



Chapter 1

Understanding of the Molecular Mechanisms of Allergy

Meinir G. Jones

Abstract

The prevalence of allergic disease has dramatically increased over the past 30 years in Westernized countries. It is unlikely that the rapid increase in the prevalence of allergic disease is the result of genetic changes, which highlights the importance of environmental factors in the development of allergic disease. The “hygiene hypothesis” was put forward in 1989 and focused attention on the notion that exposure to microbes and their products in early life can modify the risk for development of allergic disease. Infections were thought to polarize the immunological response toward a Th2-mediated immune response causing allergic disease. However, it is likely that the Th1/Th2 imbalance is too simplistic to explain the increased prevalence of allergic disease. Current research is focusing on understanding the role of T-regulatory cells in inducing a state of tolerance and the resulting modified Th2 response observed in natural and induced tolerance.

Key words Allergic disease, Hygiene hypothesis, Immunoregulation, Immunotolerance

1 Introduction

The term “allergy” was introduced in 1906 by von Pirquet [1], who recognized that in both protective immunity and hypersensitivity reactions, antigens had induced changes in reactivity. Allergy is now used to describe a hypersensitivity reaction initiated by immunologic mechanism [2]. Allergic diseases include allergic rhinitis, allergic conjunctivitis, asthma, atopic eczema, and food allergy. IgE-mediated diseases are often referred to as atopy (from the Greek atopos, meaning out of place). The definition of atopy is a personal or familial tendency to produce IgE antibodies in response to low doses of allergens, usually proteins, and to develop typical symptoms such as asthma, rhinoconjunctivitis, or eczema/dermatitis [3].

Atopic individuals have a genetic predisposition to produce IgE antibodies against common environmental allergens. Atopic disorders are thought to be due to complex interactions between

genetic and environmental factors. The prevalence of allergic disease has dramatically increased over the past 30 years [4–7], especially in Westernized countries. It is unlikely the rapid increase in the prevalence of allergic disease is the result of genetic changes, which emphasizes the importance of environmental factors in the development of allergic disease. The increased prevalence of allergy in Westernized countries highlights the potential influence of improved hygiene and better infection control on the development of allergy. An interesting observation was made after the reunification of Germany: Before reunification, allergic rhinitis and asthma were less common in East Germany compared with West Germany [8]. However since reunification, the prevalence of atopy and hay fever, but not asthma, has increased among children who spent their early childhood in East Germany [9]. Interestingly, exposure to farm animals remains a protective factor in the development of allergy [10], which is similar to that observed with hay fever at the turn of the century. These observations suggest that our Westernized lifestyle accounts for the increase in prevalence. Interestingly, hay fever was first reported back in the early nineteenth century and was thought to be essentially the disease of the upper social class and urban dwellers [3]. The rural working population was considered to have become tolerant through high-dose exposure in the fields.

2 Hygiene Hypothesis

The “hygiene” hypothesis was put forward in 1989 [11], and focused attention on the notion that exposure to microbes and their products in early life can modify the risk for development of allergic disorders. It is thought that helminth infection, mycobacteria, hepatitis A, endotoxin, and early-life infections create a milieu which protects against the development of allergic reactions. The distinction of Th1 and Th2 cells in mice (1989) [12] and in humans (1994) [13] provided a plausible immunological mechanism for the hygiene hypothesis. Th1 cells are responsible for cell-mediated immune responses against intracellular pathogens whereas Th2 cells direct immune responses against intestinal helminths [12]. Th1 cells cause autoimmune diseases, while Th2 cells cause allergic diseases and asthma. Th1 and Th2 cells cross regulate each other, and thus it was proposed that in allergic disease there were too many Th2 cells and not enough Th1 cells. Exposure to microbial products leads to a polarization toward the Th1 cells potentially suppressing the Th2 immune response involved in IgE-mediated allergy. Thus lack of exposure to microbes in early childhood may lead to a predominantly Th2 immune response causing allergic disease.

In parallel with an increase in prevalence of allergy in the last 30 years there has been a similar increase in autoimmune diseases such as type 1 diabetes, multiple sclerosis, inflammatory bowel disease, and systemic lupus erythematosus [14, 15]. An increase in the incidence of autoimmune diseases, plus the relative absence of autoimmunity in developing countries where chronic infections are endemic, has led to the theory that infection may actually inhibit the development of autoimmunity [14]. In view of increased prevalence of both Th1 autoimmune diseases and Th2 allergic disease in recent years, it is likely that the Th1/Th2 imbalance is too simplistic. It is more likely that there are other control mechanisms of T-cell regulation, which controls harmful T-cell responses.

