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Review article

Allergen-specific IgG4 in atopic disease

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Allergen-specific IgG4 has often been regarded as a two-headed creature: potentially harmful as well as potentially protective. As more is found out about these antibodies, the harmful effects are found to be hard to substantiate, at least in the allergy domain. The protective effects (with respect to allergy) are still debated, but particularly from the field of parasitology the evidence is accumulating that IgG4 does, under certain conditions, effectively interfere with allergen-induced, IgE-mediated, effector-cell triggering; i.e., it acts as a blocking antibody. It should be noted that, with respect to parasite immunity, this may be an undesirable effect (32, 36, 44, 45, 69, 70, 105).

Particularly, recent research in the regulation of B cells by T cells and T-cell-dependent cytokines helps to explain the similarities and differences between IgE and IgG4. It will be explained that a striking similarity has been found between IgE and IgG4 with respect to the type of antigen that triggers these immune responses. As will be explained in some detail later, it is now clear that this effect is a reflection of the similar T-cell help requirements: TH2-type cells need to be activated for both immunoglobulin isotypes.

In view of this similarity between IgE and IgG4 with respect to *antigen* specificity, it was unexpected to find a marked difference in *epitope* specificity between IgE and IgG4. The consequence of the differences in epitope specificity is that binding of IgG4 antibody does not necessarily interfere very effectively with the binding of IgE antibodies. In this review, we will argue that the level of allergen-specific IgG4 does not accurately reflect the level of protection obtained, because only a fraction of the allergen-specific IgG4 can interfere effectively with IgE binding.

The position with respect to the use of IgG4 antibodies to monitor immunotherapy that we will put forward in this review is that this use of IgG4 antibody assays is justifiable, but its value should not be overrated. The reasoning behind this position statement is that conventional immunotherapy depends on the immunologically specific activation or inactivation of allergen-specific T cells. Currently, this means stimulation of TH2 cells, and this process can be monitored conveniently by measuring allergen-specific IgG4. If no IgG4 antibody is induced by conventional immunotherapy, the therapy is likely to have been ineffective in reaching the allergen-specific immune system.

A future challenge for immunotherapy might well be to exploit the increasing knowledge about B-cell regulation and devise strategies to stimulate the production of IgG4 antibodies with the correct epitope specificities.

History

The interest in IgG4 in relation to atopic disease started approximately 20 years ago because of two unrelated observations. Firstly, particularly in the guinea pig, but also in other experimental animals, IgG4 antibodies proved to be important for some anaphylactic reactions. Because these sensitizing antibodies were less species-specific than what are now known as the IgE-type antibodies, the term "heterocytotropic", as opposed to "homocytotropic", was proposed for these antibodies. These non-complement-fixing, short-time-sensitizing antibodies had a relatively high electrophoretic mobility, just like IgG4 (13). In his investigations of sensitizing IgG antibodies in man, Parish (61) found suggestive evidence for involvement of IgG4 in this anaphylactic activity.

Secondly, three groups of investigators (Devey et al. (24), van der Giessen et al. (91), and Stanworth et al.; unpublished), all presenting their data at a workshop at the Second International Congress of Immunology (Brighton, UK, 1974), found a relative overrepresentation of IgG4 in patients receiving allergen immunotherapy.

Three questions have been debated by allergists ever since:

1) is IgG4 a mast cell/basophil-triggering antibody?
2) are IgE and IgG4 immune responses linked to the same type of antigen (allergen)?
3) are IgG4 antibodies particularly effective as allergen-blocking antibodies?

Is IgG4 a mast cell/basophil-triggering antibody?

The observations by Parish (61) generated considerable scientific activity (14, 57, 67, 86, 101–104), summarized in several reviews (2, 7, 20, 26, 30, 48, 55, 64, 74, 85, 87, 92, 93). Most investigators now accept that basophils cannot be triggered for histamine release by allergens via IgG4, but that basophils of some donors can be triggered by some anti-IgG4 antibodies (67, 101, 102). The dust has not completely settled on this topic, as evidenced by several recent papers on the subject of IgG4-induced histamine release. Beauvais et al. (12) present data suggesting that the basophils are triggered indirectly, via eosinophils. Jimeno et al. (39) found marked differences between two anti-IgG4 monoclonal antibodies. These authors suggest involvement of the only Fc-γ-receptor reported to be present on basophils (9, 90): the low-affinity type II γ-receptor CD32 (which is not to be confused with the low-affinity type II ε-receptor CD23). Interestingly, this low-affinity IgG receptor was recently also found to have a low affinity for IgE (8, 41, 88). The analysis of basophil triggering in the investigations by Jimeno et al. (39) is complicated by the presence of soluble IgG4, because these experiments were performed in whole blood rather than with plasma-free cells. Lichtenstein et al. (47) have presented data suggesting that IgG-IgE complexes are responsible for anti-IgG-induced histamine release. Interestingly, these investigators found most histamine-releasing activity with anti-IgG3 and least with anti-IgG4 (two monoclonal antibodies tested for each human isotype).

