

SPECIAL ARTICLE

The role of IgG subclass antibodies in the clinical response to immunotherapy in allergic disease

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Introduction

Allergen-specific immunotherapy, formerly also known as desensitization or hyposensitization, was introduced in 1911 by Noon [1] and Freemant [2] for the treatment of allergic disorders, and has been applied clinically to patients with IgE-mediated allergy to inhaled allergens such as house dust mites and pollens and to insect venoms. Its efficacy has been established by a number of studies [3–6], although some side effects have been reported [7].

Clinical improvements associated with immunotherapy have been attributed to a variety of immunological mechanisms including suppression of IgE antibody synthesis and production of IgG blocking antibodies. Thus, the recognition of four distinct subclasses of IgG has prompted attempts to identify the subclass distribution of such IgG blocking antibodies [8].

The aim of this article is to review and summarize the present 'state of the art' on the role of IgG subclass antibodies in the clinical response to immunotherapy and, furthermore, in the type I allergic reactions, on the basis of experimental data obtained by determining each immunoglobulin isotype antibody level in various allergic conditions.

The four IgG subclasses

Due to the antigenic differences both in the constant portion hinge region and at the C-terminus of the heavy chain, human IgG molecules can be divided into four subclasses, namely IgG1, IgG2, IgG3 and IgG4 [9]. The distribution of these IgG subclass proteins in normal human serum is reported to be approximately 70% for IgG1, 20% for IgG2, 6% for IgG3 and 4% for IgG4. However, it has been elucidated recently that in patients with allergic disorders the IgG subclass distribution directed against the relevant allergen is significantly different from this ratio and is composed mainly of IgG1 and IgG4.

It has been clarified that these IgG subclass proteins

possess quite different biological functions in terms of their ability to activate complement systems via coupling to C1q and to bind to Fc receptors on inflammatory cells. More precisely, complement activation through the classical pathway is most strongly exerted by IgG3 proteins, followed by IgG1 and IgG2, with IgG4 proteins reported not to bind C1q at all. IgG1 and IgG3 bind more firmly and efficiently than IgG2 and IgG4 to Fc receptors on monocytes/macrophages, lymphocytes, platelets etc. [10,11]. In contrast, these four IgG subclasses seem to possess identical affinity for Fc receptors on eosinophils [12].

In addition, it was reported that only IgG4 can bind to human basophils, as demonstrated by basophil degranulation [13,14] and histamine release [15,16] experiments employing polyclonal or monoclonal subclass-specific antibodies. It was further shown that IgE antibody-mediated basophil degranulation could be inhibited by passive sensitization with allergen-specific IgG4 antibodies in bee-venom allergy (17).

IgG subclass changes in response to house dust mite immunotherapy

As the *in vitro* experiments dealing with human basophil degranulation suggested that IgG4 antibodies might exert a blocking effect on IgE-mediated allergic reactions, allergen-specific IgG subclass antibody determination was subsequently performed utilising samples obtained from patients with bronchial asthma and/or allergic rhinitis, in order to evaluate this possibility.

Assessment in patients with bronchial asthma

Our first study involved asthmatic patients, judged to be sensitive to house dust mite (HDM) on the basis of clinical history, skin test, IgE RAST and provocation tests. The levels of IgG4 antibodies to HDM, *Dermatophagoides farinae*, were measured in the sera of 26 asthmatic patients undergoing immunotherapy with this allergen, and the results were compared with those of 16 untreated asthmatics and 20 non-asthmatics.