3 Family Size and Allergy

One of the most robust findings in allergy which has stood the test of time is the epidemiological observation of the inverse association of allergic disease with family size, which was reported with hay fever, skin prick positivity, and specific IgE [16, 17]. Other factors found to be protective against sensitization are exposure to farm animals or domestic pets, day-care attendance, and large family size [10]. The protective influence of large family size has been interpreted as evidence in support of the hygiene hypothesis. However this does not preclude other mechanisms related to birth order [18].

It is possible that pregnancy may influence the atopic status of both the mother and the fetus. Pregnancy is an immunological challenge to the mother and it is possible that the immunoregulation, which takes place to avoid fetal rejection, may also influence the allergic response in both mother and fetus [19]. There is some evidence in support of this hypothesis. Mothers of higher parity have lower levels of total IgE, as do their newborn children [20]. They report higher rates of remission from allergic rhinitis [21] and lower rates of allergic conjunctivitis [22]. Cord blood mononuclear cell responses to house dust mite are reduced in higher birth order children [23] although this may be explained by maternal age.

In a cross-sectional study, it was observed that multiparous mothers are less often atopic, although this did not hold true for the fathers [24, 25]. In a prospective study, over a period of 7 years, it was observed that the loss of maternal atopy and hay fever are associated with a higher number of intervening pregnancies [26]. Expansion of regulatory T cells has been demonstrated in both mice [27] and humans [28]. In mice the cells were alloantigen independent although capable of suppressing an aggressive response against the fetus. Their deletion led to a failure of gestation due to immunological fetal rejection. This effect was true only

for allogeneic pregnancies, the outcome of syngeneic pregnancies being unaffected. In humans, T-regulatory cells increase during early pregnancy, peaking during the second trimester and declining postpartum although remaining higher than prepregnancy [28]. The cells are capable of suppressing an allogeneic response. Allergic mothers and their neonates were found to have a stronger lymphoproliferation to alloantigen than nonallergic mothers and their neonates [29], suggesting the possibility of greater immunoregulation in nonallergic than in allergic mothers. It is not known whether the degree of regulation varies with a mother's parity nor whether it is transferred to her children.

4 Indoor Allergens

Atopic individuals are by definition sensitized to the common environmental allergens. The major allergens of the Westernized countries are Der p 1 and Der p 2, from the house dust mite (*Dermatophagoides pteronyssinus*); Fel d 1, from the cat (*Felis domesticus*); tree allergens including Bet v 1 from the birch tree (*Betula verrucosa*); and many grasses, such as Phl p 1 and Phl p 5 from timothy (*Phleum pratense*), and the ragweed allergens Amb a 1, 2, 3, 5, and 6 from short ragweed (*Ambrosia artemisiifolia*) [1]. Another allergen which has become common over the last 25 years is the glycyphagus dust mite *Blomia tropicalis*, in heavily populated tropical regions [3]. This allergen was uncommon; however it is now estimated that one billion individuals are currently sensitized.

At present there is a drive to target immunotherapy selectively to immunodominant allergens [3]. It is currently unknown whether the immunodominant allergens have intrinsic adjuvant properties that contribute to their potency. It will be intriguing to see whether removing or neutralizing the host response to immunodominant allergens will silence the allergy or redirect the immune response toward different specificities. The main allergens represent a diverse group of proteins; several are proteases which are thought to be important in the development of allergy. Currently there is much interest in the lipid binding property of immunodominant allergens [30].

Much focus has been given to understanding the mechanisms of the development of sensitization in recent years. It was proposed that exposure to indoor allergens early in life would increase the risk of allergic sensitization. If this were the case then it would follow that avoidance of allergen early in life would decrease the risk of allergic sensitization. Exposure-response studies with allergen and sensitization have demonstrated an apparent dose-response relationship with a bell-shaped curve, suggestive of a tolerance mechanism at high exposures. A bell-shaped curve was described with cat [31, 32] and house dust mite [32] allergens. Allergen avoidance trial demonstrated that reducing house dust

mite concentration did not reduce but increased sensitization rates to house dust mite [33]. Cat ownership has been associated with a reduced prevalence of sensitization [34–36] and physician-diagnosed asthma [34].

