the combined presence of allergen, elevated IgE, and the expression of IgE receptors on the surface of APCs.

IgE is a characteristic marker of atopy

IgE is central to allergic inflammation [7]. The abundance of IgE in the target organs of allergy sufferers emphasizes the importance of this immunoglobulin in promoting allergic inflammation [5,8]. IgE has the ability to bind with extremely high affinity to IgE receptors on the surface of mast cells and basophils [7]. The proximity of IgE-producing cells to tissue mast cells enables a continuous supply of antibody for cell surface receptors. Subsequent allergen exposure triggers an immediate local response. IgE promotes IgE receptor expression on effector cells, supports the survival of mast cells in mucosal tissue, and induces positive signals for IgE synthesis [9].

Overproduction of allergen-specific IgE in atopic individuals is a consequence of differential cytokine production. B cells recruited to sites of allergic inflammation encounter inflammatory mediators and Th2 cytokines, in particular, interleukin-4 (IL-4) and IL-13. These cytokines are produced locally by Th2 lymphocytes but also in high concentrations by activated mast cells and basophils [10]. Local IL-4 and IL-13 promote B cells to switch preferentially to IgE synthesis [11]. Circulating immature B cells that encounter antigen in the periphery will present processed epitopes to specific T cells (cognate recognition). Subsequent CD40/CD40L ligation at the T-cell surface results in further IL-4 production by activated Th2 T cells and initiation of heavy-chain constant region gene recombination in favor of IgG4 or IgE antibodies (isotype switching). This process has been shown to occur locally, in nasal [12,13] tissue and in bronchial tissue [14,15], enabling localization of the allergic response to a particular region with associated clinical manifestations. Following isotype switching, B cells differentiate into plasma cells and secrete antigen-specific IgE into the local environment [16]. IgE-mediated responses are inevitably dependent on IgE receptors, which exist in morphologically distinct and independent high- (Fc ϵ R1) and low-affinity (Fc ϵ R2, CD23) species.

High-affinity IgE receptor mediates immediate hypersensitivity

Fc ϵ RI is expressed widely on the surface of mast cells and basophils and has also been identified on monocytes and dendritic cells [17]. Fc ϵ RI is expressed in trimeric form (Fc ϵ RI $\alpha\gamma_2$) on APCs and is upregulated in allergic subjects, whereas it is low or absent in normal individuals [18]. A member of the immunoglobulin super family of proteins, tetrameric (Fc ϵ RI $\alpha\beta\gamma_2$) is expressed on mast cells and basophils (Fig. 1) and mediates immediate hypersensitivity reactions [19]. Cross-linking of Fc ϵ RI-bound IgE by allergen induces a cascade of complex signaling events [20] that ultimately results in the release of preformed inflammatory mediators that include histamine, tryptase, and IL-4. The parallel upregulation of CD40 ligand provides a switch factor for B cells and promotes prolonged B-cell survival. IL-4 and IgE upregulate Fc ϵ RI expression, which enables a positive feedback loop for Fc ϵ RI-associated signaling. In contrast, trimeric Fc ϵ RI on APCs is primarily involved in IgE-facilitated antigen presentation [11].

Low-affinity IgE receptor, CD23, has multiple functions

The low-affinity receptor for IgE (Fc ϵ RII/CD23) is a type II integral membrane protein. The active receptor is a trimer that comprises C-type lectin-binding

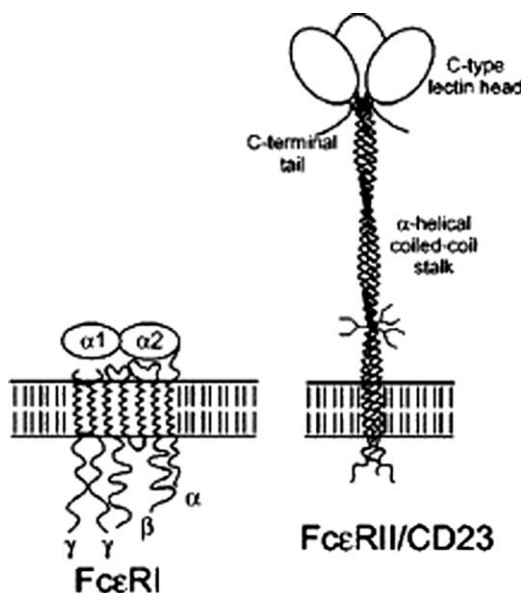


Fig. 1. Schematic representations of the IgE receptors Fc ϵ RI and Fc ϵ RII/CD23. (Adapted from Gould HJ, Sutton BJ, Beavil AJ, et al. The biology of IgE and the basis of allergic disease. *Annu Rev Immunol* 2003;21:582; with permission.)

