

Renaissance of the Blocking Antibody Concept in Type I Allergy

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Key Words

Allergy · IgE · Blocking antibodies · IgG · Immunotherapy

Abstract

Formation of IgE antibodies against per se harmless antigens (i.e. allergens) is the hallmark and key pathomechanism of type I allergy, a hypersensitivity disease affecting more than 25% of the population. Classical experiments performed more than 65 years ago demonstrated that allergen-specific IgG antibodies, termed blocking antibodies, can antagonize the cascade of allergic inflammation resulting from allergen recognition by IgE antibodies. However, controversial results have questioned the protective role of IgG antibodies in allergic diseases. Here, we review recent data demonstrating that blocking antibodies inhibit allergen-induced release of inflammatory mediators from basophils and mast cells as well as IgE-facilitated allergen presentation to T cells, thus leading to suppression of T cell activation. Furthermore, it has been reported that the development of blocking antibodies is associated with reduced boosts of allergen-specific IgE production in patients receiving allergen-specific immunotherapy. These findings suggest that blocking antibodies have protective activity by inhibiting immediate as well as

late inflammatory responses and long-term ameliorating activity on the allergic immune response by antagonizing the underlying IgE production. Induction of blocking antibodies is thus an important mechanism underlying allergen-specific immunotherapy. In addition, passive administration of blocking antibodies may be considered as a potential therapeutic strategy for allergic diseases.

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IgE Recognition of Allergens: The Key Pathomechanism in Type I Allergy

Type I allergy is caused by the recognition of allergens by specific IgE antibodies leading to a cascade of cellular inflammatory responses [1, 2]. Crosslinking of mast cell- or basophil-bound IgE by allergens induces the release of mediators (e.g. histamine, leukotrienes) and thus an immediate inflammatory response [3–5]. In addition, IgE-facilitated antigen presentation leads to a strong activation of allergen-specific T cells and may contribute to late and chronic inflammatory reactions [6–9].

A significant contribution to the understanding of type I allergy in allergic patients came as early as 1921 from the experiments performed by Prausnitz and Küstner [10]

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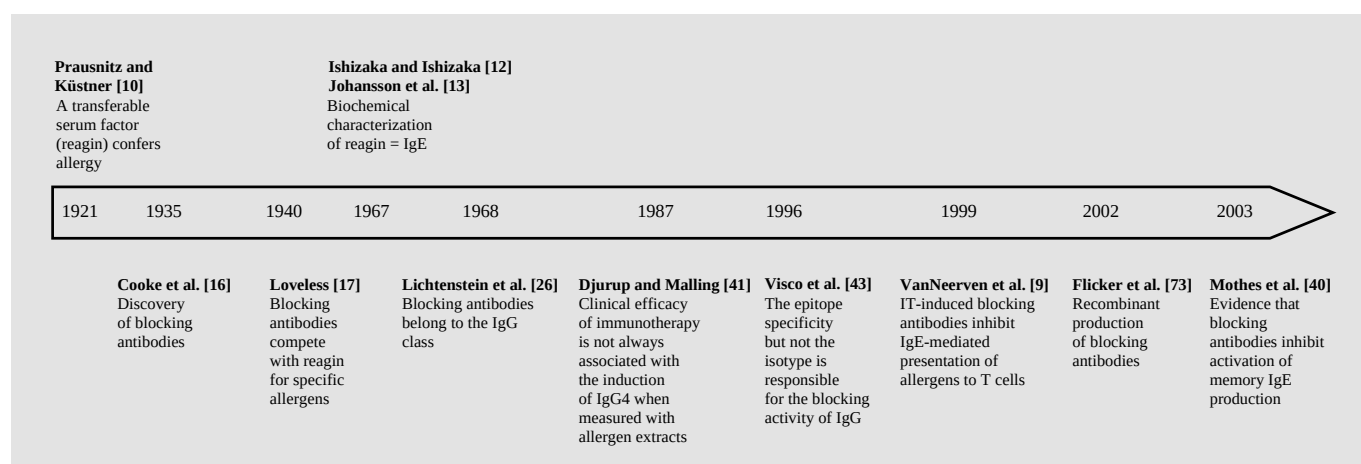


Fig. 1. Timeline: the role of antibodies in type I allergy. IT = Immunotherapy.