Immunologically, it is known that low-dose antigen exposure favors Th2 priming whereas high-dose priming leads to a Th1 type of response [3].

5 Endotoxin

Animal exposure in the home or at work may also result in high exposures to microbial and associated products including endotoxin. Conceivably this co-exposure may confer protection, manifesting as an attenuation of the immune response to allergen at high doses. Regulatory T cells selectively express toll-like receptors which are activated by endotoxin. Exposure of CD4+CD25+ cells to the Toll-like receptor-4 ligand lipopolysaccharide induces upregulation of several activation markers and enhances their survival/proliferation [37].

The host response to environmental endotoxin appears to depend in part on the time and dose of exposure. Low doses of endotoxin induce a Th2 response whereas high-level endotoxin favors a Th1 response. In an experimental model of allergy, exposure of rats to endotoxin during early sensitization protects against their development of ovalbumin-specific IgE; co-exposure of endotoxin with allergen results in dose-dependent inhibition of acute- and late-phase allergic inflammation and bronchial hyperresponsiveness; and exposure to endotoxin after allergen challenge further exacerbates the allergic response [38]. Adult pig farmers have an inverse relationship between endotoxin and sensitization to common allergens and the overall prevalence of atopic sensitization in this population is low [39].

In contrast, other studies suggest that endotoxin is not protective. Homes of cat owners did not have higher endotoxin levels than homes without cats [40, 41]. Similarly in a study of laboratory animal workers, there was no significant relation between rat-related symptoms and endotoxin levels [42]. These results argue that the effects of cat and rat ownership cannot be explained by increased exposure to endotoxin.

6 Type 2 Immune Response

Allergy is driven by a hyperactive type 2 immune response at barrier tissues, e.g., airways, gut, and skin, requiring antigen presentation and is characterized by high levels of IL-4, IL-5, IL-9, and IL-13 traditionally associated with adaptive CD4 T-helper (Th) 2

cells. Many cells are involved with orchestrating the type 2 immune response including CD4 T-helper Th2 cells, epithelial cells, dendritic cells, macrophages, eosinophils, mast cells, basophils, and the recently identified group 2 innate lymphoid cells (ILC2) [43]. ILC2s constitutively express IL-5 and promote eosinophil production; however further signals can promote IL-5 signaling and induce IL-9 and IL-13 production leading to the typical allergic pathology including stimulating mucus production, smooth muscle cell contraction, and promoting epithelial cell repair. The ILC2 signals remain largely undefined as well as the relative contribution of ILC2s and adaptive Th2 cells in allergic responses; however ILC2s and Th2 cells appear to respond to similar signals.

A recent study suggested that IgE represents an early immunological host defense against xenobiotics or large parasites that threaten tissue integrity [44]. IgE is an ancient and highly conserved immunoglobulin, and epithelial tissues such as skin, lungs, and gut have IgE-binding cells present which can rapidly mobilize its effector functions by the induction of type 2 immunity. In an animal model, IgE was rapidly induced by cutaneous exposure to a carcinogen and the IgE response required the presence of $\gamma\delta\text{TCR}^+$ intraepithelial lymphocytes, indicating that IgE contributed to early tissue immunosurveillance against environmental xenobiotics or toxins.

7 Microbiome

The human body is home to trillions of microbes that play a critical role in educating the immune system. This microbiota is a complex community of microorganisms that includes bacteria, archaea, fungi, viruses (including bacteriophages), and protozoa species. It is becoming increasingly clear that the commensal microbes that colonize our gut, lung, and skin mediate strong regulatory effects on Th2 immune responses through effects on type 2 cytokines, dendritic cells, and regulatory T cells. Some of the regulatory mechanisms of microbial colonization include production of short-chain fatty acids butyrate and propionate which inhibits lung dendritic cells from promoting type 2 immune responses leading to the protection from allergic airway inflammation. Other mechanisms in the lung suggest that the microbiota are critical in promoting tolerance to allergens through induction of programmed death ligand (PD-L1)-dependent iTreg. Furthermore, microbial-induced iTreg can also express the transcription factor retinoic acid receptor-related orphan nuclear receptor γ (ROR γ t) and iTreg co-expressing FoxP3 and ROR γ t are required to control type 2 immune responses [16]. These studies are clearly important in unraveling as they may well help us to prevent and/or treat type 2 immune responses.