regions that are attached to the cell membrane surface via α -helical stalks (Fig. 1). The receptor has two isoforms that differ in six of seven amino acids in the intracellular region, resulting in their differential expression and distinct functions. CD23a is expressed exclusively on the B cell surface following activation. CD23b expression is IL-4 inducible and is present on a variety of cells, including B cells, macrophages, monocytes, follicular dendritic cells, and eosinophils [7]. CD23 has a variety of functions. Its ligands include IgE, complement receptors CR1, CR2, and CR4, and vitronectin [7]. In mice, CD23 is involved in negative regulation of IgE synthesis [21]. Membrane CD23 is cleaved by proteases to form soluble CD23 (sCD23), which functions to promote B-cell differentiation in germinal centers via interactions with CD21 on the B-cell surface [7]. CD23 expression is upregulated by IL-13 and IL-4; therefore, it has increased expression among atopic individuals when compared with healthy individuals [22]. An important function of CD23 is its role in IgE-dependent facilitated antigen presentation.

Allergen-IgE complexes are internalized on IgE receptors

IgE-allergen complexes are captured and immobilized on the APC surface by binding to IgE receptors. Fig. 2 illustrates CD23-dependent IgE-FAP as mediated by B cells. The IgE-allergen complexes are internalized to endosomal compartments where the allergen is processed and presented to T cells on MHC class II molecules [23]. There is growing evidence for a role of IgE-FAP in activating allergen-specific T cells in vivo. Expression of the low-affinity receptor CD23 is higher in atopic individuals when compared with nonatopic individuals [22], and CD23, CD40, and HLA are all upregulated on B cells during the pollen season [24]. Interestingly, studies of B-cell lines have shown that CD23 is co-expressed in association with MHC molecules at the surface membrane of B cells [25]. Karagiannis and colleagues [26] described endocytosis of the entire CD23/IgE/allergen complex along with the associated MHC class II molecule. The association of CD23 with MHC molecules is thought to enable more efficient loading of peptide onto MHC in endosomal compartments. In the same study, CD23 was shown to be recycled to the cell surface with the peptide loaded MHC. As well as CD23-mediated IgE-FAP, Maurer and colleagues [27] isolated blood dendritic cells that expressed trimeric Fc ϵ RI (Fc ϵ RI $\alpha\gamma_2$). Despite extremely low numbers, these cells were able to efficiently stimulate IgE-dependent T-cell responses. Because FAP is known to occur via CD23 and Fc ϵ RI, any cell type expressing these receptors is able to focus allergen for more effective processing, presentation, and activation of T cells in an IgE-dependent fashion [28].

Furthermore, because Fc ϵ RI and CD23 are expressed on professional APCs, priming of naïve T cells can occur in addition to the activation of secondary responses. The potential consequences of IgE-FAP are substantial. When occurring between B cells and T cells, activation of the T cell will induce Th2 cytokine expression that further promotes IgE production by B cells. This expression, in turn, would lead to an abundance of IgE in the mucosal tissue, further

