

Mechanisms of immunotherapy: IgG revisited

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Purpose of review

This paper will review historical and recent evidence for the induction of 'blocking' IgG antibodies during successful specific immunotherapy.

Recent findings

Specific immunotherapy is frequently associated with a rise in allergen-specific IgG4 antibodies and a modest reduction in specific IgE titres, although this does not always correlate with clinical efficacy. There is accumulating evidence that specific immunotherapy also influences the blocking activity on IgE-mediated responses by IgG4, and cellular assays are commonly used to investigate these changes. Recently, a novel assay, which detects allergen-IgE binding using flow cytometry, has been used to detect 'functional' specific immunotherapy-induced changes in IgG antibody activity. Results suggest that successful specific immunotherapy is associated with an increase in IgG blocking activity that is not solely dependent on the quantity of IgG antibodies.

Summary

Successful immunotherapy is associated with quantitative and qualitative changes in the allergen-specific IgG antibody response. The induction of IgG antibodies with blocking activity may have a protective role not only through the inhibition of allergen-induced, IgE-mediated release of inflammatory mediators from mast cells and basophils, but also through the inhibition of IgE-facilitated antigen presentation to T cells. Qualitative changes in the allergen-specific IgG antibody response may possibly be an important mechanism underlying the clinical efficacy of specific immunotherapy. Monitoring changes in blocking activity using cellular assays may give an early indication of the potential success of treatment.

Keywords

antibody affinity, antibody specificity, IgE-facilitated allergen presentation, IgG blocking antibodies, specific immunotherapy

Introduction

In view of the high prevalence of allergic disease in the western world, the development of effective therapeutic approaches has become increasingly important. The majority of available treatments focus on the non-allergen-specific alleviation of symptoms, rather than treating the underlying cause. So far, the only allergen-specific treatment that has the potential for a long-term 'curative' approach is specific allergen immunotherapy. Whereas the clinical efficacy of specific immunotherapy (SIT) is well documented, its molecular mechanisms are incompletely understood. The identification and ultimately the monitoring of SIT-induced immunological changes, which precede the improvement of clinical symptoms and indicate successful treatment, is an important goal. The induction of allergen-specific IgG antibodies, particularly of the IgG4 subclass, is the most prominent immunological finding after SIT [1–3]. However, a correlation between increases in IgG4 titres and clinical improvement has not always been found [4,5]. One explanation for this is provided by increasing evidence that suggests that the biological activity as well as the quantity of IgG4 antibodies is altered during successful SIT [6–8]. SIT-induced IgG4 antibodies thus have IgE blocking activities that are not necessarily reflected in the antibody titre but require biological assays for detection [6,9–12]. These blocking antibodies can inhibit the IgE-mediated release of inflammatory mediators from mast cells and basophils and thus prevent immediate symptoms [6,9]. In addition, they have protective activity on the development of the late-phase response through the inhibition of IgE-facilitated allergen presentation to T cells [10–12]. Finally, allergen-specific IgG antibodies may also directly affect IgE production by memory B cells, either through competition with IgE for allergen binding or through the crosslinking of inhibitory IgG receptors on the surface [13,14]. We recently developed a novel flow cytometry assay, which detects IgG blocking of IgE-facilitated allergen binding to B cells [11]. Using this assay, it has been shown that the increase in allergen-specific IgG4 antibodies after SIT with grass pollen allergen was accompanied by an inhibitory activity on allergen-IgE binding [15]. This suggests that IgG blocking antibodies are at least part of the underlying mechanism of successful SIT, and that the measurement of their biological blocking activity could be a potential immunological marker for successful SIT.

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Abbreviation

SIT specific immunotherapy

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Immunotherapy and modulation of the allergen-specific antibody response

Studies of the effect of immunotherapy on different aspects of the allergic inflammatory response have demonstrated an increase in allergen-specific IgG, particularly of the IgG4 subclass. Also, the blunting of seasonal increases in allergen-specific IgE have been observed with a gradual, modest reduction in specific IgE after several years' treatment. Other known effects of immunotherapy include reduced basophil reactivity to allergen, reduced allergen-stimulated T-cell proliferation and T helper type 2 cytokine production, the generation of allergen-specific regulatory T cells, and a reduction in tissue eosinophils [1,2]. However, so far no reliable correlation could be established between any of these parameters and the clinical outcome.