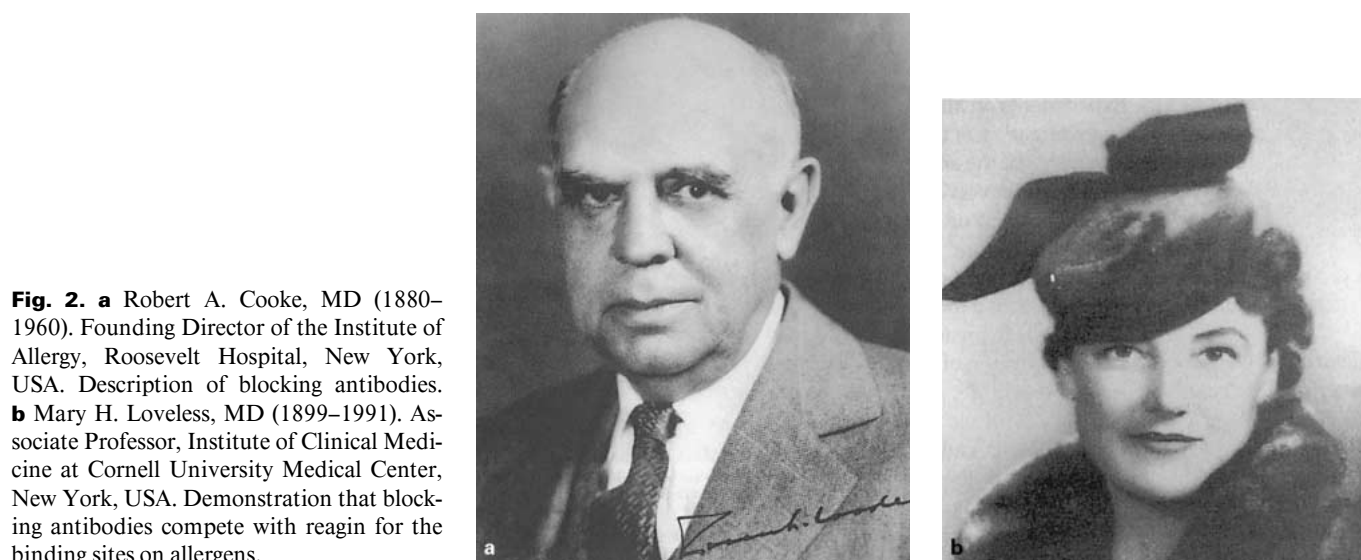


Fig. 2. **a** Robert A. Cooke, MD (1880–1960). Founding Director of the Institute of Allergy, Roosevelt Hospital, New York, USA. Description of blocking antibodies. **b** Mary H. Loveless, MD (1899–1991). Associate Professor, Institute of Clinical Medicine at Cornell University Medical Center, New York, USA. Demonstration that blocking antibodies compete with reagin for the binding sites on allergens.

(fig. 1). They transferred serum from a fish-allergic individual into the skin of a nonallergic person and observed a typical weal and erythema reaction at this site after administration of fish extract. This pivotal experiment clearly demonstrated the requirement of an allergen-specific serum component for the induction of an allergic reaction [10]. This serum factor was initially termed ‘reagin’ [11]. However, the extremely low concentration of reagin in human serum prevented its biochemical characterization for almost 50 years. The work of Ishizaka and Ishizaka [12] and of Johansson et al. [13] finally identified reagin as a novel class of immunoglobulin (fig. 1), which

was named IgE by the WHO during a conference in Lausanne in 1968 [14].

The subsequently developed radioallergosorbent test (RAST) allowed the measurement of allergen-specific IgE antibodies and thus the unambiguous diagnosis of allergy [15].

Already long before the characterization of IgE antibodies, Robert A. Cooke (fig. 1, 2a) et al. [16] demonstrated that allergic patients who were treated by allergen-specific immunotherapy (i.e. vaccination with allergen extracts) developed antibodies which blocked allergen-induced inflammation.

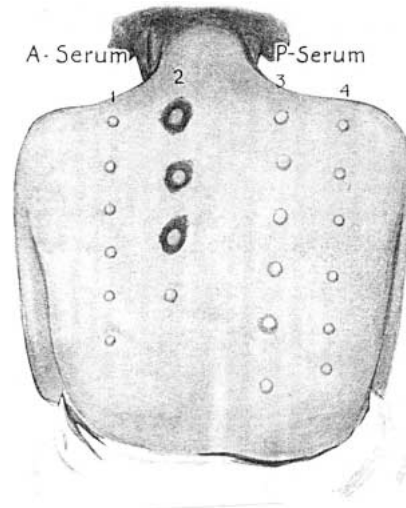


Fig. 3. Classical experiments by Cooke et al. [16] demonstrating that allergen-specific immunotherapy induces protective antibodies. Serum from a ragweed-allergic patient was obtained before (A-serum: ante-treatment serum) and after (P-serum: post-treatment serum) specific immunotherapy with ragweed pollen extract. A- and P-sera were preincubated with equal volumes of ragweed pollen extracts of different potency (50–1,000 units) and injected into the skin of a non-allergic individual (columns 2 and 3). Different dilutions of the sera (1:10–1:500; columns 1 and 4) or saline without addition of allergens (saline, control) were also injected intradermally. Results were recorded 1 h later.