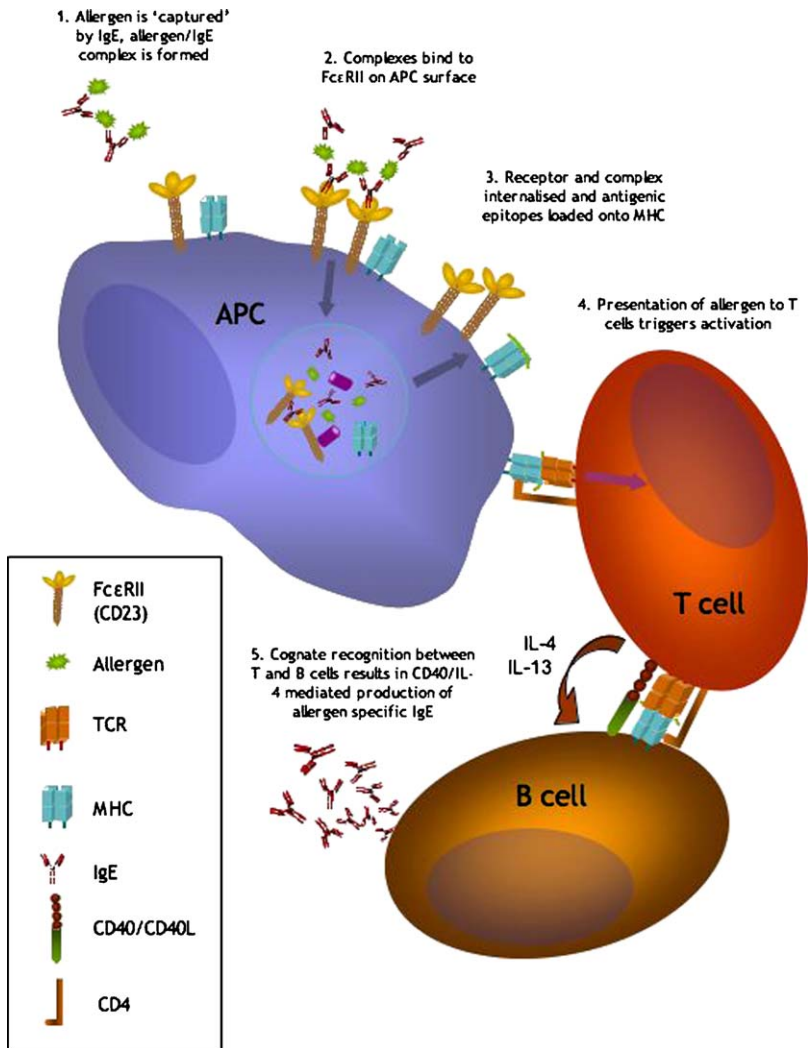


Fig. 2. CD23-mediated IgE-facilitated allergen presentation. Cartoon depicts IgE-FAP using CD23 on the surface of a B cell and resulting in cognate recognition by allergen-specific T cell. The interaction results in further production of allergen-specific IgE.

perpetuating the cycle of T-cell activation and intensifying the inflammatory response (Fig. 2).

Experimental assessment of IgE-facilitated allergen presentation

The role of IgE and IgE-CD23 interaction in antigen presentation was originally demonstrated in a murine model [29]. Trinitrophenyl (TNP)-specific IgE

facilitated the presentation of TNP by mouse B cells at 100-fold less concentrations of TNP than in the presence of TNP alone. These experiments were subsequently confirmed in humans [23].

The addition of antigen-specific IgE was shown to enhance significantly the ability of Epstein-Barr virus (EBV)-transformed B cells to present antigen to T cells with subsequent enhanced T-cell activation and proliferation. CD23-dependent FAP has since been confirmed using monomeric IgE [30] and atopic serum containing polymeric IgE when complexed with allergen [31]. In vitro experiments of IgE-FAP have used EBV-transformed human B-cell lines that express high levels of CD23 and are efficient in processing and presenting antigens to specific T cells, with resulting activation and proliferation [23]. Indicator serum from an atopic individual with high allergen-specific IgE levels is mixed