8 Cellular Response to Allergens

The cellular response of T cells in peripheral blood is often present in both healthy and sensitized individuals [45]. The epitopes recognized within allergens is also found to be similar between healthy and allergic individuals [46–50]. The cytokine profile from the *in vitro* T-cell culture is predominantly Th2 in the allergic individual. Th2 cytokines IL-4 and IL-13 stimulate the production of IgE; IL-5 and IL-9 are involved in the development of eosinophils; IL-4 and IL-9 promote the development of mast cells; IL-9 and IL-13 promote airway hyperresponsiveness and IL-4, IL9, and IL-13 promote the overproduction of mucus [1].

9 Modified Th2 Response

High-dose attenuation of risk of sensitization with cat allergen exposure is associated with high-titer IgG and IgG4 antibodies [31]. This response was described as a modified Th2 response since both IgE and IgG₄ require Th2 cytokine IL-4 for their production. The modified Th2 response is suggestive of clinical tolerance since the shift from specific IgE to IgG₄ resulted in a decrease in both sensitization and asthma [51].

We have recently established a modified Th2 response in laboratory animal workers. Within our cohort and cross-sectional studies on laboratory animal allergy we observed increasing risks of sensitization and work-related symptoms with increasing exposures to rats, except at highest exposure level where risks of both outcomes were lower [52, 53]. We established a significantly increased ratio of IgG₄:IgE in those workers most heavily exposed [52]. There was an almost twofold reduction in those who produced both specific IgG₄ and IgE as compared to those producing specific IgE only. The attenuation at high exposures and the increased ratio of IgG₄:IgE in laboratory animal workers are suggestive of a natural form of immunotherapy.

The enteric mucosal immune system plays an extremely efficient and pivotal role in the development of tolerance. Repeated exposure to allergen through the gastrointestinal tract during life leads to the development of tolerance, even in highly atopic individuals [54]. It is proposed that exposure to aeroallergens through this route may promote the local (IgA) immune responses, which promote persistent systemic tolerance, preventing the emergence of pathogenic Th2-responsive memory T cells. The information regarding the role of IgA in mediating tolerance to allergens is at present very limited. Immunotherapy studies have shown increases in the levels of TGF- β following treatment [55]. TGF- β plays a suppressor role in mucosal allergens such as house dust mite or birch pollen and this cytokine is known to induce IgA, which works

as a noninflammatory immunoglobulin isotype [55]. Levels of IgA antibodies have been shown to be upregulated following venom immunotherapy [56].

10 Natural Killer Cells

Mouse models of allergic asthma indicate that natural killer T cells are required for the development of allergen-induced airway hyper-reactivity [57]. Natural killer T cells have a unique property of responding to glycolipid antigens, rather than peptide antigens presented by the nonpolymorphic class I MHC-like protein CD1d, expressed on antigen-presenting cells. Invariant natural killer T cells rapidly produce large quantities of both Th1 and Th2 cytokines, which enhance the function of dendritic cells, natural killer cells, and B cells, as well as the conventional CD4+ and CD8+ T cells [58]. The rapid production of cytokines by invariant natural killer T cells is a manifestation of innate-like immunity and provides invariant natural killer T cells with the capacity to link innate and adaptive immune responses and critically regulate adaptive immunity and a host of inflammatory diseases [58]. Recently it was observed that CD4+ invariant natural killer T cells are abundant in the lungs of patients with chronic asthma but are virtually absent from the lungs of controls and patients with sarcoidosis [58]. These natural killer cells express an invariant T-cell receptor that recognizes glycolipid antigens, which appear to be highly conserved in both man and mice. It will be important to identify glycolipid allergens from our major allergens to determine their role in both allergy and asthma.

11 Immunoregulation

The peripheral immune response has evolved several mechanisms to maintain a state of tolerance to innocuous antigens, which involves clonal deletion, anergy, and active suppression mediated by regulatory cells. Several subsets of regulatory T cells (Tr) with distinct phenotypes and distinct mechanisms of action have now been identified [59]. Recent studies suggest that naturally occurring T-regulatory cells and antigen-induced IL-10-producing T-regulatory cells have a physiological role in protecting against human allergic disease [58]. A rare mutation in the gene encoding FOXP3 results in a disease called immune dysregulation, polyendocrinopathy, and enteropathy, X-linked (IPEX). Individuals with IPEX syndrome suffer from a range of autoimmune endocrine pathologies as well as allergic symptoms including severe eczema, increased serum IgE levels, eosinophilia, and food allergies [60]. Within allergy, there is evidence that CD4+CD25+ T cells producing IL-10 are important in regulating a nonallergic response.