A-serum		Ragweed pollen extract units		P-serum		Ragweed pollen extract units	
1	2			3	4		
1:10	○	○	50	150	○	○	1:10
1:100	○	○	100	300	○	○	1:100
1:200	○	○	150	500	○	○	1:200
1:300	○	○	Saline control	700	○	○	1:300
1:400	○			1000	○	○	1:400
1:500	○			Saline control	○	○	1:500

Discovery and Characterization of Blocking Antibodies

The studies by Cooke et al. [16] were performed to investigate the protective mechanisms underlying allergen-specific immunotherapy. Serum samples were obtained from ragweed-allergic patients before and after successful immunotherapy. These serum samples were preincubated with ragweed pollen extract and then injected into the skin of a nonallergic person. It turned out that the mixture of allergens with serum from untreated allergic patients induced strong immediate skin reactions, whereas the mixture of allergens with serum after successful treatment gave no skin reaction (fig. 3). In their classical paper, Cooke et al. [16] concluded: ‘The results have indicated to us the production of an inhibiting or immune

type of substance that prevented the allergen from reacting with the sensitized cell ...’.

A few years later, Mary H. Loveless [17] (fig. 1, 2b) found that the inhibiting antibodies bound to the very same allergen and thus prevented the allergen from reacting with sensitizing antibodies. She was able to distinguish sensitizing antibodies (i.e. IgE) from blocking antibodies by their thermostability. Heating of serum to 56°C destroyed sensitizing antibodies, whereas the blocking antibodies remained stable even when they were exposed to a temperature of 56°C for up to 24 h [17]. It was also Loveless [18] who was able to demonstrate a connection between the amount of blocking antibodies and the degree of clinical insusceptibility through detailed records of symptoms of 52 ragweed-allergic patients.

To increase the induction of IgG antibodies during immunotherapy, Loveless started to use adjuvant-bound allergen extracts for specific immunotherapy and hence pioneered the development of specific immunotherapy towards a vaccination concept. In initial studies, she used a depot medium that Freund and McDermott [19] had developed as an immunogen adjuvant in experimental animal models to administer a single large dose of pollen vaccine emulsified in oil. However, a declaration of the US Food and Drug Administration did not approve the emulsion for therapy because reports indicated that mineral oil and mineral oil adjuvants induced plasma cell myelomas in a certain strain of mice [20]. The earlier developed aluminium hydroxide-adsorbed allergen extracts [21] are, however, still in routine use today, although it is known that aluminium hydroxide-adsorbed antigen preparations also promote the induction of IgE responses followed by a large increase in IgG antibodies [22]. On the other hand, it is well established that adjuvant-bound allergens induce IgG responses more potently and cause less anaphylactic side effects than aqueous extracts [23–25].

In 1968, Lichtenstein et al. [26] analyzed sera from ragweed-allergic patients who had received immunotherapy and identified an anti-IgG-reactive serum fraction as the one containing blocking antibodies. Even more importantly, Lichtenstein et al. [26] were able to demonstrate that preincubation of the major ragweed allergen, ragweed antigen E, with the IgG fraction inhibited histamine release from the basophils of ragweed-allergic patients (fig. 1). Furthermore, Lichtenstein et al. [27] revealed for the first time a significant correlation between a rise in the IgG blocking antibody level and a decrease in specific IgE antibody levels measured over a 2-year course of immunotherapy. Investigations performed by Tada and Okumara [28] in a rat model indicated that blocking antibodies raised through immunotherapy IgE antibody synthesis may be able to decrease IgE production due to a kind of feedback inhibition.

Although hampered by the lack of pure allergens for the investigation of blocking antibodies, attempts were made to measure and quantify blocking antibody responses. Due to the fact that the assessment of blocking antibody reactivity by the inhibition of allergen-induced basophil histamine release represented a rather complicated and cumbersome technique, IgG radioimmunoassays were introduced to monitor the effects of immunotherapy [29]. These assays were further developed to determine the ratios of IgG to IgE antibody rather than the absolute amounts of IgG [30] and to measure by

RAST interference tests whether IgG competed with IgE antibodies in the same system [31]. Using such tests, Gleich et al. [32] demonstrated the effects of immunotherapy through a 6-year prospective study in nearly 50 adults with ragweed hay fever. They were again able to provide evidence that immunotherapy-induced blocking antibodies reach a plateau value after 2 years of treatment and remain at approximately that same level, whereas the seasonal IgE antibody levels were reduced by a mean of 73% over the whole study period. The inverse relationship between IgE and IgG antibodies and their allergen agreed with the former findings by Lichtenstein et al. [27] showing a correlation between the increase in blocking antibody and the decrease in allergen-specific IgE antibodies to their allergen and pointed to a role for IgG antibodies in the effects of immunotherapy.

Although it is meanwhile well established that allergen-specific immunotherapy induces high levels of allergen-specific IgG1 and IgG4 antibodies, studies in the 1980s suggested that immunotherapy leads to the predominant formation of IgG4 antibodies [33, 34]. Other studies have, however, demonstrated that allergen-specific immunotherapy also induces strong IgG1 responses, whereas little or no Th1-indicative immunoglobulin responses (i.e. IgG2, IgG3) can be detected [35–40].