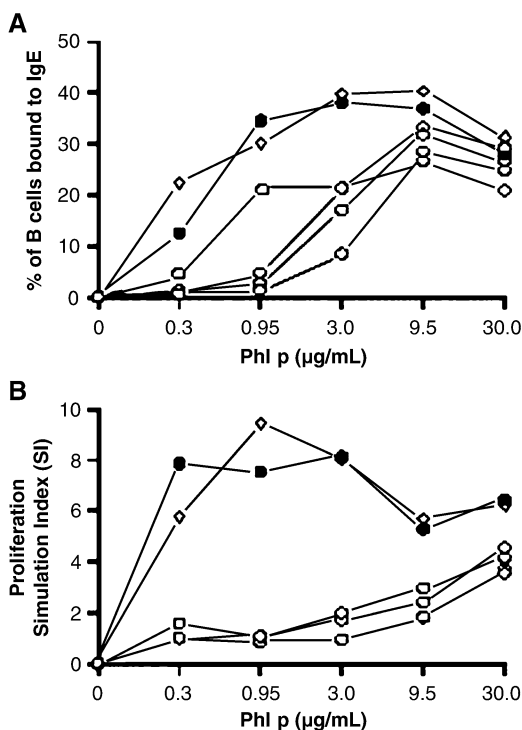


Fig. 3. Allergen-IgE binding to B cells is associated with enhanced antigen presentation to allergen-specific T cells, which is inhibited by sera from immunotherapy patients. Graph shows allergen-dependent binding of IgE in the absence (filled circles) or presence (open circles) of sera from four grass and one birch (open diamonds) immunotherapy patients. IgE-allergen complexes bound to B cells were detected with fluorescently labeled anti-IgE antibody by flow cytometry (A), or EBV-transformed B cells were irradiated and used as APCs for an autologous grass pollen-specific T-cell clone, and proliferation was measured after 24 hours by ^3H -thymidine incorporation (B). Data shown are representative of three separate experiments. (Adapted from Wachholz PA, Soni NK, Till SJ, et al. Inhibition of allergen-IgE binding to B cells by IgG antibodies after grass pollen immunotherapy. *J Allergy Clin Immunol* 2003;112:920; with permission.)

with allergen. Subsequent incubation of the allergen-IgE complexes with EBV-transformed B cells results in higher binding of IgE with CD23 [32], with internalization and processing of allergen to generate small peptide epitopes that are expressed at the cell surface in the context of MHC class II. In standardized proliferation assays, the B cells are used to present the allergen peptides to T-cell clones. Elevated T-cell activation is measured as proliferation of clones [31]. Van der Heijden and colleagues [33] showed that Der-p 2 (house dust mite major allergen)-specific T-cell clones proliferated at 100- to 1000-fold lower concentrations of Der-p 2 when the allergen was complexed to high IgE-containing serum compared with uncomplexed allergen. The investigators were able to demonstrate a direct correlation between FAP-mediated T-cell proliferation and the subject's serum IgE levels.

The use of T-cell assays as a readout of efficient IgE-FAP is complex and time-consuming and requires the generation and maintenance of long-term allergen-specific T-cell clones. The assay can be greatly simplified by measuring only the IgE-dependent binding of allergen to CD23 on the surface of B cells, which has been shown to correlate closely with subsequent T-cell activation and proliferation (Fig. 3). In this modified assay, surface binding of the allergen-IgE complexes is performed at low temperatures to prevent internalization of the complex [3], thereby allowing detection by flow cytometry of the allergen-IgE complexes to the B-cell surface. Detection of the allergen-IgE complexes is most easily measured by use of anti-IgE antibodies, although allergen binding may also be confirmed by use of biotinylated allergen [34]. Using sera from subjects with grass pollen allergy, the authors have confirmed that specific IgE levels correlate with the efficiency of allergen-IgE complex binding ($r = 0.79$, $P < .0001$). This association strongly supports a role for IgE-FAP in the pathogenesis of allergic disease *in vivo*.

Immunotherapy induces changes in humoral responses to allergen

The clinical benefits of specific allergen immunotherapy are well documented [35]. The mechanisms of successful treatment are not fully understood, although immune deviation or the induction of T regulatory cells is likely to be central to disease remission. Chronic antigen exposure results in high-titered IgG4 responses [36], and because immunotherapy involves the long-term administration of high-dose allergen, it is not surprising that levels of IgG4 increase dramatically following treatment [34,37,38]. Far from being a simple bystander effect, IgG4 antibodies induced by immunotherapy have been shown to have functionally relevant “blocking” activity.

The concept of blocking antibodies, whereby competition with IgE for binding to allergen prevents IgE-mediated responses, has existed since Cooke and co-workers [39] demonstrated in 1935 that postimmunotherapy serum prevented inflammation that had been induced by preimmunotherapy serum. In 1968, this inhibitory activity was shown to be elicited by IgG “blocking” antibodies [40],

suggesting that these IgG antibodies could inhibit histamine release from sensitized basophils induced by atopic serum. More recently, IgG4 has been shown to be the prominent antibody isotype that is induced by immunotherapy.

