

The Subclass Nature and Clinical Significance of the IgG Antibody Response in Patients Undergoing Allergen-Specific Immunotherapy

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The purpose of this paper is to discuss the methodological difficulties in quantitation of human IgG subclass antibodies to allergens, to describe the subclass nature of the IgG antibody response in patients undergoing allergen-specific immunotherapy, and to discuss the possible immunological functions and clinical significance of allergen-specific IgG antibodies of different subclasses. Based on results obtained by use of assays with documented specificity it is concluded that the IgG antibody response during allergen-specific immunotherapy is IgG1 and IgG4 restricted, although low levels of IgG2 and IgG3 antibodies to some allergens may occur. In most patients the early IgG antibody response is IgG1 dominated and the late IgG4 dominated. A too early or too pronounced IgG4 dominated antibody response seems to indicate a poor clinical outcome of immunotherapy with inhalant allergens, whereas a pronounced early IgG1 antibody production has been found to be associated with a decrease in synthesis of IgE antibodies to an insect venom. It is therefore proposed that an early IgG1 dominated response is necessary to induce suppression of the ongoing IgE antibody production, which in its turn may be a prerequisite for long-lasting clinical effect. The possibility of induction of an early IgG1 dominated response in every patient by use of alternative immunotherapy procedures is discussed.

Key words: allergen-specific immunotherapy; asthma; IgE antibodies; IgG subclass antibodies; rhinitis; solid-phase immunoassays.

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Introduction

Patients with IgE antibody-mediated allergy to inhalant allergens (e.g. pollens, molds, dust) or to insect venoms are often treated with allergen-specific immunotherapy (formerly called desensitization or hyposensitization). At present, routine immunotherapy is carried out by repeated subcutaneous injections of aqueous, alum-adsorbed/aqueous-extracted or alum-adsorbed/pyridine-extracted allergen preparations (20). The two latter extracts will be called alum-extracts and pyridine-extracts, respectively. Several studies have documented the clinical efficacy of immunotherapy with aqueous extracts of inhalant allergens (55, 61, 62)

and insect venoms (24, 32, 38). Thus, immunotherapy with aqueous extracts of inhalant allergens results in clinical improvement in about two-thirds of patients with allergic rhinitis, whereas placebo treatment only improves one-third (62). Although the beneficial effect of immunotherapy on allergic asthma for many years was considered largely undocumented (62), more recent studies seem to have established that immunotherapy is effective for this condition, too (57, 59). Immunotherapy with high-dose aqueous insect venom preparations seems to protect almost every treated patient (24, 32), whereas only about one half of the untreated patients experienced some spontaneous improvement (24, 73). The available

evidence suggests that immunotherapy with alum-extracts of both inhalant allergens (56, 57) and insect venoms (48) and with pyridine-extracts of inhalant allergens (62) demonstrates the same clinical efficacy as treatment with aqueous extracts. It should be noted that most studies of the clinical efficacy of immunotherapy have focused solely on the short-term effect of the treatment, i.e. on the symptomatic improvement during *ongoing* immunotherapy. What happens when immunotherapy is discontinued, is largely unknown (57, 62).

The present form of immunotherapy is costly, time-consuming and inconvenient to the patient, and carries a significant risk of adverse reactions. Furthermore, although immunotherapy with inhalant allergens offers some improvement to most of the patients, almost no patients become completely asymptomatic, and many patients do not improve at all (59, 61, 62). These drawbacks of the current form of immunotherapy have led to a search for more convenient, less costly, more efficient and safer forms of immunotherapy, and the clinical efficacy of modified extracts (e.g. polyethylene glycol-conjugated extracts, polymerized extracts), other routes of allergen administration (e.g. oral), and modified dose-regimens of subcutaneous injection therapy (e.g. clustered injections) is at present under investigation. In this context it has been recognized (59, 61) that unless the immunological basis for the symptomatic improvement can be more clearly defined, attempts to improve immunotherapy will probably have limited success.

Today, the mechanisms responsible for the clinical efficacy of immunotherapy are only incompletely understood (61, 62). During immunotherapy several immunological changes may be observed (summarized in (68)):

- i) a rise in the concentration of allergen-specific IgG antibodies (asIgG) in serum
- ii) a fall in the concentration of allergen-specific IgE (asIgE) in serum after *prolonged* treatment
- iii) a suppression of the IgE antibody response to seasonal allergen exposure in patients with seasonal inhalant allergy

- iv) a rise in the concentration of allergen-specific IgA (asIgA) and asIgG in nasal secretions
- v) a fall in sensitivity of the basophil leucocytes to the offending allergen
- vi) a fall in lymphocyte proliferation and lymphokine production on exposure to the allergen
- vii) an increase in the activity of allergen-specific T suppressor cells.