A retrospective survey of six immunotherapy studies based on reported clinical outcomes and the sole analysis of IgG4 antibody responses, reporting an association between IgG4 and clinical improvement in two and lack of association between IgG4 and clinical improvement in four studies, is frequently cited as the main argument against the concept that induction of allergen-specific IgG antibodies represents a mechanism underlying allergen-specific immunotherapy (fig. 1) [41]. In the next section, we will however discuss why true blocking antibodies with protective activity cannot be measured with raw allergen extracts and present recent data obtained with purified allergens reconfirming the important role of blocking antibodies in allergen-specific immunotherapy.

Several Reasons Why Allergen Extracts Cannot Be Used for a Precise Measurement of Blocking Antibody Activity

Allergen extracts are prepared from allergen sources by various procedures and accordingly may vary greatly with regard to their composition [42]. Furthermore, many other factors (e.g. maturation stage of pollen, culture conditions of moulds, different cultivars of plants) influence the

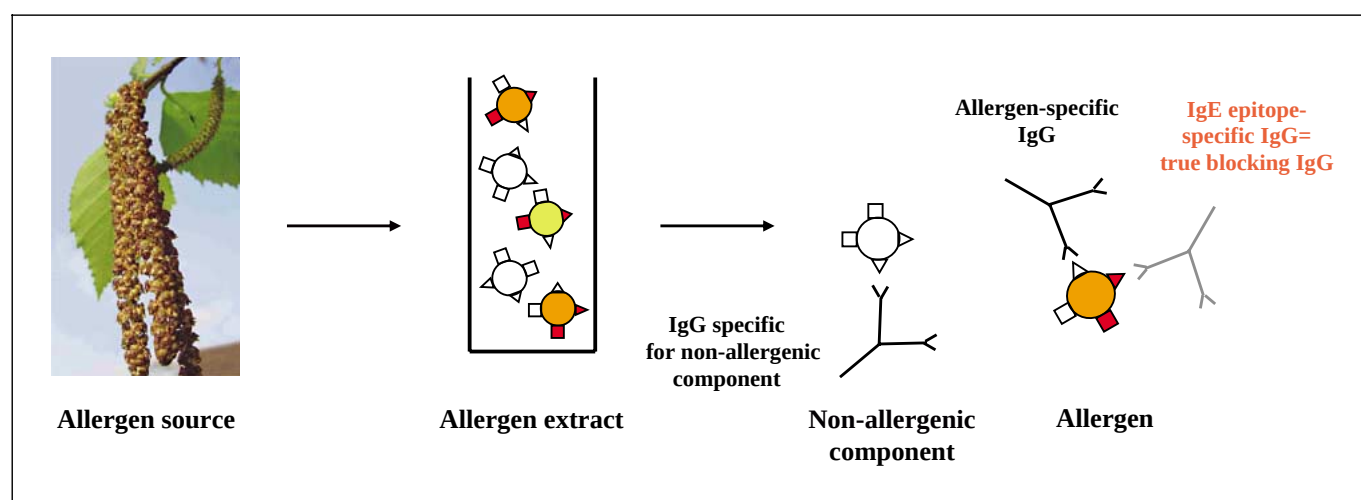


Fig. 4. The importance of allergen and epitope specificity for the blocking activity of IgG antibodies. Raw allergen extracts prepared from allergen sources consist of allergens (orange, yellow) and non-allergenic components (white). IgG antibodies reacting with allergen extracts may recognize non-allergenic components or allergens. Allergens contain epitopes which are recognized by patients' IgE antibodies (red) as well as other epitopes (white). Only those IgG antibodies which inhibit IgE binding belong to the 'blocking/protective' antibodies.

amounts of allergens in the allergen sources. The obtained allergen extracts therefore consist of an unpredictable mixture of different allergens and non-allergenic components. Despite attempts to estimate the contents of certain allergens in the raw extracts with the help of antibody-based standardization methods, the allergen contents cannot be influenced nor is there any control regarding the ratios of individual allergens in an extract. Therefore, IgG antibody measurements performed with allergen extracts cannot discriminate between IgG antibodies specific for non-allergenic components and allergens (fig. 4). Even if pure allergens are used for the measurement of specific IgG antibodies, it is not possible to distinguish with certainty between allergen-specific IgG antibodies with blocking activity (i.e. those which inhibit IgE binding to the allergen) and those reacting with irrelevant epitopes (fig. 4). Several other aspects requiring attention in the evaluation of blocking antibodies are summarized in table 1.