The levels of mite-specific IgG4 were evaluated by

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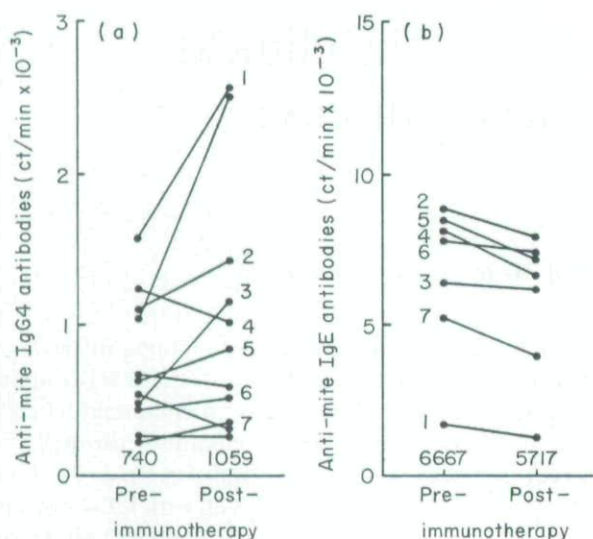


Fig. 1. Comparison of mite-specific IgG4 (a) and IgE (b) antibody levels before and after immunotherapy with house dust mite in 11 asthmatic patients. Mean values for each group studied are indicated. Reproduced with permission from ref. [18].

radioimmunoassay before and after immunotherapy, and were found to be significantly higher in treated asthmatics as compared with the other two groups. Mite-specific IgG4 increased in seven out of 11 patients, most of whom appeared to have benefited from the treatment. Levels of mite-specific IgE, however, tended to decrease with treatment (Fig. 1) [18].

The second study involved 11 HDM-sensitive asthmatic patients and was performed in collaboration with Drs T. Yamai, T. Fukuda and S. Makino of the Dokkyo University School of Medicine. The IgG1 and IgG4 antibodies were determined by ELISA using polyclonal anti-human IgG1 and monoclonal anti-IgG4 antibodies, respectively, in the sera obtained before immunotherapy, and 18–24 months after. Clinical efficacy was also evaluated by following symptom medication scores.

Levels of HDM-specific IgG1 and IgG4 antibodies increased during immunotherapy; IgG4 antibodies showed a sharper and more prominent increment as compared to IgG1 in this respect. It is interesting to note here that the asthmatic patients with clinical improvement showed a significantly higher ratio of the increased rate of IgG4 antibodies *vs* that of IgG1 antibodies, than those who did not respond to immunotherapy (Fig. 2).

These two studies involved asthmatic patients receiving conventional immunotherapy, but a similar increase in IgG4 antibodies and its association with clinical improvement in patients receiving rush immunotherapy was also reported in recent Japanese publications [19,20].

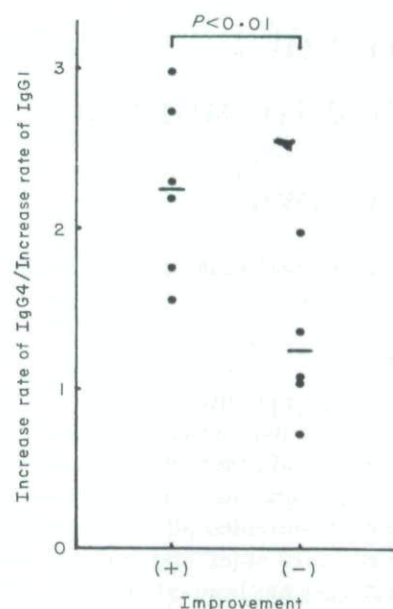


Fig. 2. Comparison of the rate of increase of IgG4/the rate of increase of IgG1 antibodies, as compared to pretreatment basal values, between asthmatic patients with and without clinical improvement following immunotherapy with house dust mite.

Assessment in patients with allergic rhinitis

IgE and IgG subclass immune responses during immunotherapy were evaluated in patients with allergic rhinitis caused by HDM.

The first study involved 70 patients and was performed in collaboration with Drs H. Kozeki, J. Katagiri and Y. Fujita of Chiba University School of Medicine. Levels of HDM-specific IgG4 increased steadily throughout treatment, but those of HDM-specific IgG1 reached a plateau at 6 months (Table 1), as was the case for grass pollen- and bee venom-sensitive subjects reported earlier [21,22]. Mite-specific IgE also tended to increase during treatment.