Several studies have shown that a marked rise in serum level of asIgG is the most consistent immunological change during immunotherapy with both inhalant allergens and insect venom (12, 24, 32, 42, 43, 44, 57, 85). Furthermore, prolonged immunization with high doses of seasonal inhalant allergens rather consistently leads to a suppression of the secondary IgE antibody response to seasonal inhalant allergen exposure or even to a more permanent decrease in serum IgE antibody level (12, 33, 34, 41, 42, 52, 57). Also, a decrease in serum asIgE seems to be the rule after long-term immunotherapy with insect venoms (11, 25, 85).

Although asIgG and asIgA do occur in nasal secretions from allergic patients, immunotherapy does not result in impressive changes in the concentrations of these antibodies (10, 13, 64, 65).

Turning to the changes in cellular immunity during immunotherapy, a decrease in basophil cell sensitivity to the offending allergen is seen in an appreciable number of the immunized patients (41, 42). The changes in lymphocyte proliferation and lymphokine production and in suppressor/helper T cell activity have been examined in a few studies only (as recently reviewed by Rocklin (68)) and will not be discussed in any details here.

In several of the just cited studies a possible statistically significant association between the immunologic parameters and clinical improvement was sought for. In a few studies (24, 43) serum level of asIgG or the ratio of asIgG to asIgE (32) was found to correlate significantly with symptomatic improvement, but most studies have failed to demonstrate this relationship. At present, there is no evidence that any other

immunological parameter correlates better with clinical improvement than serum level of asIgG does.

Despite the lack of significant correlation between serum level of asIgE (or decrease herein) and symptomatic improvement, several authors have suggested that the often observed fall in asIgE might contribute to the beneficial effect of immunotherapy (25, 32, 35, 41, 50, 85), and that this change even might be the most important mechanism underlying the symptomatic improvement in some patients (38). It has also been suggested that a persistent fall in asIgE may be the most desirable response to immunotherapy with insect venoms (11).

In contrast, there is at present no evidence that local antibodies (asIgG and asIgA in secretions) contribute appreciably to clinical improvement (10, 13, 64, 65). Neither has any significant correlation between decrease in basophil sensitivity and clinical improvement been found (42), although decreased cell sensitivity may contribute to symptom amelioration in some patients.

It has been shown that asIgG formed during immunotherapy can inhibit the IgE antibody-mediated histamine release from basophils *in vivo* and *in vitro* (41), and also, that a rise in asIgG precedes clinical improvement, fall in cell sensitivity and fall in serum asIgE (50). Based on these observations and the occasionally observed significant correlation between serum level of asIgG and symptom amelioration, production of asIgG is considered the most important immunological change during immunotherapy, and it is generally accepted that production of asIgG in some way underlies the clinical improvement experienced by the immunized patients. The presumed beneficial effect of asIgG antibodies has traditionally been explained by their capacity to neutralize the offending allergens before these react with cell-bound asIgE and trigger the allergic reaction. In accordance with this view, asIgG antibodies are often named as "blocking antibodies" (34, 44, 62).

The possibility that asIgG antibodies may exert their beneficial effect by induction of a suppression of the specific IgE antibody produc-

tion has also been considered by several authors (4, 33, 34, 35, 41, 44, 55, 62, 68). However, such a regulatory role for asIgG has not been generally accepted (12, 44, 68).

The understanding of the protective role of asIgG antibodies has been further complicated by the recognition that asIgG antibodies may sensitize some patients. Sensitizing asIgG antibodies are named short-term sensitizing IgG antibodies (STS IgG). Although the existence and clinical significance of such STS IgG has been questioned (86), there is now ample evidence that sensitizing antibodies of the IgG class do occur in some allergic patients (2, 19, 58, 79). However, it should be noted that most, if not all, studies of STS IgG have been limited to asIgG antibodies formed as response to natural exposure to allergens. Thus, it has not been documented that immunotherapy may result in appearance of sensitizing IgG antibodies, although this possibility must clearly be taken into consideration.

It thus appears that asIgG antibodies may take part in at least three different processes: i) allergen-neutralizing, ii) inhibition of IgE antibody production, and iii) sensitizing of the effector cells for the allergic type I reaction. Animal experiments have shown that IgG antibodies of different subclasses may possess different immunological functions (7, 89). It is therefore reasonable to assume that human IgG antibodies of different subclasses may differ in immunological functions, too. This possibility has stimulated several investigators, including ourselves, to attempt to characterize the IgG subclass antibody response in patients undergoing allergen-specific immunotherapy (1, 14, 15, 16, 17, 18, 23, 77, 87, 88, 91).

In this review I shall briefly summarize the more important biological properties of the four human IgG subclass proteins, discuss some of the technical difficulties in quantitation of serum asIgG subclass antibody levels, and outline the available data concerning the subclass nature of the IgG antibody response to various inhalant allergens and insect venoms. Thereafter, some of our own results concerning the relationship between the IgG subclass antibody response during immunotherapy and the

clinical effect of this treatment, and between the antibody response in the IgG subclasses and the IgE class, will be discussed. Finally, I will present some speculations about the possible roles for asIgG antibodies of different subclasses and also discuss the usefulness of asIgG and asIgG subclass quantitation in the evaluation of new kinds of immunotherapy and in the monitoring of the treatment of the individual patient.