Epitope Specificity

The importance of epitope specificity for the blocking activity of allergen-specific IgG antibodies has been demonstrated in detail for the major birch pollen allergen,

Table 1. Aspects which are important for the blocking/protective activity of antibodies

Epitope specificity [22, 43–49]
Affinity [44]
Isotype [33–40]
Binding to Fc receptors [57–62]
Availability in the organs where allergen encounters occur [55, 56, 63]
Anti-idiotypic antibodies [64–69]

Bet v 1 [43]. When monoclonal IgG antibodies specific for Bet v 1 were established from a patient who had received birch pollen-specific immunotherapy, three types of antibodies could be discriminated. The blocking antibody type, represented by Bab 1, inhibited IgE binding to Bet v 1 and Bet v 1-induced histamine release. Antibodies without relevant blocking activity did not cause relevant inhibition of IgE binding to Bet v 1 nor reduction of Bet v 1-induced histamine release. A third type of antibody, represented by Bab 2, even enhanced IgE recognition of Bet v 1 and Bet v 1-induced skin reactions, most likely due to the induction of conformational alterations in the Bet v 1 molecule (fig. 1) [43, 44]. These findings empha-

sized that the epitope specificity of IgG antibodies is decisive for their blocking and protective activity. Furthermore, these data suggest that it may be very important to select allergens/allergen derivatives/epitopes for specific immunotherapy which induce the correct type of antibodies (i.e. antibodies which inhibit IgE binding to the allergen) and hence exert blocking/protective activity. The importance of the latter principle was already recognized in the 1970s, when chemically modified allergens were evaluated regarding their suitability for allergen-specific immunotherapy [45]. Also, today, assays analyzing whether recombinant allergens, i.e. genetically modified or synthetic allergen derivatives, induce a favorable type of antibody response in animals are used [46]. These assays investigate whether the antibodies induced in animals inhibit IgE binding to allergens and allergen-induced basophil degranulation in allergic patients [47–49].

Affinity

High affinity to the allergen certainly represents a desirable feature of a potential blocking antibody. In principle, it would be of great advantage if IgG antibodies induced by specific immunotherapy were to have higher affinity than IgE antibodies and compete for the IgE epitopes. Measurements of antibody affinities to allergens have so far been hampered by the lack of pure allergen molecules and the fact that IgE as well as IgG antibody responses in allergic patients are of a polyclonal nature. The analysis of the above-mentioned Bet v 1-specific human IgG antibodies has, however, revealed that IgG antibodies induced by immunotherapy can have high affinity and are characterized by the presence of somatic mutations in their complementary determining region, indicating that prolonged immunization may even increase the affinity of IgG through affinity maturation [43, 44]. Regarding the blocking/protective role of IgG, epitope specificity seems, however, to be more important than affinity because even a high-affinity IgG antibody directed against the wrong epitope will not be able to block IgE binding to the allergen.

Isotype

Despite earlier reports that IgG4 may represent the most important isotype induced by allergen-specific immunotherapy [33, 34], recent studies performed with purified allergens and their epitopes have clearly shown

that common forms of injection immunotherapy induce allergen-specific IgG1 and IgG4 antibodies [37–39]. Even when Th1-driving adjuvants were used, few or no allergen-specific IgG2 or IgG3 responses were induced [40]. The isotype of the induced antibodies is clearly of no or minor relevance regarding their blocking activity, which is conferred by the antibodies' variable domains. Since IgG2 antibodies against environmental antigens are known to mediate type III hypersensitivity diseases (e.g. farmer's lung), it is also questionable if indeed a shift towards allergen-specific Th1 immunity is a desirable goal of allergen-specific immunotherapy [50]. Whether it may be of importance to induce allergen-specific IgA antibodies rather than IgG antibodies is another issue to be discussed. Allergic inflammation mainly occurs at mucosal sites which are exposed to allergen contact, and it has been argued that IgA antibodies at mucosal sites may represent a shield against intruding allergens [51]. On the other hand, IgA antibodies may activate eosinophils [52, 53], and IgA responses to wheat antigens occur in patients suffering from celiac disease and thus may even be linked to hypersensitivity to foreign antigens [54]. Furthermore, it has been demonstrated that allergen-specific IgG antibodies can be found in sufficient amounts in mucosal secretions and hence IgG may act equally as well as IgA as a protective antibody shield at mucosal sites [55, 56].

Epitope specificity is more important than antibody isotype with respect to the blocking activity of allergen-specific IgG antibodies, but it has also been demonstrated that the isotype of allergen-specific antibodies may modulate allergen-specific effector cell activation via co-cross-linking events mediated by Fc receptors [57].

Binding to Fc Receptors

Allergen-specific IgG antibodies can bind to Fc γ receptors, which are present on a variety of immune cells (e.g. macrophages, monocytes, NK cells, B cells, granulocytes, mast cells) [57–59], and hence may cause a cascade of immunomodulatory activities. Classical work by Ravetch and Bolland [57] demonstrated that depending on the pairing of activation (Fc γ RIIA, Fc γ RIII) and inhibitory receptors (Fc γ RIIB), immune responses are enhanced or suppressed. With regard to mast cells, it has been reported that co-crosslinking of Fc ϵ RI and Fc γ RIIB receptors may downregulate effector cell activation [60]. Recently, these findings were utilized by Zhu et al. [61], who showed that a human bifunctional fusion protein consisting of the γ hinge region + CH γ 2 CH γ 3 and CH ϵ 2 + CH ϵ 3 + CH ϵ 4

inhibited FcεRI-mediated degranulation of human basophils and mast cells by binding simultaneously to both receptors, FcεRI and FcγRIIB. Since mast cells can express Fcγ receptors [59, 62], it may be possible that allergen-specific IgG antibodies may modulate mast cell functions if they can bind to inhibitory Fcγ receptors or co-crosslink Fcγ- and FcεRI receptors.