Lichtenstein et al.'s paper (47) touches upon the interesting subject of IgG anti-IgE autoantibodies, which are, to a substantial extent, of the IgG4 isotype (e.g., references 17, 78). However, this topic is outside the scope of this review.

One of the recurrent arguments in favor of a pathogenic role for IgG4 antibodies is that they are occasionally found in patients with a history suggestive of immediate-type allergy without demonstrable specific IgE (e.g., reference 43). The value of these observations is, however, very limited, because IgG4 antibodies in nonallergic control subjects are usually also found, particularly if an exposure-matched control is tested. Statistically, however, patients with a food allergy have been found to have higher mean IgG4-antibody levels than nonallergic controls (10, 11, 34, 37, 40, 52, 56, 73, 75), possibly reflecting increased exposure to the allergen as a consequence of a permeability-increasing local allergic reaction in the gut. In very young food-allergic children (less than 18 months old) with a physiologically unresponsive IgG4 system, the IgG1- rather than the IgG4-antibody level to the food is often in the upper percentiles for that age group. A similar explanation presumably holds for the increased IgG-antibody levels found in atopic eczema patients (2, 19, 29, 31, 34, 51, 68, 72, 73, 76, 77, 106) or patients with celiac disease (37).

Biologic properties of IgG4 antibodies

In large antibody excess, IgG4 antibodies form multi-antibody complexes just as IgG1 antibodies do. This can be shown, for example, by the binding of these complexes to the rheumatoid factor. In contrast to the IgG1 complexes, however, these IgG4 complexes do not bind to the first complement component C1q. IgG4 antibodies, therefore, do not activate the classical complement route. In fact, IgG4 antibodies were found to interfere with the complement activation by IgG1 antibodies (95).

Upon lowering the antibody/antigen ratio, researchers noted another difference between IgG1 antibodies and IgG4 antibodies. With IgG1 antibodies, the size of the immune complexes increases dramatically, whereas the size of the IgG4 complexes

decreases. Further experiments indicated that IgG4 antibodies are functionally monovalent (6, 80, 94). Yet, physicochemical evidence indicates the presence of two antigen-binding sites in IgG4. These results can be reconciled by assuming that binding of one antigen sterically prevents the binding of a second antigen. A practical consequence of this functional monovalency is that IgG4 antibodies are not detected in antibody assays that depend on bivalency, such as the reverse sandwich assay (100). A related issue is the apparently low avidity of IgG4 antibodies in some assays (23, 65). IgG4 complexes are too small to precipitate in polyethylene glycol, and this may result in spuriously low avidity values. Using anti-immunoglobulin reagents (protein A or anti-IgG4) and avoiding the pitfall of competing "invisible" antibodies in the other isotypes, a pitfall which also leads to an underestimation of the avidity, researchers have found the avidity of IgG4 antibodies to be of the same order of magnitude as that of IgG1 or IgE antibodies.

It is occasionally assumed that the functional monovalency of IgG4 makes it unsuitable to act as a cytophilic antibody, like IgE, to become cross-linked by antigen.

As indicated above, cross-linking of two IgG4 antibodies by a single antigen is not necessarily a problem, although the restricted hinge flexibility might impose some barriers. However, the engagement of Fc-receptors would be limited to two, whereas IgE-mediated cross-linking seems to be considerably more effective at slightly higher aggregation ratios. This is presumably an academic discussion, because the only Fc-receptor known to be present on basophils that bind IgG4, the type II Fc-γ-receptor, binds only polymeric, i.e., antigen-complexed, IgG4.