The four IgG subclasses

Based on antigenic differences in the heavy chain, human IgG molecules can be divided into four subclasses (IgG1, IgG2, IgG3, and IgG4) (54). The IgG subclasses are isotypes, i.e. all members of the human species have IgG proteins of all four subclasses in their blood. (In contrast, a given IgG allotype occurs in the blood of only some individuals). The antigenic differences between the four subclasses may be due to differences in primary amino acid sequence or carbohydrate composition (45). The available evidence seems to favorize the former possibility (45). Also, genetical analysis has shown that each of the four different human γ -chains is encoded by a single, separate gene (21, 74). Thus, these genetic experiments strongly suggest that "undiscovered" IgG subclasses do not exist.

The distribution of the concentrations of the IgG subclass proteins in normal human serum is approximately: 66 % IgG1, 23 % IgG2, 7 % IgG3, and 4 % IgG4 (54). If the distribution of IgG antibody activity on subclasses is significantly different from this ratio, the IgG antibody response is said to be subclass selective or subclass restricted (54).

Differences in biological activities of the four subclasses have been excellently reviewed by others (54, 78) and only a few points need to be mentioned here. Complement activation through binding of C1q is most efficiently exerted by IgG3 proteins, and the affinity for C1q decreases in the order IgG3 > IgG1 > IgG2 > IgG4. In most experimental systems, IgG4 does not bind C1q at all. IgG1 and IgG3 bind more efficiently than IgG2 and IgG4 to

Fc-receptors on lymphocytes, monocytes, and neutrophils (54, 78). In some assay conditions IgG2 and IgG4 apparently do not bind to these cell types at all. In contrast, the four subclasses have identical affinity for the Fc-receptors on the eosinophils (37). Recent experiments have provided strong evidence that IgG4 proteins can bind to basophils (19), whereas no definitive evidence for binding of the other three subclasses to this cell type has been presented (2, 19).

Quantitation of allergen-specific IgG subclass antibodies

Whereas most studies of asIgG antibodies have made use of liquid-phase assays, the published methods for measurement of allergen-specific antibodies of all four IgG subclasses have been based almost entirely on solid-phase techniques (14, 17, 18, 23, 88, 91), and most of the following discussion will therefore be confined to solid-phase assays. In this type of assays, the allergen extract is made insoluble by chemical coupling or physical adsorption to a solid surface (e.g. red blood cells, Sepharose particles, paper discs, plast tubes).

Ideally, an assay for measurement of asIgG subclass antibodies should possess at least the following characteristics: i) allergen-specificity, ii) subclass-specificity, iii) equipotency, iv) high sensitivity, v) good precision, and vi) lack of competition between antibodies of different subclasses.

Allergen-specificity is best documented by preincubation of positive sera with solutions of increasing concentrations of the relevant allergen (i.e. the allergen immobilised on the solid surface) and of several other irrelevant allergens. Among the latter, closely related allergens should be included (i.e. honey bee venom when testing the specificity of an assay for yellow jacket venom). In some cases soluble complexes formed during the preincubation may interfere in the solid-phase assay and make interpretation of the results and documentation of the allergen-specificity difficult. This problem can usually be overcome by polyethylene glycol

precipitation of the complexes before testing of the supernatant for any remaining allergen-specific IgG antibodies (Djurup, unpublished results). To further elucidate the specificity of the assay, data concerning the asIgG subclass antibody level in sera from untreated patients and from normal subjects should be presented.

The subclass specificity of a solid-phase assay may be assessed by soluble inhibition with purified myeloma proteins of the four subclasses, using insolubilized myeloma proteins as antigen for the subclass-specific antibodies. However, it is more straightforward and gives more valuable information to test this kind of specificity in the same type of assay (i.e. a solid-phase assay) as that used for the IgG subclass antibody quantitation (17). Also, this approach has been considered more correct (8). Thus, antibodies which have been found specific in, e.g. liquid-phase assays need not be specific in solid-phase assays (8, 17), in which high antigen density may uncover cross-reactivity not apparent at lower antigen density (e.g. in liquid-phase assays) (70).

The development of reliable methods for quantitation of asIgG subclass antibodies has largely been restricted by the scarcity of good animal antibodies with absolute specificity for the four human IgG subclasses (2). Appearance of commercially available subclass-specific, monoclonal antibodies (mAbs) has meant a great advance in this respect. We have tested the reagents from one commercial supplier rather extensively and found them truly subclass-specific in two different solid-phase-assays (15, 17) and a liquid-phase assay (to be published). However, it should be noted that mAbs to human IgG subclass proteins may behave very differently in different assays (27, 76), for which reason their specificity (and presumed reactivity with the complementary subclass protein) should be confirmed in any new or modified assay.