Changes in HDM-specific IgE and IgG1 did not correlate with effectiveness of treatment, but there was a positive correlation between the increase in HDM-specific IgG4 levels and the extent to which treatment was effective (Table 2). Thus, it was possible to assess the clinical efficacy of immunotherapy by following changes in the level of HDM-specific IgG4 or IgG4/IgE [23].

In another study performed in collaboration with Drs Y. Watase and M. Okuda of Nihon Medical College, IgG subclass antibody levels were determined both in sera and in nasal discharges obtained from 25 rhinitic patients (Figs 3 and 4). HDM-specific IgG1 and IgG4 antibodies could be detected also in nasal discharges, besides sera, and they tended to increase during immunotherapy [24]. As no significant correlation was found between IgG

Table 1. Changes of IgG and IgG subclass antibodies during immunotherapy in patients with allergic rhinitis caused by house dust mite

Antibody	Pretreatment (n = 70)	6 months (n = 58)	12 months (n = 46)	24 months (n = 25)
IgG	70.3 ± 30.8	85.7 ± 35.1*	84.2 ± 32.2*	81.9 ± 31.0
IgG1	89.3 ± 27.4	105.4 ± 28.5*	107.5 ± 28.0*	108.3 ± 28.7*
IgG4	23.1 ± 14.1	46.2 ± 28.1*	67.7 ± 47.2*	80.1 ± 44.4*

Expressed as mean ± s.d. of the antibody levels in percent of reference serum.

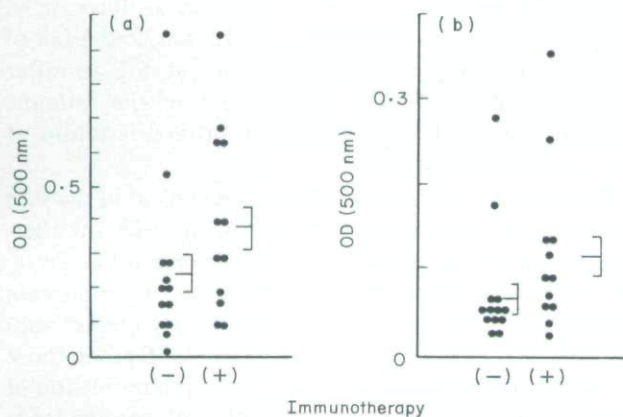
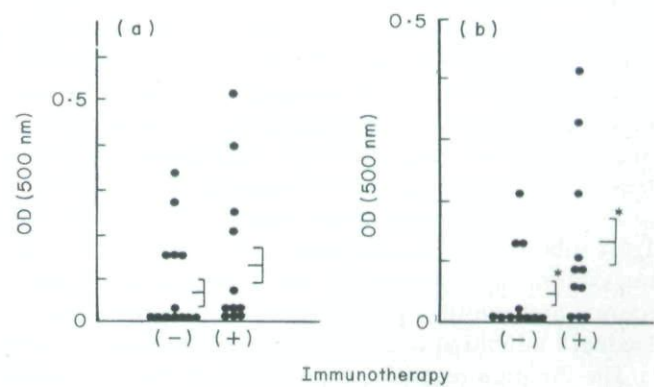
* $P < 0.01$ as compared with the pretreatment value (reproduced with permission from ref. [23]).

Table 2. Clinical efficacy of immunotherapy and changes of IgE, IgG1, and IgG4 antibodies in patients with allergic rhinitis caused by house dust mite

Antibody	Immunotherapy		
	Effective (n = 28)	Slightly effective (n = 26)	Ineffective (n = 13)
IgE	1.66 ± 1.35	2.12 ± 1.83	1.65 ± 1.14
IgG	1.38 ± 0.66	1.37 ± 0.51	1.32 ± 0.63
IgG1	1.22 ± 0.24	1.29 ± 0.35	1.19 ± 0.26
IgG4	3.98 ± 3.15*	2.77 ± 2.35	1.95 ± 1.55
IgG4/IgE	3.71 ± 3.41*	1.69 ± 1.29	1.45 ± 1.23

Expressed the rate of increase as compared with pretreatment basal value.