In a study of chronic parasitic filarial infection, a significant correlation was demonstrated between high IgG4 titers and patients who were asymptomatic despite chronic infection. IgG1 and not IgG4 levels were associated with allergic responses to the parasite, and depletion of IgG4 from the serum removed blocking activity as defined by basophil histamine release [41]. Serum from post allergen-immunotherapy patients has been shown to inhibit basophil histamine release, a marker of Fc ϵ RI-mediated immediate hypersensitivity reactions [42,43]. In a study of modified birch pollen immunotherapy, serum from immunotherapy-treated but not placebo-treated patients could inhibit Bet v 1 (birch pollen major allergen)-mediated basophil histamine release but not grass pollen-mediated release, demonstrating the allergen-specific nature of immunotherapy [43].

IgE-facilitated allergen presentation is inhibited by postimmunotherapy serum

In 1999 van Neerven and colleagues demonstrated the induction of blocking antibodies during immunotherapy that were capable of inhibiting IgE-FAP-mediated T-cell activation [3]. Serum from a patient treated with long-term birch pollen immunotherapy was incubated with birch pollen allergen and serum containing high levels of birch-specific IgE. The inhibition was shown to be allergen-specific, because serum from grass-treated immunotherapy patients was unable to inhibit IgE-facilitated presentation of birch pollen. The researchers showed that this inhibitory activity was present 3 to 6 months after starting treatment. The induction of inhibitory activity following immunotherapy has since been confirmed in other trials [3,34]. van Neerven further demonstrated that blocking IgG antibodies induced by birch immunotherapy and isolated by fractionation could inhibit IgE-FAP-mediated activation of CD4⁺ T cells [3]. Wachholz and co-workers confirmed these findings using IgG-fractionated sera from grass pollen-immunotherapy patients [34]. Nouri-Aria and colleagues [44] confirmed that immunotherapy induced increases in serum inhibitory activity for allergen-IgE complex binding (Fig. 4) and increases in allergen-specific IgG4 antibody levels. These observations occurred in parallel with increases in IL-10 production. Furthermore, blocking activity for IgE-FAP demonstrated in postimmunotherapy serum co-purified with IgG4-containing fractions [44].

Lack of correlation between IgG4 and clinical improvement

The main argument against a functional role for IgG4 is that the serum levels of the antibody do not correlate well with clinical efficacy. For example, in a trial

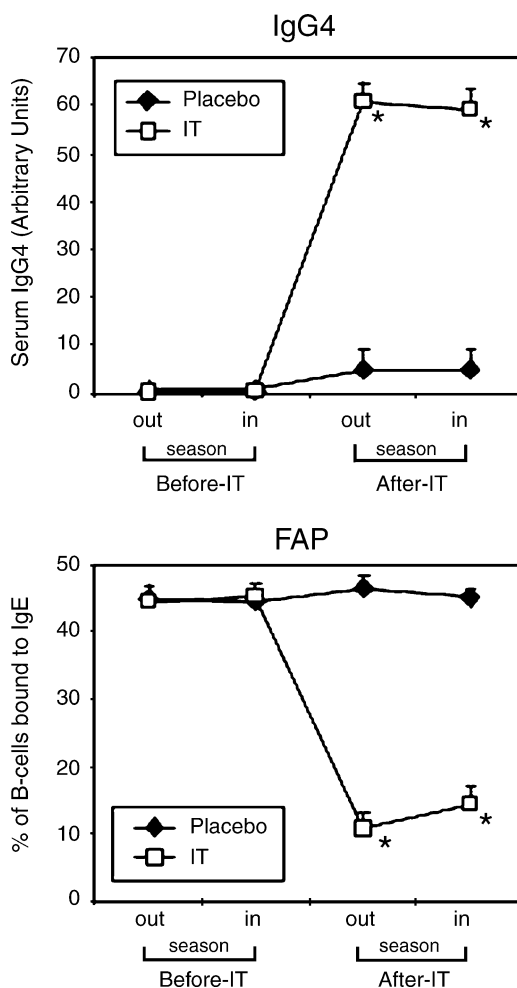


Fig. 4. Increased serum allergen-specific IgG4 concentrations and inhibition of allergen-IgE binding to B cells by sera from immunotherapy-treated but not placebo-treated patients before (*Before IT*) and after 2 years of treatment (*After IT*) before (*out*) and during (*in*) the pollen season. Results are expressed as mean $\pm$ SE. * $P < .05$ as determined by Wilcoxon matched pairs test. (Adapted from Wilcock LK, Wachholz PA, Hae-Won D, et al. Inhibition of IgE-facilitated allergen presentation: a longitudinal study of grass pollen immunotherapy. ACI International 2006, Supplement 2, 244–7, with permission. © 2006 Hogrefe & Huber)