IgG4 as an indicator of chronic antigenic stimulation

The marked contribution of IgG4 to the immune response induced by repeated allergen injections has been known for a long time. This suggested some link with allergy. However, we soon found that also in conditions unrelated to allergy repeated antigen stimulation resulted in an IgG4-dominated antibody response. This was particularly clear in a study of the immune response in novice beekeepers (6). During the first few months of beekeeping, we found predominantly IgG1 antibodies. In more than 50% of the beekeepers, IgE antibodies also appeared, usually with large local reactions, but without severe generalized allergic reactions. IgG4 dominance became apparent particularly during the second year of beekeeping. Professional beekeepers had extremely high IgG4 antibody levels, sometimes even resulting in increased total IgG4 levels. Since these professional beekeepers had virtually no reaction to bee stings, it is likely that the immune response was protective in the conventional way: antitoxic immunity comparable to immunity to diphtheria toxin.

Although these findings might be described as a switch from IgG1 (and possibly IgE) to IgG4, this would be confusing, since it suggests a class-switching event on a cellular level. It is improbable that the cells that originally produce IgG1 antibodies themselves switch to IgG4 production. It is more likely that repeated antigenic stimulation results in the maturation of previously resting B cells (presumably memory cells) that subsequently mature and undergo a class switch to become IgG4-secreting plasma cells.

Are IgE and IgG4 immune responses linked?

All IgE-inducing antigens investigated so far have proved to be excellent IgG4-inducers, including not only many different atopic allergens, but also parasite allergens and castor bean allergens. The reverse does not seem to be necessarily the case, as evidenced by a food protein that induces strikingly high IgG4 responses without a marked contribution to food allergy: the banana protein BanLec 1 (42). Analysis by Western blotting of complicated allergen extracts (22) or parasite extracts (35) reveals the IgG4 response to be more similar to the IgE response than the other immunoglobulin isotypes. On the level of the total allergen molecule, IgE and IgG4 responses are thus found to be associated (48). Presumably, the explanation is that both isotypes are IL-4 dependent and thus require the stimulation of TH2-type T-helper cells (27, 28, 38, 49, 59, 81–84).

Antigens that, for whatever reason, are effective TH2-inducers will thus promote both IgE and IgG4 responses, whereas antigens that induce TH1 cells are more likely to stimulate IgG1 and IgG3 responses. (Note the awkward nomenclature problem: the codes for the human isotypes do not correspond to the murine isotypes; human IgG4 is, to some extent, comparable to mouse IgG1, whereas human IgG1 is more like mouse IgG2a; human IgG2 behaves like mouse IgG3.)

Because of this similarity between IgE and IgG4 on immunoblots, it was tempting to suggest that IgE and IgG4 are descended from the same parental B cell, diverging upon class switching in the daughter cells. One possible scheme would be to postulate that nonatopic B cells switch to IgG4 because of a relatively low IL-4 production by TH2 cells, whereas a significant fraction of atopic B cells switch to IgE because of higher IL-4 production in these patients. The concentration of IL-4 required for switching to

human IgG4 (or mouse IgG1) is likely to be lower than that required for IgE switching.

It has even been suggested that IgG4 antibodies that are produced after immunotherapy result from switching IgE-producing cells to IgG4-producing cells. This is unlikely for several reasons. One reason is that the induction of IgG4 antibodies occurs even in patients in whom the IgE antibody level does not decrease. Another reason is that a switch from IgG4 to IgE rather than the other way around would be expected from what is currently known about isotype switching. Indeed, in mice there is good evidence for isotype switching from (murine) IgG1 to IgE (50, 79).

According to this common parental cell scheme, the *epitope* specificity of IgE and IgG4 antibodies would be expected to be very similar if not identical. Recent data from our laboratory, however, indicate a marked difference between IgE and IgG4 in epitope specificity. These results were obtained in three different test systems, all three using variants of natural allergen molecules.

In one approach, we analyzed *phylogenetic variants* of allergens. We compared the IgE and IgG4 responses to the major birch allergen *Bet v* I and the homologous protein in apple (21). The cross-reactivity of these two proteins for IgE is well known (16). We found that in one patient clear IgG4 reactivity with apple was induced upon immunotherapy with birch pollen extract, but that, in contrast to the situation with IgE antibodies against apple, most IgG4 reactivity with apple extract was, in general, not related to birch pollen. These data suggested that IgE and IgG4 antibodies against the birch allergen did not fully overlap.