* $P < 0.05$ as compared to the ineffective group (reproduced with permission from ref. [23]).

**Fig. 3.** House dust mite-specific IgG1 (a) and IgG4 (b) antibody levels determined in sera of patients with allergic rhinitis.**Fig. 4.** House dust mite-specific IgG subclass antibody levels determined in nasal discharges obtained from patients with allergic rhinitis.

subclass antibody levels in sera and those in nasal discharges, it was suggested that these IgG1 and IgG4 antibodies might be produced locally to act some role in preventing the occurrence of type I allergic reactions.

It should be noted that these studies mentioned above utilize the crude mite extract, but the sharp increase of IgG4 antibodies against *Der p* I in the actively treated group was reported by Rowntree *et al.* [25] from the placebo-controlled trial with HDM-sensitive rhinitic patients.

Recently, a similar assessment employing serum samples obtained from patients with Japanese cedar pollinosis were performed in collaboration with Drs H. Nagakura and T. Endo of Jikei Medical College. The results obtained so far demonstrated a resembling pattern of IgG subclass immune responses in these patients and suggested the association of good clinical outcome with the rate of increase of IgG4, but not IgG1 and IgG3, antibodies during immunotherapy.

Table 3. Proposed mechanisms of action of immunotherapy

1 Suppression of IgE antibody formation
2 Production of IgG blocking antibodies
(a) IgG1 subclass antibodies early in the course
(b) IgG4 subclass antibodies predominantly in the late phase
3 Induction of anti-idiotypic antibodies
4 Activation of antigen-specific suppressor T cells
↓
Improvement in bronchial/nasal hypersensitivity

IgG subclass changes in response to immunotherapy with other inhaled allergens

IgG subclass responses observed during immunotherapy with grass/tree pollens [26–30] and dog/cat dander extracts [31,32] seem to be much the same as those found with HDM. Thus the antibody response occurs predominantly in the IgG4 subclass, especially in the late phase and correlates to some extent with the effectiveness of the treatment. It should be noted that early clinical benefits produced by immunotherapy seem to involve also the IgG1 subclass, since at least one report [33] indicated the association with a decrease in symptom medication scores, and another [34] suggested a possible role in feedback inhibition of IgE antibody production.

The situation regarding mould immunotherapy, however, seems to be different. Malling and Djurup [35] reported that although an increase in IgG1 and IgG4 antibody levels upon immunotherapy with *Cladosporium herbarum* was observed, their increase seemed to indicate poor or deleterious clinical outcome. In addition, a direct correlation between the magnitude of the IgG1 response and the number of side effects was also noticed. Since these results are based on one single allergen system involving a small number of patients, this has to be re-evaluated by further investigation.

IgG subclass changes in response to immunotherapy with insect venoms

Once again, immunotherapy with bee and wasp venoms results in a predominantly IgG4 response [36–39]. IgG1 antibodies are also evoked initially, but prolonged immunization appears to produce a shift to a predominantly IgG4 antibody response [22]. In connection with this, a recent publication [40] indicates a similar increase in IgG4 antibodies against imported fire-ant whole body extract and venom upon immunotherapy. It should also be noted that Djurup *et al.* [41] emphasized the role of IgG1 antibodies in causing feedback inhibition of IgE antibody production in patients treated with yellow jacket venom.

It is also interesting to note that bee keepers who are

repeatedly stung by bees exhibit a similar IgG subclass response [42], namely IgG4 antibodies steadily increased with a direct correlation to the number of bee stings experienced by the bee keepers, while IgG1 antibodies reached a plateau after a certain number of bee stings. In Europe, gammaglobulin fractions of such beekeepers were administered intravenously to subjects sensitive to bee venoms and were proven to prevent systemic side effects evoked by active immunization with bee venom [43,44].