of venom immunotherapy, IgG4 levels were markedly elevated in patients who responded to treatment but also in those who did not [45]. This lack of correlation has been confirmed in trials of immunotherapy with venom [46] and inhalant allergens [47]. More recently, the role of IgG4 as a blocking antibody induced by immunotherapy has been reviewed. The findings suggest that impure or irregular allergen extracts used for determining IgG titers fail to distinguish true blocking antibodies and may be responsible for the reported lack of positive correlations [48].

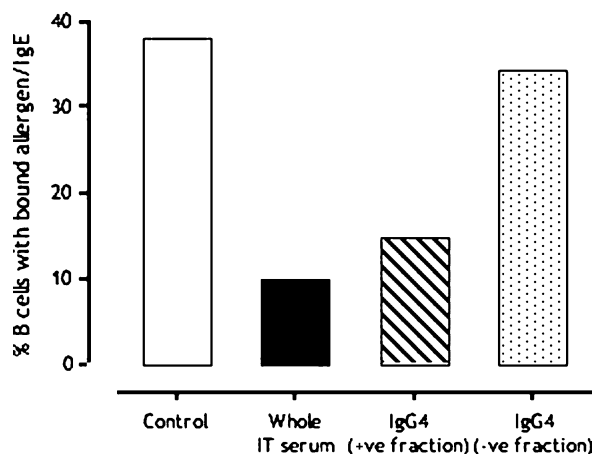


Fig. 5. Serum obtained following grass pollen immunotherapy was separated by affinity chromatography. The IgG4 fraction, the eluted fraction from which IgG4 had been removed, and the whole serum were compared for their ability to inhibit the binding of allergen-IgE complexes to B cells, as detected by flow cytometry (*Adapted from* Nouri-Aria KT, Wachholz PA, Francis JN, et al. Grass pollen immunotherapy induces mucosal and peripheral IL-10 responses and blocking IgG activity. *J Immunol* 2004;172:3257.)

The authors recently demonstrated that IgG4 contributes most of the inhibitory activity contained within postimmunotherapy sera [44]. Serum from one patient who had received 2 years of grass pollen immunotherapy was fractionated to separate IgG4. The IgG4 fraction was able to inhibit the binding of allergen and IgE with a similar capacity as whole serum (Fig. 5). In contrast, the fraction in which IgG4 had been removed displayed dramatically reduced inhibitory activity. Further studies involving larger numbers of patients are required to confirm these findings. Nonetheless, these data support a role for IgG4 as the principal inhibitory activity in postimmunotherapy serum.

IgA as a blocking antibody

IgA is also increased in response to allergen immunotherapy [38]. IgA is the predominant antibody isotype at mucosal surfaces; the active antibody is polymeric and is associated with a secretory component. IgA functions as a sequestering agent by binding to antigens and preventing their passage into the tissue where activation of immune responses may occur. IgA from nasal secretions of immunotherapy patients has been shown along with IgG to block IgE-mediated basophil histamine release [49]. To investigate the potential role of IgA in blocking facilitated antigen presentation, the authors fractionated the serum from three immunotherapy patients for IgA and IgG. The fraction containing IgG was able to inhibit binding of allergen-IgE complexes to EBV-transformed B cells. In

contrast, IgA displayed no inhibitory activity in its polymeric or secretory form (C. Pilette, unpublished observations, 2005).