The kinetics of the induction of IgG and IgG4 immunoglobulin subclasses during SIT has been studied with the aim of using this as an immunological marker to predict therapeutic success. Studies of bee venom SIT suggested that the quantity and kinetics of allergen-specific IgG antibodies induced in response to the treatment could predict the risk of systemic allergic reactions [16]. In particular, the production of low levels of venom-specific IgG early in the maintenance phase of low-dose venom SIT was associated with a significant risk of systemic allergic reaction to sting challenge, whereas higher IgG levels were associated with successful treatment. Furthermore, it has been suggested that naturally occurring immunity is associated with the development of a specific IgG subclass. For example, immunity to bee venom in beekeepers that develops after frequent bee stings is associated with the preferential induction of IgG4 antibodies, whereas individuals who have been stung only a few times predominantly developed an IgG1 response [17]. These findings suggest that IgG4 antibodies become prominent upon chronic antigenic stimulation, whereas IgG1 antibodies are produced after limited antigenic stimulation. Protection from allergy by the preferential development of specific IgG antibodies has recently been the subject of much attention. Platts-Mills and colleagues [18–20] have suggested that the development of IgG and IgG4 antibodies to cat allergen in the absence of IgE may be associated with the absence of symptoms. This 'modified Th2 response' (so called because both IgG4 and IgE are dependent on the T helper type 2 cytokine IL-4), could be regarded as a form of immunological tolerance.

Although the efficacy of SIT seems to be partly correlated to the increased synthesis in IgG antibodies, a clear correlation between IgG4 antibody titres and clinical efficacy has not always been found [1,2]. In a retrospective study of six SIT studies based on reported

clinical outcome and IgG4 antibody responses, only two found an association between IgG4 and clinical outcome, whereas four did not find any such correlation [21]. There is thus increasing evidence that a simple quantitative measurement of the antibody response is not a suitable predictor for successful SIT.

Immunotherapy-induced changes in IgG specificity

It has been reported that prolonged exposure to high allergen concentrations during SIT may increase the affinity of IgG through affinity maturation [22]. An increase in IgG affinity during SIT would result in enhanced competition with IgE for allergen binding and potentially block IgE-mediated mechanisms. By generating five B cell lines from a birch pollen SIT-treated donor that produced high-affinity monoclonal IgG antibodies to the major birch pollen allergen Bet v 1, Visco *et al.* [23] identified one antibody (BAB1) that completely inhibited allergen–IgE binding from allergic patients, as well as IgE-mediated histamine release by basophils. Surprisingly, another antibody (BAB2) actually enhanced allergen–IgE binding. As all antibodies exhibited similar affinities, this suggests that IgE blocking activity cannot be explained solely by an increase in affinity. Interestingly, molecular analysis of the antibodies revealed that they all recognized different epitopes on the Bet v 1 molecule, which were distinct from the IgE epitopes [24].

Similarly, Michils *et al.* [7] reported that an increase in specific IgG during venom SIT was associated with a rapid change in antibody specificity. Interestingly, the allergen binding of pooled IgG fractions from allergic individuals was inhibited to a greater extent by sera from other allergic patients than by sera from SIT patients, despite higher antibody titres in the latter. This suggests that IgG antibodies induced in response to SIT do not compete for the same epitopes but have rather developed distinct epitope specificity.

The preferential expression of a dominant IgG response with distinct specificity has also been linked to allergic status or immunoprotection. For example, it has been reported that IgG antibodies from atopic individuals are directed towards different epitopes on the allergen compared with those derived from normal non-atopic individuals [25,26]. Duchateau *et al.* [25] compared the epitope specificity of IgG antibodies directed towards the house dust mite allergen Der p 1 in mite-allergic individuals and healthy subjects. The authors reported a significant increase in a specific IgG fraction in mite-allergic patients with different epitope specificities compared with healthy individuals. A similar finding has also been reported in grass pollen-allergic individuals, when it was shown that allergic and clinically

unreactive states towards inhaled allergens were associated with different patterns of IgG antibody specificity and were unrelated to subclass expression [26]. Collectively, these data suggest that SIT-induced changes in antibody specificity are as likely to play a role in predicting and mediating clinical efficacy, as are changes in antibody titres.

IgG as blocking antibodies

A potential change in antibody specificity during SIT is an important factor because it may influence the biological effects of the antibodies, and would also explain why a positive correlation between the quantity of IgG and the level of protection through SIT is not always found. The biological effects of allergen-specific IgG antibodies, particularly of the IgG4 isotype, as a 'blocking antibody' in immunotherapy and protection from atopic disease in general has been the subject of much attention. Cooke *et al.* [27] were the first to demonstrate that heat-stable, non-reaginic antibodies were able to block allergic serum-mediated skin reactions when incubated with the allergen extract before injection into the skin. Subsequently, Loveless [28] showed that these inhibitory antibodies bound to the same allergen as sensitizing antibodies. The author also demonstrated a connection between the amount of these thermostable blocking antibodies [29], later identified by Lichtenstein *et al.* [30], as antibodies of the IgG isotype, and the degree of clinical protection. According to a number of studies, potentially blocking antibodies are found mainly in the IgG4 subclass, although IgG1 might also be blocking [31]. Interestingly, it has been demonstrated that not only IgG4 and IgG1 but also IgA2 antibodies have blocking activity, which might be important in allergy, considering that IgA antibodies are abundant at mucosal surfaces [32].