IgG subclass antibodies and their association with late asthmatic or cutaneous responses

Asthmatic patients usually manifest two patterns of bronchial response when exposed to the relevant allergen. One pattern is known as the immediate asthmatic response (IAR) in which a fall of FEV_{1.0} is observed at 15–20 min after challenge. The other is known as the late asthmatic response (LAR), where a fall of FEV_{1.0} is observed several hours after challenge, in addition to IAR.

The relationship between the presence of IgG subclass antibodies and the appearance of the LAR was evaluated in several laboratories including ourselves. Gwynn *et al.* [45] reported at first that IgG4 antibodies might produce LAR and might contribute to the development of severe asthma. However, following reports could not confirm this association [46,47]. Subsequently, we have examined the association with IgG1 antibodies and found that there is close correlation between the presence of high titres of IgG1 antibodies and the propensity of LAR to develop in HDM-sensitive asthma [48].

In addition, we have experienced one interesting case with egg-sensitive bronchial asthma. After oral provocation with two eggs, he demonstrated a decrease in ovalbumin-specific IgG1 antibodies and an increase of C1q-binding immune complexes at 15 min, and a fall of FEV_{1.0} with dyspnoea and wheezing at 6–8 hr after challenge, indicating the involvement of the immune complex-mediated allergic reaction in manifestation of his clinical symptoms [49].

The late-phase response can also be evoked in the skin following an intradermal injection of the relevant allergen. In this respect, it should be noted here that Fling *et al.* [30] recently reported that patients receiving mountain cedar immunotherapy showed significantly greater suppression of the late cutaneous response (LCR) than those receiving placebo injections, and that this suppression of the LCR correlated significantly with both specific IgG4 antibody levels and the cumulative dose of the allergen received.

Possible roles of allergen-specific IgG subclass antibodies produced during immunotherapy

The experimental data accumulated up to now indicates that the IgG antibody response during allergen-specific immunotherapy is mainly composed of IgG1 and IgG4 subclasses, although low levels of IgG2 and IgG3 antibodies can be detected in certain instances. In most of the cases, IgG1 antibodies seem to dominate early in the course, but as immunotherapy treatment goes on IgG4 antibodies become more prominent.

It is now well established that IgG4 antibodies increase upon prolonged exposure to the relevant allergen in atopic subjects, as primarily suggested by Aalberse *et al.* [21,22]. In this connection, there is no doubt concerning the elevation of IgG4 antibodies during immunotherapy, as demonstrated in almost all of the publications dealing with this subject. However, there seems to remain some space for discussion regarding their role in the type I allergic reactions. In our hands, the association was observed between the development of IgG4 antibodies and objectively measured clinical improvements resulting from immunotherapy with the inhaled allergens, which suggested a role as blocking antibodies. Several lines of evidence seem to be in accordance with this notion, but a few do not, as mentioned here.

One of the roles of IgG4 antibodies formed during immunotherapy may be neutralizing the allergens before they interact with cell-bound IgE antibodies on mast cells and basophils (50). Interference with IgE binding to Fc receptors on the cell also seems to be possible [17,51]. The other role may be protecting the host from immunological damage induced by immune complexes composed mainly of IgG1 antibodies and the relevant allergens, by competing with IgG1, since the IgG4 subclass does not activate the complement system and does not have the capacity to bind to Fc receptors on certain inflammatory cells. In support of the latter role of IgG4 it was demonstrated [52,53] that immune complex formation and complement activation by IgG1 antibodies were inhibited *in vitro* by the addition of IgG4 antibodies to the experimental system. Thus IgG4 antibodies seem to act as blocking antibodies by reducing the effects of complement-fixing IgG1 (and maybe IgG3) antibodies and/or interfering with the actions of IgE antibodies.