Healthy beekeepers show a substantial increase in IL-10-producing CD4+CD25+ T cells and monocytes, suggesting that these regulatory T cells induce tolerance to bee stings [61].

Recent interest has focused on the role of T-regulatory cells in allergic disease and immunotherapy. Seasonal atopic patients were found to have a diminished suppressive effect of T-regulatory cells compared with nonatopic controls and this was more pronounced during the hay fever season [62]. The degree of allergen exposure is likely to alter the suppressive effect of T-regulatory cells in allergic patients. At high grass pollen exposure, regulatory cells from nonatopic donors retained the ability to inhibit both proliferation and cytokine production while allergic donors failed to inhibit proliferation. At high allergen doses of wasp venom, T-regulatory cells from both allergic and healthy controls lose their regulatory function, whereas nonallergic donors retained their regulatory function at low allergen doses [63]. A study investigating regulatory differences before and after cat peptide immunotherapy demonstrated that antigen-specific proliferative responses of memory T cells were reduced after peptide immunotherapy compared with preimmunotherapy samples [64]. The CD4 cells isolated after peptide immunotherapy suppressed the proliferative response of baseline CD4-negative cells in the co-culture experiments, suggesting that peptide immunotherapy induces a population of CD4 T cells with suppressive/regulatory activity.

Grass pollen immunotherapy is associated with an increase in T-regulatory cells with IL-10+CD4+CD25+ phenotype [65]. Similarly bee venom immunotherapy demonstrated a significant increase in levels of IL-10 after therapy [66]. In a study of cat peptide immunotherapy they were able to demonstrate reduced antigen-specific proliferative responses in memory T cells following treatment. IL-10 is a potent suppressor of both total and allergen-specific IgE, whereas it simultaneously increases IgG4 production [67]. Specific IL-10 secreting T reg cells consistently represent the dominant subsets against common environmental allergens in healthy individuals, in contrast to the high frequency of allergen-specific IL-4 secreting T cells in allergic individuals [68].

In a recent pilot study, in which we investigated the role of CD4+CD25+ T cells in laboratory animal allergy, CD4+CD25- cells proliferated to a significantly greater degree than either PBMCs ($p < 0.01$) or CD25+ cells ($p < 0.001$) in all individuals (Hayley Jeal personal communication). Inhibition of proliferation to rat by CD4+CD25+ T cells did not differ between cases and controls. IL-5 suppression was increased in controls than cases although not significantly. Results from this pilot study indicate that the ability of CD4+CD25+ T regulatory cells to suppress proliferation in rat sensitized individuals compared with controls does not differ, but failure of these cells to suppress IL-5 production in cases could contribute to the development of their allergic disease.

12 Genetics

HLA class II antigens are involved in the presentation of epitopes of foreign proteins at the antigen-presenting cell surface and are important in the development of a hypersensitivity response. Epitope mapping of the major cat allergen Fel d 1 has revealed several T-cell epitopes [69]. One of these T-cell epitopes located in the carboxy-terminus of chain 2 of Fel d is associated with HLA-DRB1*0701 with preferential IL-10 induction. Production of IL-10 was enhanced in PBMC from DR-7-positive modified responders compared to their DR-7-negative counterparts. Expression of HLA-DR7, coupled with high-dose exposure to cat allergen, could favor optimal induction of T-regulatory cells in parallel with Th1 cells. When this occurs during the initiation phase of the immune response, it may result in imprinting of the regulatory mechanism necessary to prevent expansion of Th2 cells. A second immunodominant region within the chain, induced IL-5 and a strong T-cell proliferation, in allergic and modified Th2 responders but not in control subjects [69]. Thus the ratio between TR1 and Th2 cells may determine the development of a healthy or allergic immune response. The generation of allergen-specific T reg cells and increased production of suppressive cytokines IL-10 and TGF- β are essentially early events in allergen-specific immunotherapy [67, 68].