Availability of Blocking Antibodies in the Organs Where Allergen Encounters Occur

The majority of allergic inflammatory reactions occur at mucosal surfaces (e.g. nasal mucosa, respiratory tract, gastrointestinal tract, conjunctiva) [1]. In order to exert protective activity, allergen-specific IgG antibodies need to be present in these tissues. It has indeed been demonstrated that mucosal secretions contain allergen-specific antibodies of a similar specificity and isotype as those found in the peripheral blood of allergic patients [55, 56]. Moreover, it has been shown that allergen-specific immunotherapy increased allergen-specific IgG not only in serum but also in nasal secretions, probably even through local production [63]. In this context, the development of immunotherapy protocols which induce high levels of blocking antibodies not only in serum but also in mucosal secretions may be considered. Furthermore, local administration of blocking antibodies to the target sites of allergen encounter may represent a promising therapeutic strategy.

Anti-Idiotypic Antibodies

The theory of Jerne [64] postulates that anti-idiotypic antibodies are formed spontaneously and play an important role in the regulation of immune responses. Several studies have also suggested that atopic as well as nonatopic individuals produce anti-idiotypic antibodies against allergen-specific IgG and IgE antibodies [65–68]. However, since the demonstration that human auto-anti-idiotypic antibodies to mite allergen-specific IgE can degranulate basophils, little information has been available regarding the biological importance of such anti-idiotypic antibodies in allergy [69].

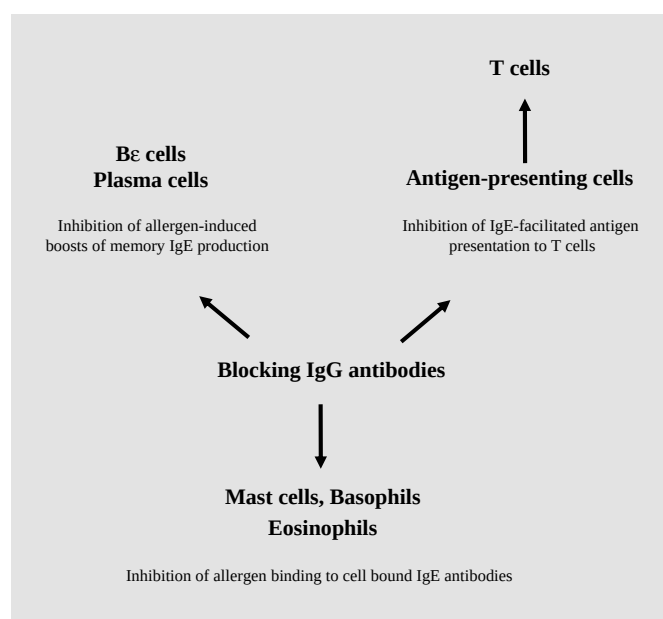


Fig. 5. Anti-allergic effects of allergen-specific blocking antibodies.

Recent Studies Performed with Pure Allergens and Epitopes in Defined Cellular Systems Demonstrate Mechanisms for the Antiallergic Effects of Blocking Antibodies

Using defined allergens (i.e. recombinant allergens), their epitopes and the corresponding antibodies, the protective activity of blocking antibodies has been investigated in defined cellular systems. These studies have demonstrated that allergen-specific IgG antibodies which block IgE binding to allergens inhibit allergen-induced release of inflammatory mediators from basophils [38–40, 70] and IgE-facilitated antigen presentation to T cells [9, 71] (fig. 5). Furthermore, old observations made by Gleich et al. [32] showing that increases in allergen-specific IgG during specific immunotherapy are accompanied by reduced IgE production have been confirmed at a molecular level (fig. 5) [40].