Equipotency implies that equivalent concentrations (e.g. in mol/l) of allergen-specific antibodies in the different subclasses result in the same direct signal (e.g. bound radioactivity (cpm)) or the same transformed signal (e.g. U/l) (8, 14, 17). In animal model systems it has

been possible to obtain perfect equipotency over the whole dose-response range (8). We have shown that it is possible to equalize the maximal signals by use of different dilutions of mAbs in an assay for human IgG subclass antibodies (17). Also, preliminary results indicate that equalizing of the maximal signals ensures equipotency in the whole working range of the assays for IgG1 and IgG4 antibodies (15). However, as we have not yet found significant amounts of IgG2 or IgG3 antibodies to allergens (see below), we do not know whether it is possible to obtain equipotency in all four subclass assays with these mAbs which have widely different affinities. Development of mAbs with identical affinity for each of the four subclass proteins should be possible and use of such antibodies should ensure equipotency over the whole dose-response range.

To obtain equipotency, Wahlgren et al. (90) have used another approach: They adsorbed increasing concentrations of a mixture of two myeloma proteins (one κ and one λ -protein) of each of the four subclasses to separate microtiter wells and used the thus physically adsorbed myeloma proteins to produce a standard curve for each subclass assay. To give valid results, this approach requires that the affinity between an allergen-bound IgG subclass antibody and a subclass-specific mAb on one hand, and between the physically bound IgG myeloma proteins and the mAb on the other, is identical. Also, the reaction kinetics of the two different kinds of interaction must be identical (92). These assumptions are probably not satisfied: Thus, when we compared the affinity between covalently bound IgG subclass proteins and the mAbs, and between physically adsorbed IgG subclass proteins and mAbs, we found that physical adsorption led to a marked decrease in the relative affinity of the mAb with specificity for IgG4 (unpublished results). Also, the relative affinities of the other mAbs were changed, although less dramatically. In agreement with this observation, Bird et al. (5) have recently found that a monoclonal antibody with specificity for the human IgG allotype G1m(f) lost its specificity when tested against IgG adsorbed directly on a polystyrene surface (but not when

tested against IgG bound through an anti-IgG antibody). Probably, such change in affinity or loss of specificity is due to conformational changes induced by adsorption of the IgG to the surface (5). In this context it should be borne in mind that a monoclonal antibody usually is specific for one single epitope, and that affinity and/or specificity may be severely altered by even a small change in conformation.

High sensitivity is necessary to ensure detection of low levels of asIgG subclass antibodies, i.e. to distinguish between absolute and relative subclass-restriction. Also, high sensitivity permits use of high dilutions of patient's sera, which is necessary to ensure allergen excess (a prerequisite for achievement of parallel serum dilution curves) and to avoid competition between antibodies of different subclasses (17, 90). Sensitivity is related to non-specific binding, and achievement of high sensitivity requires minimizing of non-specific binding. We have found (15) that use of a non-porous, smooth sorbent (e.g. a polystyrene surface) and a buffer with heterologous IgG proteins and moderate to high ionic strength results in very low non-specific binding. Inclusion of Tween in the incubation buffer may further lower the non-specific binding to some allergens and possibly increase precision (unpublished results). The use of a blocker (e.g. gelatine, bovine serum albumin) is *not* recommended, as this may increase non-specific binding, decrease specific binding, and cause unwanted cross-reactions (unpublished results).

None of the assays for asIgG subclass antibodies which thus far have been developed, permits quantitation of the asIgG subclass level in absolute units (e.g. pmol/l). The well-known methods for such standardization of solid-phase assays are: i) Use of a reference serum calibrated by a liquid-phase assay (40, 46), ii) purification of allergen-specific antibodies on an allergen-sorbent column (63), iii) batchwise elution of solid-phase bound asIgG (the RAST elution technique (28, 72), and iv) use of insolubilized non-Ab IgG as reference (90, 92). However, each of these methods has its limitations. Thus, liquid-phase and solid-phase assays may measure different populations of antibodies

(46, 66). Elution from allergosorbents in column experiments is almost always incomplete (63), and the eluted fraction cannot be considered representative of the whole antibody population. Use of non-Ab immunoglobulin bound to the solid-phase by an anti-immunoglobulin may in certain conditions serve as a valid reference system for quantitation of IgG antibodies in absolute units (92), but the validity of this approach for absolute quantitation of asIgG subclass antibodies has not been explored. By use of the RAST elution technique (28, 72) or some modification hereof (47), it should be possible to standardize solid-phase assays for measurement of asIgG subclass antibodies. However, in preliminary experiments we found no significant elution of IgG4 antibodies at the alkaline pH applied in (28). Very likely, modification of the elution conditions may overcome this technical problem.

Before standardization of the assays has been obtained, the sensitivity of the various assays cannot be quantitatively compared. However, as a provisional measure for the sensitivity, the titer of the most potent human serum found should be indicated. Also, the ratio of specific to non-specific binding may give an impression of the sensitivity of the assay.