One way in which IgG may block IgE-mediated mechanisms is by inhibiting the release of inflammatory mediators from mast cells and basophils either by direct competition with IgE for allergen binding or possibly by the binding of IgE-allergen-IgG complexes to the low-affinity IgG receptor FcγRIIb with resulting co-aggregation with the high affinity IgE receptor (FcεRI) and the consequent inhibition of IgE receptor triggering [13,14,33].

IgG as blocking antibodies of IgE-facilitated antigen presentation

In addition to their role in mediator release, IgE antibodies are involved in facilitating allergen presentation to T cells. IgE bound to the high-affinity IgE receptor (FcεRI) or the low-affinity IgE receptor (FcγRII, CD23) on antigen-presenting cells can efficiently capture and focus the antigen, such that very low

concentrations of antigen are able to stimulate responding T cells maximally [34,35,36*,37]. After binding of antigen the complex is internalized, processed and then presented on the surface in association with the major histocompatibility complex II (MHCII) for presentation to T cells [38].

IgE-facilitated antigen presentation via FcεRII has recently been used in a cellular assay to investigate the induction of blocking IgG antibodies during SIT. Van Neerven *et al.* [10] showed that IgE antibodies from birch pollen allergic patients facilitated the presentation of Bet v 1, the major birch pollen allergen, to allergen-specific T-cell clones by a factor of more than 100. Moreover, blocking antibodies induced by birch pollen SIT inhibited IgE-facilitated antigen presentation, resulting in decreased T-cell proliferation as well as the reduced production of cytokines by Bet v 1 specific T cells. Reduced T-cell proliferation and cytokine production are often accompanied by successful SIT, as is a reduction in allergen-induced late-phase responses. The fact that blocking antibodies are involved in these changes could provide a valuable clue to the underlying molecular mechanisms of the treatment. We have recently established a simplified version of this cellular assay, which measures IgE-allergen binding to FcεRII on B cells using flow cytometry [11]. This allergen-IgE binding was associated with enhanced stimulation of allergen-specific T-cell clones, suggesting that the binding of complexes translates functionally into enhanced antigen processing and presentation. Using this assay we were able to show that sera collected after a double-blind placebo-controlled trial of grass pollen immunotherapy were able to block allergen-IgE binding, in contrast to sera collected at the start of treatment or from placebo-treated patients. We confirmed in a recent study that this inhibition was caused by allergen-specific IgG antibodies of the IgG4 subclass, because serum levels of these antibodies increased after treatment, and the inhibitory activity could be co-purified with IgG4 [15]. Moreover, the increase in blocking IgG4 antibodies was associated with IL-10, a cytokine that favours B cells switching to IgG4 in the presence of IL-4 [39]. The increase in blocking IgG4 antibodies during SIT was thus associated with an increase in IL-10 messenger RNA-expressing cells in the nasal mucosa, and the induction of IL-10-producing allergen-specific cells in the peripheral blood. We observed a trend for those patients with the lowest overall assessment scores after SIT to show a lower inhibition of IgE-allergen binding to B cells. Overall, the results raised the possibility that parallel increases in IL-10 and IgG4 antibodies with 'functional' blocking activity may possibly be predictive or surrogate of the clinical response to immunotherapy.

It could be argued that increases in blocking activity in sera from immunotherapy-treated patients is simply parallel to the associated increase in immunoreactive serum IgG4 antibodies. However, although the increase in IgG4 antibodies paralleled the blocking activity, a uniform correlation between these two parameters could not be found in our studies. This supports previous findings suggesting that the inhibition of IgE-mediated mechanisms cannot solely be explained by increased serum levels of IgG4 *per se*, but are more likely the result of changes in specificity or affinity. These observations stress the importance of studying the functional activity of allergen-specific IgG, as a blocking antibody or otherwise, as opposed to measuring total antibody levels in sera.

Although blocking activity has been mainly attributed to allergen-specific IgG4 antibodies, autoantibodies to IgE could also play a role. For example, anti-idiotypic antibodies of the IgG4 and IgG1 isotype are produced during any immune response are directed against the antigen-binding site of the first antibody produced in response to allergen exposure, and have been shown to inhibit the antigen-specific human IgE response *in vitro* [40,41]. Therefore, the inhibition of allergen–IgE binding as detected by our assay could be caused by at least two different mechanisms. The first is a truly allergen-specific blocking mechanism, in that allergen-specific non-IgE antibodies compete with IgE for binding of the allergen, thus decreasing the amount of allergen–IgE complexes detectable on the surface of B cells. The other is non-allergen specific and mediated via autoanti-IgE antibodies. Unfortunately, little is known about the contribution of autoantibodies on blocking IgE-mediated mechanisms.