IL-10 induces isotype switching to IgG4

The induction of T regulatory cells is a recognized feature of allergen immunotherapy, along with the associated production of the regulatory cytokine IL-10. IL-10 induces anergy by blocking co-stimulation; however, a secondary function that has more relevance to IgE-FAP is its ability to induce isotype switching to IgG4 antibodies [50]. The authors recently showed that grass pollen immunotherapy induced local increases in IL-10-positive cells in the nasal mucosa. This induction was associated with increases in IgG4 and blocking activity for IgE-FAP [44]. Further evidence comes from Satoguina and colleagues [51] who demonstrated that when T regulatory-1 (Tr-1) cells producing IL-10 were added to B-cell cultures, they induced preferential production of IgG4. Increased production of IL-10 seems to be a consistent feature of allergen immunotherapy [38], and it can be concluded that increases in IgG4 may be at least partially explained by the associated increases in IL-10. Time course studies before and after immunotherapy may further test the possible causal association between these events.

IgG4-blocking activity may result from direct competition with IgE

The mechanism by which IgG4 blocks IgE-facilitated antigen presentation is not completely understood. The lack of correlation between IgG4 levels and clinical correlates of successful treatment may indicate that a distinct subpopulation of IgG4 antibodies is important. Chronic antigen stimulation induces high titered IgG4 responses that may not have a fundamental role in inhibiting IgE-mediated responses. In contrast, a component of T regulatory/IL-10 induced “high-affinity” IgG4 may comprise the major inhibitory component. This effect is supported by data showing an increase in the binding capacity of IgG4 and a concurrent decrease in the binding capacity of IgE following ragweed immunotherapy [52]. In contrast, a separate study by Jakobsen and coworkers [53] reported that immunotherapy with birch pollen had no effects on the affinities of IgG4, IgE, or IgG1 as measured by surface plasmon resonance. Blocking IgG4 antibodies may compete directly with IgE for epitopes. IgG4 is functionally monovalent; therefore, it does not have the capacity to cross-link antigen [36]. IgG4 is also noninflammatory in that it is unable to activate complement or bind with high affinity to Fc γ receptors. It is therefore likely that IgG4 is only able to compete with IgE for binding of allergen and is unlikely to have other inflammatory and potentially deleterious effects other than inhibition of IgE-mediated allergic responses and possibly helminthic infections.

In addition to the concept of IgG4 as a scavenging receptor competing with IgE for allergen binding, inhibitory activity may result from the binding and co-aggregation of Fc ϵ RI with adjacent Fc γ RIIb on the surface of effector cells. Fc γ RII receptor signaling via the inhibitory cytoplasmic domain has been shown to block IgE-mediated degranulation of human basophils [54] and mast cells [55]. It is suggested that IgE bound to its receptor may bind allergen complexed to (immunotherapy-induced) allergen-specific IgG, and that this results in the co-aggregation of IgG receptors, with subsequent postreceptor inhibitory signaling events downregulating IgE-mediated responses [56].

Clinical relevance of IgE-facilitated allergen presentation inhibition

The presence of IgE bound to the high-affinity receptor on the Langerhans cells of patients with atopic dermatitis [57], the high expression of the low-affinity receptor among atopic individuals [58], and its differential expression during the pollen season [24] all point toward a role for IgE-FAP in local Th2 cell activation and associated allergic responses.

The potential role of IgG4-blocking antibodies in preventing IgE-mediated pathology is apparent. IgG4 antibodies are known to block Fc ϵ RI-mediated immediate hypersensitivity reactions and may be important in preventing the early phase responses of allergic disease. The role of IgG4-blocking antibodies in inhibiting IgE-FAP also proposes a role in the inhibition of late phase allergic responses as mediated by T cells. Inhibition of this pathway could also prevent the overproduction of IgE antibodies. Paradoxically, IgE initially increases after immunotherapy with subsequent blunting of seasonal increases, followed by a gradual and prolonged fall in IgE after 3 to 5 years to below baseline IgE levels. Long-lived IgE-producing plasma cells may be responsible for the persistence of IgE in the serum despite long-term immunotherapy. Nevertheless, the effects of IgE could be minimized by parallel elevations in “functional” high-affinity IgG4 antibodies that block IgE-mediated responses and prevent the induction of new IgE-producing cells.

Current data concerning the contribution that IgE-FAP has in allergic disease suggest that this is an important process that contributes to activation and exacerbation of inflammatory pathways. The role of blocking antibodies in inhibiting this process in vivo is now emerging.

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