The precision of the assay should be evaluated according to established criteria. As immunotherapy is often carried out for several years, it is usually impossible to use the same allergosorbent batch for monitoring of the antibody response during the whole course. Therefore, the interassay coefficient of variation should be evaluated by use of at least two different batches of allergen preparation and/or allergo-sorbents.

The observation that IgG1 binding did not reach a plateau in presence of very high IgG4 antibody level (and vice versa) (15, 17) suggests that competition between asIgG antibodies of different subclasses for the available antigenic determinants does not occur in highly sensitive solid-phase assays, in which the patient's sera are tested in high dilution (1:100 or more (15)).

Several methods for measurement of asIgG subclass antibodies have been published. In Table 1, I have summarized the performance

characteristics of these assays as regards some of the above discussed criteria. We have applied our own assay (named TIRA (three-layer immunoradiometric assay)) to detailed studies of the IgG subclass antibody response in patients undergoing immunotherapy with grass pollen extract (16) or yellow jacket venom (15). By use of this technique and a crossed-radio-immunoelectrophoresis technique (CRIE) (77), we have also performed preliminary studies of the IgG subclass antibody response in patients immunized with mite extract, birch pollen, and the mold *Cladosporium herbarum*. Based on the results gained by these studies and the available evidence from other authors, the subclass nature of the IgG antibody response during allergen-specific immunotherapy will now be discussed. However, only results from studies in which the subclass specificity of the animal anti-human IgG antibodies was documented, or at least commented upon, will be included. Also, only results from studies dealing with the response in all four subclasses will be discussed.

The subclass nature of the IgG antibody response during immunotherapy

We have studied the IgG subclass antibody response to an inhalant allergen, timothy grass pollen (16), and to a parenteral allergen, yellow jacket venom (15), in some details.

In 19 patients immunized according to a conventional dose-increase schedule with an alum-extract of timothy grass pollen for 2 years and 3 months, we found an IgG1 and IgG4 restricted response (16). Thus, no IgG2 or IgG3 antibodies were found in any of the serum samples. As some carbohydrate antigens and glycoproteins, according to some authors (26), may bind poorly to plast surfaces, we considered it of interest to confirm the observed subclass restriction by use of a technique not relying on physical adsorption to plast. Using a recently developed CRIE technique for characterization of the IgG subclass antibody response to the individual allergens in complex allergen mixtures (77), we have confirmed that the IgG antibodies to timothy grass pollen formed in these patients belonged solely to subclass one

Table 1
Characteristics of published assays for measurement of asIgG subclass antibodies¹

Authors	Assay type ²	Allergen	Specificity		Equipotency	Precision ³
			Allergen	Subclass		
Devey et al. (14)	RCLAAR	grass pollen	+	+	+	n.m.
Djurup et al. (15, 17)	TIRA	grass pollen	+	+	+	18-27
		yellow jacket venom	+	+	+	15-44
Etievant et al. (18)	ELISA	house dust	n.d.	n.d.	n.e.	n.m.
Giessen et al. (23)	DASS	grass pollen	IgG1 & IgG4: + IgG2: (-)	+	(+)	n.m.
Urbanek et al. (87, 88)	ELISA	bee venom	(+)	n.d.	n.e.	n.m.
Zanussi et al. (91)	ELISA	grass pollen	n.d.	n.d.	n.e.	n.m.

¹ Only assays for measurement of antibodies of all four subclasses have been included.

² Abbreviations: DASS: defined antigen substrate spheres; ELISA: enzyme-linked immunosorbent assay; RCLAAR: red cell linked antigen-antiglobulin reaction; TIRA: three-layer immunoradiometric assay.

³ Precision is stated as interassay coefficient of variation (per cent).

n.d.: not documented; n.e.: not evaluated; n.m.: not mentioned.

+ : The assay has the property.

(+): The assay probably has the property, but documentation was inadequate.

(-): The assay probably does not have the property.

and four (Søndergaard, Djurup, Østerballe & Weeke, to be published).

Before initiation of treatment, one patient had low levels of both IgG1 and IgG4 antibodies, whereas another two patients had low levels of IgG1 antibodies only (16). During immunotherapy all except one patient had an increase in both IgG1 and IgG4 antibodies. In the early course the antibody response was IgG1 dominated. Thus, the median of the IgG1 antibody concentration peaked after only 12 weeks, when the ratio between IgG4 and IgG1 antibody medians was 0.44. A second, slightly lower peak of IgG1 antibody level was reached after 2 years and 3 months, i.e. at the end of the immunotherapy. At this time the IgG4/IgG1 antibody ratio was 2.32. The IgG4 antibody level continued to rise as long as allergen-specific immunotherapy was carried out, and accordingly the IgG4 antibody level reached its peak at the end of the treatment. One year after termination of immunotherapy, six patients still had significant levels of both IgG1 and IgG4 antibodies, whereas seven patients had IgG1 antibodies only, and three patients IgG4 only. The antibody levels at this time were significantly lower than at the end of treatment (16).