Nevertheless, there is conclusive evidence that IgG4 is the main antibody involved in the inhibition of allergen–IgE binding to B cells and IgE-facilitated allergen presentation after immunotherapy, whether this is truly allergen specific or mediated by autoantibodies [10,12,15]. In summary, the inhibition of allergen–IgE binding to B cells by SIT sera is mediated via IgG4 antibodies, some of which might be autoantibodies directed against allergen-specific IgE, whereas the majority will be allergen specific.

New approaches to immunotherapy: induction of blocking antibodies using recombinant allergens

Although the administration of increasing doses of allergen during immunotherapy has been shown to reduce the majority of allergic symptoms, the treatment also carries the risk of inducing systemic reactions, caused by an increase in IgE antibodies during the induction phase. Because of the severity or frequency of

systemic reactions, some patients cannot receive sufficient amounts of allergen to achieve protection. Therefore, several attempts have been made to bypass or block IgE recognition of the antigen in order to reduce the side-effects associated with conventional immunotherapy.

With the aid of recombinant techniques, new allergens have been generated that display decreased allergenicity while retaining their immunogenicity. The principal approach of allergen modification is to modify B-cell epitopes in order to prevent IgE binding and effector cell cross-linking while preserving T-cell epitopes. Modification of the secondary allergen structure has been successfully used to reduce IgE binding of Der p 2, the major house dust mite allergen, while retaining T-cell reactivity [42]. Similar results have been obtained by using synthetic recombinant hypoallergenic variants of the major grass pollen allergen, Phl p 1, and the major birch pollen allergen Bet v 1 [43,44]. The Bet v 1 hypoallergenic variant also showed increased immunogenicity in animals *in vivo* and increased the induction of T helper type 1 cytokines by human peripheral blood T cells *in vitro* [44]. Similarly, in venom allergy, the cloning and expression of recombinant venom allergens from *Hymenoptera* has improved diagnosis and opened new treatment options [45]. For example, recombinant venom allergens can be used to characterize patients' individual sensitization patterns and provide the basis for tailor-made immunotherapy treatments [46,47].

In addition to introducing conformational changes to the B-cell epitopes of allergens in order to reduce allergenicity, another approach is to reduce the density of the B-cell epitopes. Recently, King *et al.* [48] presented a method in which small portions of an allergen are merged with a homologous but poorly cross-reacting host protein. By creating hybrids of 10–49 residues of Ves v 5, a major allergen from yellow jacket wasp, and Pol a 5, a paper wasp protein of similar size with low antigenic cross-reactivity as the host protein, the investigators could reduce allergenicity by 100–3000-fold as shown in a histamine release assay with basophils from yellow jacket-sensitive patients.

Recombinant allergens have also been shown to be very efficient in inducing blocking IgG antibodies when administered *in vivo*. Linhart *et al.* [49] used genetically engineered hybrid molecules of the major timothy grass pollen allergens for immunization in mice. The investigators were able to show that immunization with the recombinant hybrid molecules induced stronger and earlier IgG responses than immunization with individual allergens or allergen extract. Moreover, these IgG antibodies were able to inhibit the binding of allergic patients' IgE to grass pollen allergen [49]. Recently, the

generation of hybrid antibodies by grafting the variable portions of murine IgE with specificity for the major timothy grass pollen allergen, Phl p 2, onto a complete human IgG1 backbone resulted in the inhibition of IgE binding to Phl p 2, as well as allergen-induced histamine release from basophils [50].

Conclusion

Identification of immunological parameters that manifest themselves early during SIT, even before clinical changes become apparent, would be an important tool for predicting the success of the treatment. Although the induction of allergen-specific IgG antibodies is usually associated with successful SIT, evidence suggests that their biological effect on allergen/IgE-mediated mechanisms is not necessarily associated with their absolute quantities in serum. Measuring antibody titres during SIT with the aim of correlating them with clinical outcome may simply be too crude a measure. An alternative approach is to assess functional changes in IgG antibodies, in terms of their 'blocking' activity, specificity, or avidity. A simple flow cytometry-based assay capable of detecting IgE blocking activity of IgG antibodies induced during the course of SIT may be such a tool, although more detailed studies in large numbers of patients and during both the up dosing and withdrawal phases of immunotherapy are needed to test this hypothesis. In addition, identifying and choosing allergen epitopes with the selective capacity of inducing blocking activity in the absence of IgE binding is an important goal in the development of novel approaches to SIT.

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