Similar, but more quantitative, data were obtained with another well-characterized major allergen, the cat allergen *Fel d* I (99). In this case, we compared the response to the cat molecule with that to the ocelot homologue. The ocelot was more suited than some of the other wild members of the cat family we tested, e.g., lion and tiger, which proved to be too cross-reactive for the present purpose.

In order to exclude any contribution of feline allergens distinct from *Fel d* I and its ocelot equivalent, we used affinity-purified human antibodies to study the cross-reactivity in a quantitative way. Allergen-specific antibody from sera of cat-allergic patients was purified on a *Fel d* I affinity column. The purified antibodies were tested in IgE and IgG4 RAST for antibody activity to cat and ocelot. As with the birch-apple study, we found a higher cross-reactivity for IgE than IgG4.

A second approach was the use of *recombinant chains* of the same allergen, *Fel d* I. The primary structure of this allergen has been established, and *E. coli*-derived recombinant protein has been produced (71). The allergen is a dimer of a two-chain molecule; it is called chain 1 and chain 2. We found more immune reactivity with chain 1 than with chain 2, but for both chains we found higher reactivity for IgE than for IgG4 (97).

The third approach was to compare IgE and IgG4 binding to small *chemically synthesized peptides* (98). By testing a panel of overlapping peptides, we first identified several IgE-binding peptides. These peptides were subsequently used to isolate by affinity chromatography peptide-reactive antibodies from human sera. Then, we measured IgE- and IgG4-antibody activity among these peptide-reactive antibodies. For this assay, we used purified radiolabeled allergen (i.e., ^{125}I-labeled *Fel d* I). The ratio (IgE antiallergen/IgG4 antiallergen) was higher in the affinity-purified material than in the starting serum. We did not find indications for a lower recovery of IgG4 antibodies in such affinity purification procedures. These results indicate that IgE is more reactive with partially processed allergen than IgG4.

IgE responses to atopic allergens in atopic and nonatopic subjects

The nature of the immunologic difference between atopic and nonatopic subjects is one of the basic problems in allergy. Platts-Mills has shown that, for the grass pollen allergen *Lol p* 1 (66) and for the house-dust mite allergen *Der p* I (18), not only are the IgE antibody levels higher in atopics, but often also the IgG antibody levels. Nonallergic persons usually have little or no detectable antibody against these two allergens. This effect seems to be a reflection of the exposure level, because this difference at the allergen-specific IgG level between allergic and nonallergic subjects disappears at higher exposure levels. Natural exposure to *Lol p* I or *Der p* I is very low, at least 10-fold lower than exposure to the cat allergen *Fel d* I when a cat is in the house. It is not uncommon to find substantial levels of IgG antibodies against the cat allergen without concomitant IgE antibodies (33, 92, 96) (Fig. 1). Even higher levels of exposure are found for experimental animals (e.g., rodents) in the case of occupational exposure. In this situation, virtually everyone develops IgG antibodies (46). The IgG isotype follows the usual pattern: it starts with an IgG1-dominated response and, upon continued exposure, gradually shifts, in most cases, towards an IgG4-dominated response.

These findings suggest that the difference between allergic and nonallergic responses might be antigen-dose-related: allergic responses might be induced at immunizing doses too low to stimulate effective IgE-control mechanisms, and atopic subjects might be more inclined towards an immune response (both

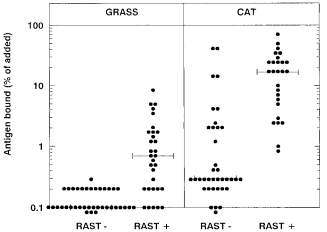

Fig. 1. IgG antibodies against two purified allergens in allergic and nonallergic donors. *Lol p* 1 from grass pollen and *Fel d* 1 from cat were iodinated and used in antigen-binding test with protein A Sepharose as IgG-binding reagent. Per test, 2.5 μl serum was used. Allergic patients had not received immunotherapy. Under these conditions, no binding of allergen via IgE was found. These results illustrate that 1) IgG antibodies against purified allergens are more common in allergic than in nonallergic donors; 2) IgG antibodies against high-exposure allergen (*Fel d* 1) are more common in nonallergic donors than IgG antibodies against low-exposure allergens (*Lol p* 1). Contribution of IgG4 to IgG response in these sera is usually less than 50%.

IgG and IgE) at a low antigen dose. In other words, the antigen threshold may be lower in atopy.