Our results concerning the subclass restriction of the IgG antibody response to grass pollen are in partial agreement with those of Devey et al. (14), who found very low, probably insignificant, levels of IgG2 and IgG3 antibodies. Van der Giessen et al. (23) also found an IgG4 dominated response in patients immunized for 2 years with grass pollen. In this study, low levels of both IgG1 and IgG2 antibodies were seen in some sera. The data presented indicated that the IgG2 antibodies detected were allergen-nonspecific. Thus, the binding of the IgG2 antibodies in the only serum with high IgG2 antibody level could not be inhibited by soluble grass pollen allergen, and the same IgG2 binding was found, when the serum was reacted with Sepharose without allergen (23). In this context it is of interest that Hamilton & Adkinson (29), in another solid-phase assay using Sepharose-based allergosorbents, have found that about 10% of normal

sera display non-specific binding. Apparently, this binding is due to IgG anti-Sepharose antibodies (30). As human IgG antibodies to pure carbohydrate antigens are mainly, if not only, of the IgG2 subclass (22), the IgG2 antibodies found in the study of van der Giessen et al. (23) might have been directed against the Sepharose-sorbent. In summary, the available evidence indicates that the IgG antibody response in patients undergoing immunotherapy with grass pollen is IgG1 and IgG4 restricted, and in the late course dominated by IgG4 antibodies.

In 31 patients undergoing three different kinds of immunotherapy with aqueous- and alum-extracts of yellow jacket venom we also found an IgG1 and IgG4 restricted response (15). Before initiation of therapy, 12 patients had significant levels of both IgG1 and IgG4 antibodies, 11 of IgG1 antibodies alone, and two of IgG4 antibodies alone. In some patients pretreatment levels of IgG1 and/or IgG4 antibodies were high. This indicates that natural exposure to yellow jacket venom in the form of a single sting may induce a marked antibody production. It is especially interesting that several patients had high IgG4 antibody levels before the artificial immunization was begun. Thus, as will be discussed later, a marked IgG4 antibody production is usually the result of prolonged and persistent antigen stimulation. The presence of high IgG4 antibody level before initiation of immunotherapy might be due to a peculiarity of the yellow jacket venom allergen, to the natural way of exposure (i.e. subcutaneous injection), or to a genetic predisposition to form IgG4 antibodies in the patients concerned. Our data seem to favour the latter possibility. Thus, all four patients with a high initial IgG4 antibody level (arbitrarily defined as more than 10 times the detection limit of the assay) experienced a further marked increase during artificial immunization. The "final" level (the patients are still undergoing immunotherapy) was thus from 1.4 to 20 times higher than the initial level, and – with one exception – the highest final levels in the treatment group in question. The low rise was observed in patients given a treatment

favouring IgG1 antibody production (see below). One of the patients with high initial IgG4 antibody level had an atopic predisposition, whereas the other three patients were non-atopic.

In the 10 patients receiving a conventional dose-increase regimen with alum-extract, both IgG1 and IgG4 antibody levels have been rising steadily in the 2 years we have thus far followed these patients. After 6 months of treatment the IgG response became IgG4 dominated, and after 2 years the IgG4/IgG1 antibody ratio was 2.4.

In the nine patients receiving clusters of aqueous venom fortnightly during the dose-increase phase, the IgG1 antibody response was very pronounced during the first 2 months, and after 6 months was still dominating (IgG4/IgG1 antibody ratio 0.7) (15). In another group, receiving clusters of aqueous venom weekly during the dose-increase phase, the IgG1 and IgG4 antibody response closely paralleled each other during the first 6 months (15). (The patients in the two latter groups have at present been followed for 6 months only.)

Although the patients receiving clustered injections fortnightly during the initial phase did not match perfectly with the patients in the two other groups (15, 48), our results indicate that it may be possible to modify the balance between IgG1 and IgG4 antibody production by variations in the form of the allergen presentation (aqueous or alum-bound) or – more likely – by variation in the dose regimen. Especially interesting is that of the four patients with high initial IgG4, the two given clustered injections fortnightly had the least pronounced increase in IgG4 antibody level. This observation, although based on only four patients, invites exploration of the possibility that clustered injections given every second week (or with longer intervals) may override the apparent predisposition of some individuals to preferential IgG4 antibody production.

As mentioned, we have up to now only performed detailed studies of the IgG subclass antibody response to grass pollen and yellow jacket venom. However, our preliminary studies of the antibody response to birch pollen,

mite extract, and the mold *Cladosporium* have indicated that the IgG antibody response to these allergens is dominated by IgG1 and IgG4 antibodies too, although low to moderate levels of IgG2 and IgG3 antibodies to *Cladosporium* have been found in a few sera. However, as the performance characteristics of the assays for IgG subclass antibodies to mite extract and *Cladosporium* have not yet been rigorously tested and only a small number of sera examined, these preliminary results must be cautiously interpreted.