Blocking Antibodies Inhibit Allergen-Induced Mediator Release from Mast Cells and Basophils

The immediate symptoms of type I allergy are caused by allergen-induced crosslinking of mast cell- and basophil-bound IgE antibodies and the consequent release of

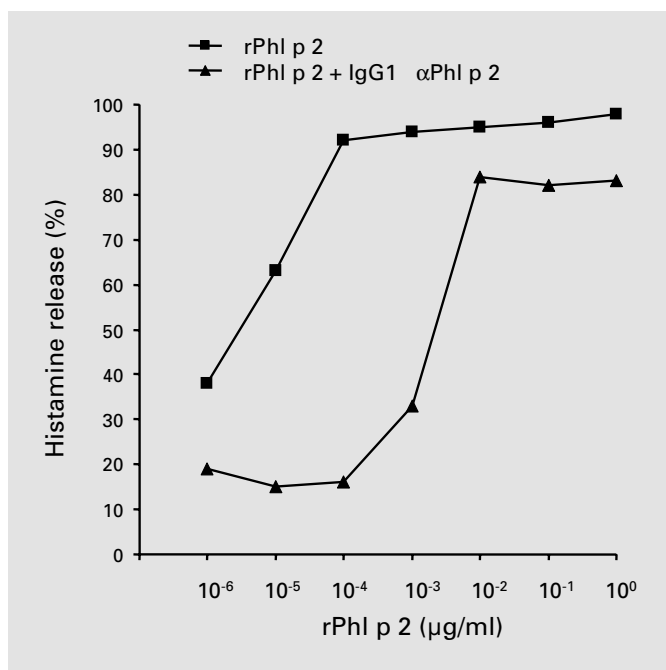


Fig. 6. A recombinant monoclonal allergen-specific IgG antibody inhibits allergen-induced basophil histamine release. Various concentrations of the major timothy grass pollen allergen rPhl p 2 (10^{-6} – 10^0 $\mu\text{g/ml}$) were preincubated with (triangles) or without (squares) rPhl p 2-specific IgG1 antibodies (x-axis). Histamine release is expressed as the percentage of total histamine (y-axis). Reproduced with permission [74].

inflammatory mediators (e.g. histamine, leukotrienes). In recent years, several studies have demonstrated that allergen-specific IgG antibodies induced by immunotherapy prevented the release of histamine from basophils of allergic patients. A human monoclonal antibody, Bab 1, specific for the major birch pollen allergen, Bet v 1, inhibited the binding of allergic patients' IgE antibodies to Bet v 1 and Bet v 1-induced histamine release from basophils [43]. Interestingly, a single monoclonal antibody was sufficient to yield strong inhibition of polyclonal IgE reactivity and histamine release. The analysis of sera from patients who had received grass pollen-specific immunotherapy also demonstrated that immunotherapy induced allergen-specific IgG1 and IgG4 antibodies which inhibited allergen-induced basophil degranulation [38–40]. That immunization of mice with defined recombinant allergens also induced IgG antibodies which blocked the binding of allergic patients' IgE and allergen-induced histamine release was demonstrated in another study [72].

Furthermore, we recently isolated human allergen-specific IgE Fabs with specificity for major grass pollen allergens from a combinatorial library constructed from lymphocytes of a grass pollen-allergic patient [73]. IgE Fabs with specificity for the major timothy grass pollen allergen, Phl p 2, were converted in human IgG1 antibodies by grafting the variable portions of the Fabs onto a complete human IgG1 backbone. The resulting Phl p 2-specific IgG1 antibodies therefore recognized IgE epitopes on the Phl p 2 allergen and hence inhibited IgE binding to Phl p 2 as well as Phl p 2-induced histamine release from basophils (fig. 6) [74]. Several explanations for the beneficial activity of allergen-specific IgG may be considered in this context. First, it is possible that IgG simply competes with IgE for the same epitopes and hence prevents IgE recognition of allergens by competition [38, 39]. Second, IgG antibodies bound to IgE epitopes targeting the allergens in proteolytic cell compartments may reduce effector cell activation via Fc γ receptors [57]. Third, when IgG antibodies bind to different epitopes than IgE epitopes on the allergen, it has been reported that co-crosslinking of Fc ϵ RI and Fc γ RIIB receptors may downregulate effector cell activation [60]. Recently, this fact was confirmed by the results of Zhu et al. [61], who showed that a human bifunctional fusion protein consisting of the γ hinge region + CH γ 2 CH γ 3 and CH ϵ 2 + CH ϵ 3 + CH ϵ 4 inhibited Fc ϵ RI-mediated degranulation of human basophils and mast cells by binding simultaneously to both receptors, Fc ϵ RI and Fc γ RIIB.

All of the latter results suggest that recombinant allergen-specific antibodies with blocking/protective activity can be obtained by molecular cloning techniques and produced as defined reagents with therapeutic potential [75].

Blocking Antibodies Inhibit IgE-Facilitated Allergen Presentation to T Cells

It is well established that antibody-mediated presentation of antigens to specific T cells leads to far greater T cell proliferation and the induction of cytokine release compared to non-antibody-mediated allergen presentation [76, 77]. In this context, it has been demonstrated that IgE-facilitated allergen presentation can be mediated by both the high- (Fc ϵ RI) and low (Fc ϵ R2)-affinity receptor for IgE via monocytes, dendritic cells and B cells [6–8]. In a recent study, it was shown that IgE antibodies specific for the major birch pollen allergen, Bet v 1, facilitated the presentation of Bet v 1 via CD23 to Bet v 1-specific CD4+