Despite the fact that both IgE and IgG responses are often induced by allergens that enter the body via the mucosal membranes, these responses are not of mucosal type: the usual IgA domination of the classical mucosal immune response is absent and the antibodies are not preferentially excreted. The antibody levels in the secretions are usually a fairly close reflection of the plasma levels.

To accommodate the data currently available on the antiallergen immune response, we have proposed a two-compartment hypothesis (van Milligen (96)). According to this hypothesis, the normal immune response to allergens occurs when the antigen is taken up by a dendritic cell and transported to the lymphoid organs and there induces a well-orchestrated immune response. This response may include some IgE, but this IgE is relatively harmless because of the excess of IgG antibodies with the same specificity. An example is the human response to tetanus toxoid immunization. Under some conditions (particularly infestation by certain parasites), an unusually large amount of IL-4 is produced, resulting in a larger production of IgE. This IgE is still not as harmful as might have been expected, because of the presence of even larger quantities of IgG antibodies (particularly IgG4) with exactly the same epitope specificity.

An allergic immune response occurs (still according to this two-compartment hypothesis) when isolated, solitary immune cells are confronted by an antigen outside the lymphoid organs. Usually such an encounter would not trigger an immune reaction, because the microenvironment is unsuitable. Occasionally, the local situation is permissive, probably in relation to some transient, special kind of inflammatory reaction that attracts and activates the types of cells required for the triggering of a few B and T cells. In many cases, these signals also activate the regulatory circuits, and then no harm is done.

Sometimes, however, the signals are not sufficiently strong to activate suppressive mechanisms, and IgE is produced in quantities quite similar to those of IgG antibodies that are locally induced. Compared to "ordinary" IgG responses, this is a negligible response. Most of the IgG produced by allergic patients is from the lymphoid compartment, and thus of different epitope specificity. This lymphoid IgG synthesis might be triggered via the IgE-mediated, CD23-dependent, antigen-presentation pathway (53, 54). The basic immunologic problem in atopy might then be related to an increased risk of mounting these "ectopic" immune responses; e.g., because of enhanced antigen trapping in extra-lymphoidal sites. The preferential recognition by IgE antibodies of partially unfolded epitopes suggests that allergens are perhaps sticking to a tissue matrix component at the time of the first B-cell encounter. Another factor contributing to enhanced antigen trapping might be a preexisting IgG antibody response to cross-reacting structures on the allergen. This would fit with two other observations (15):

1) for some foods it has been shown that cross-reactivity exists with inhalant allergens
2) a higher than normal immune response to foods is a risk factor for the development of inhalant allergy.

Apparently, IgG4 production results not only from the activation of B-cell precursors that give rise to the IgE-producing B cells, but also from precursors that yield few or no IgE-producing B cells.

Can IgG4 antibody assays be used for diagnosing allergies?

As discussed above, IgG4 antibodies are found independently of atopic background in virtually everyone with sufficient exposure to TH2-stimulating antigens. Nevertheless, a statistical association is found between allergic symptoms and high IgG4 antibody levels to the offending allergen. For mite and pollen allergens, and, to a lesser extent, for cat allergens, more allergen-specific IgG4 antibodies are found in donors who also make IgE antibodies. These results are probably best explained by assuming that in atopy an increased reactivity of the immune system

to TH2-type responses exists. A contributing factor might be the enhanced, IgE-mediated antigen presentation (54). This difference is much more marked for the most potent allergenic components in an allergen extract than for nonallergenic components, and it is often (but not always, e.g., not in young children (40)) more marked for IgG4 than for other isotypes.

An additional problem is that in some cases the elevated IgG antibody titers are a consequence rather than a cause of the disease. It is well known that conditions such as wheat intolerance may result in elevated IgG antibody levels not only against gliadin but also chicken ovalbumin and other food antigens, presumably secondarily to malfunctioning of the intestinal mucosa.

Despite the statistical difference between allergic patients and nonallergic controls that can be found in some conditions, it is hard to define a condition where the assay of allergen-specific IgG4 would be advisable (52). In most of these conditions, a better discrimination can be found with specific IgE assays.

Investigations in which allergic patients without detectable allergen-specific IgE were identified with allergen-specific IgG4 did not include the two types of reference values that are essential for the correct interpretation of an IgG4 antibody assay: 1) a sufficient number of age- and exposure-matched controls; 2) responses of the same patient to other antigens with similar exposure characteristics.

Can IgG4 antibody assays be used to monitor immunotherapy?