Aalberse et al. (1) have found an IgG1 and IgG4 restricted response to the purified major allergen (phospholipase A₂) of honey bee venom. Furthermore, these workers also found a shift from an IgG1 dominated response to an IgG4 dominated response in bee keepers who were repeatedly stung.

Clinical significance of allergen-specific IgG subclass quantitation

Clinical efficacy of immunotherapy with inhalant allergens is difficult to evaluate (3, 62), but may be assessed by comparison of symptom scores in a placebo group and in a treatment group, or by comparison of symptom scores before, during, and after treatment, using the patients as their own controls. Clinical efficacy of immunotherapy with insect venoms has in recent years been evaluated by a deliberate sting challenge.

To evaluate the predictive value of allergen-specific IgG subclass antibody levels in patients undergoing immunotherapy with an inhalant allergen, we have calculated the coefficients of correlation between preseasonal levels of IgG subclass antibodies to grass pollen and the symptom scores in the subsequent pollen season (16). At the 5 % level, no significant correlation between these parameters was observed. However, at the 10 % level, the preseasonal levels of IgG4 antibodies after 1 and 2 years of immunotherapy showed a positive correlation to the symptom scores in the subsequent seasons. As another study of the same patients had indicated that the very early immunological changes might have predictive value for the

final clinical result (57), we also assessed the interrelationships between the levels of IgG subclass antibodies reached after 12 weeks and the symptom scores in the first season after termination of therapy (16). A high IgG4 antibody level or a high IgG4/IgG1 antibody ratio at this time was found to be a highly sensitive and specific marker for a poor clinical result.

Preliminary studies of the IgG subclass response to *Cladosporium herbarum*, using the semi-quantitative CRIE technique, have indicated that patients with the most pronounced IgG4 antibody response have the poorest clinical result after 1 year of treatment (Malling et al., to be published).

In summary, these findings indicate that both an early achievement of a high IgG4 antibody level (or a high IgG4/IgG1 antibody ratio) and a high and persisting IgG4 antibody production against an inhalant allergen may augur a poor clinical result. The possible immunological mechanisms underlying these associations will be discussed in the next paragraph.

Various regimens of high-dose insect venom immunotherapy offer 95–100 % protection against anaphylaxis as long as the treatment is continued (24, 48). As pointed out previously (15), this fact renders any laboratory evaluation of the clinical efficacy of ongoing high-dose venom immunotherapy almost superfluous, but monitoring of the IgG antibody response may be valuable in patients undergoing low-dose regimens (15, 24).

Recently, it has been proposed that a persistent fall in IgE antibody level might well be the most desirable result of venom immunotherapy (11), and that it may be justifiable to discontinue immunotherapy, at least temporarily, when IgE antibodies to the venom cannot be detected *in vitro*. Also, the just cited study and studies by Kemeny et al. (38) and Graft et al. (25) indicate that some patients undergoing venom immunotherapy actually may be cured of their disease, as the IgE antibodies may disappear by prolonged immunization. It would therefore be of interest to identify the factors which determine the suppression of the IgE antibody level. In this

context we have found that a high IgG1 antibody level early in the course of immunotherapy was associated with a rapid decline in IgE antibody level (15). At present we do not know whether this association was induced by the immunotherapy regimen (clustered injections of aqueous extract fortnightly) or was due to a genetic predisposition in this group of patients. However, this observation calls for further studies of the relationships between the IgG subclass antibody levels and the IgE antibody level in patients undergoing clustered and single injection immunotherapy, given with different time intervals.

Possible functions of allergen-specific IgG1 and IgG4 formed during immunotherapy

Aalberse et al. were the first to suggest that chronic antigen stimulation generally results in an IgG4 dominated antibody response (1, 36). Several lines of evidence seem to confirm this hypothesis. Thus, we (15, 16) and others (23) have found that prolonged immunotherapy with both inhalant and parenteral (e.g. insect venoms) allergens result in an IgG4 dominated response in most of the patients. Also, IgG4 antibodies are disproportionately increased in patients with chronic parasite infestations (36), and antibodies to factor VIII in hemophilic patients are almost only of the IgG4 isotype (67). Furthermore, recent studies have indicated that autoantibodies to thyroglobulin also mainly belong to the IgG4 subclass (84), but apparently this does not apply to all kinds of autoantibodies (60). Thus, in general, prolonged and persistent (16) antigen stimulation of the immune system seems to cause an IgG4 dominated antibody response. As the IgG4 antibodies do not have appreciable affinity for complement or for Fc-receptors on lymphocytes, neutrophils or macrophages, they are unable to initiate processes that actively lead to disposal of the offending antigen and to an inflammatory reaction. As long-lasting inflammatory reactions may be deleterious to the host organism, it is tempting to speculate that one biological function of IgG4 antibodies may be to protect the organism against immunological