Turning to the role of IgG1 antibodies, their situation is much more complicated and controversial. It seems likely that all subjects, including non-atopic people, can recognize and produce IgG1 antibodies to inhaled and to food allergens [54,55] and this IgG1 antibody response seems to decline after reaching a certain level of allergen exposure. There is also no doubt concerning the elevation of IgG1 antibodies and their contribution to clinical

benefits early in the course of immunotherapy to neutralize the allergens. However, it is possible that IgG1 antibodies may become gradually harmful to the organism as their level increases, through the mechanisms described previously.

It is interesting to note the association between IgG1 antibodies and the LAR reported by our laboratory [48]. It is also well known that the frequency and magnitude of the LAR after bronchial provocation tests decrease following immunotherapy [56,57]. It is assumed at present that the LAR is produced by a localized type III allergic reaction in the bronchial mucosa involving inflammatory cells like eosinophils, monocytes and neutrophils, which are activated by IgG1-containing immune complexes [58]. Considering that IgG1 antibodies might be involved in producing the LAR and that IgG4 antibodies become more predominant in the late-phase of immunotherapy, it seems reasonable to speculate that the changes in the ratio between the amounts of IgG4 antibodies *vs* IgG1 antibodies (and maybe IgE antibodies) may affect the decrease in the LAR.

The association with IgG anaphylactic antibodies should be discussed here. IgG short-term sensitizing antibodies (IgG S-TS) with anaphylactic capacity were first described by Parish [59] and were supposed to be related to the IgG4 subclass in the early 1980s [60]. However, it should be pointed out that IgG S-TS were reported to be produced as a response to natural exposure to allergens in most studies. Considering the roles of IgG1 and IgG4 antibodies and the experimental data showing that the interaction between IgG4 antibodies and allergens does not induce basophil granulation [17], it might be justified to say that IgG1, but not IgG4 antibodies are related to IgG anaphylactic antibodies.

The final point to be discussed is the effect of IgG subclass antibodies on the suppression of IgE antibody production. As mentioned earlier, Djurup *et al.* [34,41] proposed that IgG1 antibodies formed early in the course of immunotherapy take part in this respect. They did not indicate the IgG4 subclass as a possible candidate, but this should be clarified further. In addition, the effect of auto-anti-idiotypic antibodies must also be taken into account, since it was reported that not only IgE but also IgG4 antibody formation was suppressed after long-term immunotherapy [61] and that these kind of antibodies were detected in the sera of patients receiving ragweed immunotherapy [62].

Conclusions

Allergen-specific immunotherapy, formerly called desensitization or hyposensitization, has been applied clinically for the past 80 years in the treatment of patients

with various allergic disorders. Its effectiveness has been established, and its immunological mechanism of action may be summarized as presented in Table 3.

The overall results obtained so far suggest that specific IgG antibodies increased during immunotherapy, especially the IgG1 subclass early in the course followed predominantly by the IgG4 subclass, play an important role in blocking the IgE-mediated allergic symptoms. As the treatment continues for a longer period, other mechanisms such as suppression of IgE antibody formation, induction of anti-idiotypic antibodies and activation of allergen-specific suppressor T cells might also be involved in producing clinical improvements, and these immunological changes would result collectively in a decrease of bronchial and/or nasal hypersensitivity in patients.

It should be emphasized, however, that many questions remain to be answered regarding the roles of each IgG subclass antibody formed during immunotherapy in type I allergic reactions, especially their association with 'allergic inflammation.' Their predictive values for the clinical outcome of immunotherapy should also be clarified further, since this kind of determination will allow selection of appropriate patients for immunotherapy. The available data relating to this also seem to be controversial.

After establishment of the beneficial role of the IgG subclass antibodies, the possibility to produce human 'monoclonal' antibodies with the aid of biotechnology should be pursued in future, since these might be able to provide a new and fundamental approach to suppress IgE-mediated type I allergic reactions.

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