Several studies have demonstrated HLA associations with low-molecular-weight chemical sensitizers [70–73]. It is likely to be more difficult to establish a HLA association with larger allergen as multiple T-cell epitopes are likely to be presented by different HLA molecules, diluting any single-HLA association. We have established in laboratory animal allergy that HLA-DR7 was associated with sensitization to rat allergen and respiratory symptoms at work whereas HLA-DR3 was protective against sensitization [74]. The major rat allergen, Rat n 1, is a relatively low-molecular-weight protein with just five identical T-cell epitopes.

13 Intradermal Exposure

The high-dose attenuation of risk of sensitization and symptoms demonstrated with laboratory animal allergens is similar to both natural tolerance seen with beekeepers that have been frequently stung by bees and the induced tolerance by subcutaneous injection of natural allergen in immunotherapy. One common theme with bee keepers, laboratory animal workers, and cat owners, who develop tolerance at high exposures, is that they are at high risk of being bitten and scratched. We suggest that the intradermal exposure to the allergens is responsible for the induced tolerance. In

support of our hypothesis is the observation that repeated inhalation of cat peptides as an experimental method of immunotherapy was not—in contrast to an intradermal method—associated with the induction of hyporesponsiveness (“tolerance”) in the skin or the lung. Other groups of workers at risk of developing occupational asthma, for example bakers and detergent workers, do not develop attenuation at high exposure—these workers are less likely to have an intradermal exposure.

14 Immunotherapy

Specific allergen injection immunotherapy is an effective treatment for IgE-mediated allergic diseases, which may confer long-term benefit for at least 3 years after discontinuation [75]. Immunotherapy is generally administered by either subcutaneous injection or sublingual administration of allergen.

The mechanisms associated with immunotherapy are thought to involve both humoral and cellular responses. Following immunotherapy there is a modest reduction in allergen-specific IgE and increases in allergen-specific IgG antibodies, particularly the IgG4 subclass. Effector cells are reduced at the site of allergic inflammation, including a reduction in the numbers of mast cells, eosinophils, and basophils and the release in mediator secretion. There is thought to be an immune deviation toward a Th1-type response or the induction of T-regulatory cells producing IL-10 and transforming growth factor beta.

More recently regulatory B cells (Breg) are thought to be important in maintaining and restoring tolerance to allergens, particularly the Breg subset CD73⁻ CD24⁺CD71⁻ BR1 cells [76]. In those patients receiving immunotherapy with bee venom, the allergen-specific B cells increased two- to fivefold, reaching a level comparable to healthy beekeepers during the season. In parallel with IL-10, allergen-specific B cells also upregulated C-C chemokine receptor (CCR)5, a receptor for macrophage inflammatory protein 1a and b. Interestingly, there is a link between IL-10-producing BR1 cells and IgG4 production suggesting that BR1 cells may promote tolerance through IL-10-mediated suppressive effects and through directing the humoral response toward IgG₄.

Ratios of IgE to IgG4 have been found to change during specific immunotherapy [61]. Specific serum levels of both IgE and IgG4 increase during the early phase of therapy, but the increase in specific IgG4 is more pronounced and the ratio of specific IgG4:IgE is increased 10- to 100-fold, suggesting a protective role for IgG4 [61]. IL-10 is a potent suppressor of both total and allergen-specific IgE, whereas it simultaneously increases IgG4 production [67].

Successful immunotherapy is associated with increases in allergen-specific IgG₁ and IgG₄ antibody concentrations occur that correlate with the clinical outcome [77–80]. It is not known whether allergen-specific IgG or IgG₄ antibodies associated with the modified Th2 response exhibit functional activity. It is possible that IgG antibodies may play a protective role by blocking leukocyte histamine release, inhibiting signal transduction and mediator release through the high-affinity IgE receptor (FcεR1) and IgG (FcγRIIB) receptors [81–84] and blocking allergen-induced IgE-dependent histamine release by basophils [85]. IgG antibodies may exert their effect by competitive inhibition of allergen-IgE complexes, which prevents complexes binding to the low-affinity IgE receptor, CD23, and subsequent antigen presentation. Others have suggested that not only quantitative changes occur with the IgG antibody but also the spectrum of the specificity of the IgG is altered [86]. One grass pollen immunotherapy study demonstrated a blunting of seasonal increases in serum allergen-specific IgG and IgG₄. Further examination showed the postimmunotherapy serum to exhibit inhibitory activity, which co-eluted with IgG₄ and blocked IgE-facilitated binding of allergen-IgE complexes to B cells. Increases in IgG and IgG blocking activity correlated with the patients' overall assessment of improvement [79].

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