T lymphocytes by a factor of >100 [9]. Moreover, the authors of this study demonstrated that blocking IgG antibodies induced by allergen-specific immunotherapy inhibited IgE-facilitated allergen presentation, resulting in decreased T cell proliferation and reduced production of IL-4, IL-5, IL-10 and IFN- γ by Bet v 1-specific T cells [9]. These results provide an explanation for the reduction of T cell activation and cytokine release as well as for the decrease of allergen-induced late-phase responses observed after successful immunotherapy [78]. Very recently, these results were confirmed for blocking antibodies induced by grass pollen-specific immunotherapy, and a flow cytometric assay was developed based on detection of allergen-IgE binding to the low-affinity IgE receptor on B cells to examine the blocking effects of specific serum IgG [71]. The latter study concluded that allergen-specific IgG antibodies induced by immunotherapy can disrupt IgE-dependent allergen processing by antigen-presenting cells.

Blocking Antibodies Inhibit Allergen-Induced Boosts of Memory IgE Production

Using quantitative allergen-specific IgE determinations, it has been demonstrated that seasonal allergen contact (e.g. during the pollen season) induces a large increase in allergen-specific serum IgE levels, presumably due to the activation of IgE memory cells [79]. Increases in allergen-specific IgE production were also observed when allergic patients were exposed to allergen contact by nasal provocation [80]. Based on these findings, it is tempting to speculate that repeated allergen contact is a strong driving force in the boosting of memory IgE production, which may be linked to the well-known observation that repeated allergen contact leads to clinical aggravation of allergic disease (e.g. progression from mild forms of allergy such as rhinitis to severe forms such as asthma). By parallel measurements of allergen-specific IgE and IgG antibodies, Gleich et al. [32] demonstrated that the seasonal increases in allergen-specific IgE antibody levels were reduced during immunotherapy lasting 6 years, whereas allergen-specific IgG levels were high. Similar findings were made using recombinant timothy grass pollen allergens to monitor patients who had received grass pollen-specific immunotherapy [40]. Actively treated patients who developed allergen-specific IgG antibodies, but not patients who received placebo treatment, showed reduced increases in allergen-specific IgE levels induced by allergen contact in the grass pollen season [40]. These

findings suggest that allergen-specific IgG antibodies may reduce boosts of IgE production in IgE memory cells, perhaps by inhibiting allergen binding and activation of IgE memory cells.

Possible Therapeutic Use of Blocking Antibodies

The concept of passive vaccination was developed more than 100 years ago by Emil von Behring [81, 82] for the neutralization of bacterial toxins, and is meanwhile also used for prophylaxis against viral infections (e.g. hepatitis). Today, passive vaccination is routinely used in the form of specific immunoglobulins, which then confer protection against toxins or viral infections, albeit for a limited period of time. Provided that sufficient amounts of human allergen-specific IgG antibodies can be produced under reproducible and sterile conditions, it may be envisioned that such antibodies could be administered to allergic patients either locally in the target organs of atopy or systemically, similarly to the currently used passive vaccines. Blocking antibodies may be obtained by conventional tissue culture technology (e.g. hybridoma technology) [83] or by using combinatorial cloning techniques [73, 84, 85]. In the first case, it is necessary to humanize the antibodies, whereas in the second case, lymphocytes from individuals containing blocking antibodies may be used. Allergen-specific IgG antibodies may exert their beneficial activity via at least two mechanisms. First, it is possible that they exhibit immunomodulatory activity via certain Fc receptors or receptor co-crosslinking events [57]. Second, they may simply compete with allergic patients' IgE antibodies for their epitopes. For the latter type of antibodies, in vitro and in vivo assays have been developed which would allow prescreening and selection of the most promising antibodies for clinical applications. Since it has been demonstrated in the last decade that the epitope complexity of allergens is less complex than anticipated, and the most common and relevant allergens have been produced as recombinant allergen molecules, it should be feasible to obtain blocking antibodies for the treatment of the most common allergies [86].

Summary and Conclusion

The concept that immunoglobulins other than IgE (i.e. allergen-specific IgG antibodies) can neutralize IgE-mediated inflammatory reactions was demonstrated in nu-

merous experiments long ago. The reinvestigation of the blocking antibody concept with defined reagents (i.e. recombinant allergens, epitopes, monoclonal antibodies) in defined cellular and in vivo systems shows that the induction of blocking antibodies represents a major mechanism underlying allergen-specific immunotherapy [87]. Beneficial effects of blocking antibodies are (1) inhibition of allergen-induced release of inflammatory mediators from mast cells and basophils and thus of immediate symptoms, (2) inhibition of IgE-facilitated allergen presentation to T cells and thus reduction of late-phase symptoms and (3) inhibition of allergen-induced boosts of IgE production in memory cells.

Due to the rapid progress made in the elucidation of the structures of disease-eliciting allergens and in the field of antibody technology, another exciting area for a potential therapeutic application of blocking antibodies is emerging, i.e. passive administration of protective antibodies.

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