The question of whether IgG4 antibody assays can be used to monitor immunotherapy brings us back to the last question mentioned in the beginning: is IgG4 antibody particularly effective as an allergen-blocking antibody? The current answer to the latter question is that, presumably, it is not, but it is the best we have. The next question then might be this: is an IgG4 antibody assay relevant to monitor the effects of conventional immunotherapy? (1, 3, 58, 60, 63, 89). There is a tendency to push aside completely the concept of blocking antibodies as a factor in immunotherapy. Undoubtedly, there is more to immunotherapy than blocking antibodies alone. It is now abundantly clear that the late inflammatory sequelae of the acute allergic reaction are a major factor in the pathology, and it is highly probable that T-cell activation plays an important role in this inflammatory process. Downregulation of these antigen-specific, proinflammatory T cells is an important issue in immunotherapy. It should be kept in mind that these T cells are antigen-specific; i.e., they have to be activated by the allergen. One of the possible effects of blocking antibody might be on T-cell activation, or rather on prevention of antigen presentation to T cells. Antigen presentation to allergen-specific T cells has been shown to be greatly enhanced by allergen-specific IgE, probably via the low-affinity IgE receptor CD23 on the antigen-presenting cell (54). Competition between IgE and blocking antibody at this level (IgE on the low-affinity receptor on an antigen-presenting cell rather than IgE on the high-affinity receptor on the mast cell) could explain the effects of immunotherapy in the late reaction.

However, conventional blocking activity between mast-cell-bound IgE and soluble blocking antibody is also a mechanism to keep in mind. Immunotherapy usually does have an effect on the immediate skin reactivity that cannot be attributed to a decrease in allergen-specific IgE.

Furthermore, allergen-neutralizing activity can be shown to be present in post-treatment serum and in parasitic infections (36, 69), by *in vivo* (skin test; e.g., reference 62), *in vitro* (basophil histamine release), and RAST inhibition/RAST interference (1, 3–5). Binding of IgG4 antibody to an allergen does not necessarily interfere with IgE binding. This can easily be shown *in vitro* by adding a small amount of allergen to a postimmunotherapy serum that contains both IgE antibodies and IgG4 antibodies (IgG4 being in excess), and analyzing by RIA the immune complexes formed. A large fraction of the IgG4-containing immune complexes has been found to contain IgE. This IgG4 is unlikely to be bound directly to IgE (i.e., as IgG4 autoanti-IgE or as IgE anti-IgG4), because this binding is allergen-dependent (Fig. 2). The implication is that a large fraction of allergen-specific IgG4 induced by immunotherapy has an epitope specificity different from the epitope specificity of IgE. These results thus confirm the conclusion of the direct epitope-mapping data mentioned earlier.

What do we know about the association between efficacy of immunotherapy and IgG4 antibody levels? Few investigators would deny that the overall association is strong: conventional immunotherapy is usually effective and usually results in a marked increase in allergen-specific IgG4. On the other hand, the association is certainly not perfect, and, on an individual patient basis, many exceptions can be found. Some patients do very well without much IgG4 response, whereas other patients show an impressive allergen-specific IgG4 response without much effect on their symptoms.

The first category of patient should not come as a surprise, considering the appreciable placebo score found for any therapeutic modality in allergy. The issue of whether this category is larger than can be explained by the placebo effect has, to our knowledge, not been addressed adequately.

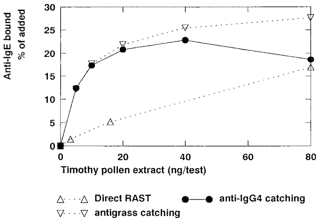

Fig. 2. IgG4 binding to allergen does not necessarily interfere with binding of IgE antibodies. Serum of grass-allergic patient with high titer of IgG4 antibodies against *Lol p* I and other grass pollen allergens was incubated with graded doses of grass pollen extract. Allergen-serum mixture was incubated with monoclonal anti-IgG4 coupled to Sepharose. After washing, labeled anti-IgE was added. Results illustrate formation of immune complexes containing, apart from allergen, both IgG4 and IgE. Even at very low allergen doses, no indication was found for lag phase that would suggest formation of IgE-free immune complexes. This is indicated by results of parallel experiments with allergen bound either directly to CNBr-activated Sepharose ("direct RAST") or to rabbit antiallergen antibodies coupled to Sepharose ("antigrass catching"). These results support view that, because of epitope specificity different from IgE antibodies, no large fraction of allergen-specific IgG4 antibodies interferes effectively with IgE binding. However, RAST-interference studies show that small fraction of IgG4 antibodies does interfere (1, 3, 5).

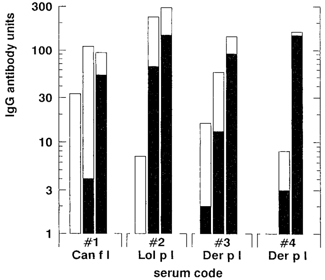

Fig. 3. Follow-up study of patients receiving immunotherapy with dog (patient no. 1), grass pollen (patient no. 2), or house-dust mites (patients nos. 3 and 4). For each patient, the IgG antibody assays are shown before and during immunotherapy. Open bars represent allergen-specific pan-IgG (i.e., strictly speaking, protein A-binding isotypes: IgG1, 2, 4) antibodies; filled bars represent allergen-specific IgG4. Assay was performed with radiolabeled purified allergens (*Can f* I for dog, *Lol p* I for grass pollen, *Der p* I for house-dust mites) in combination with anti-immunoglobulin (protein A or monoclonal anti-IgG4) coupled to CNBr-activated Sepharose. Note that, provided one uses purified allergens, increase in antibody titer, particularly for IgG4, is well over 10-fold.

The second category, patients with high IgG4 titers without clinical benefit, needs more discussion. One subgroup of such patients was identified by Djurup (25): patients with a high IgG4 titer before the start of the immunotherapy. This investigator found that these patients did not improve on subsequent treatment. One possible explanation for this phenomenon might be related to the answer to the question of why these patients had high levels of preexisting IgG4. In view of what is now known about IgG4, it is likely that these patients were highly exposed to the allergen in their environment. It would not be unexpected if patients with high allergen exposure would have less benefit from immunotherapy.

The lack of a tight correlation between IgG antibody titers and a decrease in symptom scores is undoubtedly also caused by the imprecision of determination of the two variables. Symptom scoring is not only imprecise, but it is also easily influenced by other factors, such as infections and additional allergies. IgG antibodies can be measured with a high precision, but these results are often confounded by the inclusion of irrelevant specificities, in measuring the pan-IgG response to a total allergen extract. A far more informative assay is obtained by testing the IgG4 response to purified allergen. This is best illustrated by analyzing the "stimulation index" that can be found upon immunotherapy. With the first type of assay, a high background antibody response is found, which is present in allergic as well as in nonallergic patients. After immunotherapy, some increase can usually be found, but usually not more than three- to fivefold over pretherapy levels. This contrasts to the results obtained with the other procedure: IgG4 against a purified allergen (Fig. 3). Here the background is usually low, and upon successful treatment the antibody level usually increases more than 10-fold and occasionally more than 100-fold.

Using an assay with a high "stimulation index" enables a clear distinction between effective and ineffective treatment in the immunologic sense. Moreover, as indicated before, the response measured is an unmistakable sign that the TH2-arm of the immune system has been reached.

Conclusions

1) Allergen-specific IgG4 is unlikely to be a direct cause of allergic reactions.
2) IgG4 antibody responses are often associated with IgE antibody responses. This is presumably

a reflection of the requirements of IgE-inducing antigens to stimulate TH2-type immune responses.

3) Without immunotherapy, IgG antibodies against atopic allergens are not restricted to the IgG4 isotype: in many cases, IgG1 antibodies are predominant.
4) IgG4 antibodies against allergens are particularly prominent after prolonged immunotherapy, in line with the place of the IgG4 antibody response against other TH2-stimulating antigens.
5) IgG4 antibodies are often found also in nonallergic subjects, particularly after chronic exposure to protein antigens. Early in these responses, IgG1 antibodies usually predominate.
6) Despite the similarity between IgE and IgG4 with respect to the type of antigens recognized, the epitope specificity of IgG4 antibodies is often found to differ from that of IgE antibodies. These two apparently conflicting observations can be explained by assuming that the IgE and IgG4 responses are regulated by the same type of T cells but that distinct B cells may be involved.
7) From both the therapeutic and diagnostic points of view, it is important to be able to focus on IgG4 antibodies with the correct epitope specificity.
8) Classical immunotherapy can be considered to be immunologically effective if a substantial (10–100-fold) increase in allergen-specific IgG4 is induced.

Acknowledgments

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