damage at continued antigen exposure (16, 36, 51). In accordance with this view, dominance of the late phases of a humoral immune response by IgG4 antibodies may be considered a normal phenomenon, occurring in both atopic and non-atopic persons. The beneficial role of IgG4 antibodies formed during allergen-specific immunotherapy may be to neutralize the allergens before they reach the cell-bound IgE antibodies, i.e. IgG4 antibodies may serve as blocking antibodies in the traditional sense. The available evidence suggests that IgG4 antibodies formed during immunotherapy with *insect venoms* actually perform such function. Thus, it has been demonstrated that all patients achieving a certain level of IgG antibodies are protected against sting-induced anaphylaxis during ongoing immunotherapy (24). This indicates that the antigen neutralizing capacity *per se* is responsible for the clinical protection obtained during *ongoing* venom immunotherapy. As most of the antigen neutralizing capacity apparently is due to IgG4 antibodies, it is reasonable to assume that these antibodies perform a beneficial, blocking role during ongoing insect venom immunotherapy.

In contrast, as reviewed at the beginning of this paper, most studies have failed to demonstrate a statistically significant association between the IgG antibody level (or the antigen neutralizing capacity) obtained by immunotherapy and the degree of symptom amelioration in patients suffering from *inhalant allergy*. In this kind of allergy the allergic reactions mostly take place on the mucosa of the eyes, nose, and lungs. The effector cells in the mucous membranes are probably largely inaccessible to serum IgG antibodies. Thus, at least in nasal secretions, the rise in asIgG is not appreciable and does not parallel the rise in asIgG in serum (10, 64), indicating that asIgG in nasal secretions may be locally produced. Also, the antigen neutralizing is too slow and of too low capacity to be effective in preventing allergens from releasing histamine *in vivo* (10). It is therefore unlikely that the antigen neutralizing capacity *per se* of serum IgG antibodies of either subclass plays any significant role in symptom amelioration during immunotherapy with inha-

lant allergens. As further support for this assertion, we failed to observe any significant negative correlation between preseasonal levels of either subclass and the seasonal symptom score (16). It may therefore be concluded that the allergen neutralizing capacity of IgG4 antibodies largely is without beneficial effect in patients undergoing immunotherapy with inhalant allergens.

In contrast, the results summarized in this review suggest that IgG4 antibodies formed during immunotherapy with grass pollen or *Cladosporium* may be deleterious in patients demonstrating: (i) high initial IgG4 antibody production, (ii) an initial IgG4 dominated response, or (iii) a very pronounced IgG4 antibody production during the whole course (see above). At least two different explanations for these findings are apparent. Thus, accepting that production of IgG1 antibodies may mediate a beneficial immunoregulation, a too early or too marked IgG4 antibody production may indirectly act deleteriously, e.g. by preventing formation of regulatory (tolerogenic) IgG1 antibody-allergen complexes (as discussed below). Another possibility is that IgG4 antibodies formed during immunotherapy may sensitize mast cells and basophils in some patients. Thus, it is now almost generally accepted (2, 19, 79) that IgG4 immunoglobulins may bind to basophils in some individuals and cause histamine release when cross-linked by anti-IgG4 antibodies. However, it still remains to be unequivocally shown that formation of IgG4 antibody-allergen complexes on the surface of mast cells and/or basophils can induce histamine release (58).

Turning to the role of IgG1 antibodies formed during allergen-specific immunotherapy, I first want to review very briefly the evidence from animal experiments that IgG antibodies with affinity for Fc-receptors on lymphocytes and macrophages (to be called cytophilic IgG antibodies) may take part in suppression of the IgE antibody response. Thereafter, the evidence that immunoregulation of this kind actually occurs in allergic patients undergoing immunotherapy will be discussed.

Studies performed by Borel's group have

shown that only cytophilic subclasses of IgG efficiently mediate tolerance when tolerance induction is attempted by use of low doses of tolerogen (7). Also, these and other studies have shown that the Fc fragment is essential for some kinds of tolerance induction (7, 75). There has been some controversy as to whether T cells participate in such isologous IgG-induced tolerance. However, recent experiments within the field of contact allergy have provided evidence that haptens bound to isologous IgG may induce formation of T suppressor cells (49). It has also been shown that injection of antigens (allergens) coupled to isologous IgG may induce suppression of an ongoing (secondary) IgE antibody production, and that T suppressor cells take part in this process (39). While these experiments combined provide strong evidence that injection of haptens or antigens coupled covalently to isologous IgG of cytophilic subclasses may specifically suppress the IgE antibody production to the hapten or antigen in question, they do not elucidate the possible immunoregulatory role of IgG antibodies formed *in vivo*. As support for such regulatory role for IgG antibodies it has been shown that passive administration of IgG antibodies can suppress the IgE antibody production (80, 81). Furthermore, Takatsu & Ishizaka (82) noted a significant rise in the IgG antibody titer, occurring simultaneously with or before the IgE antibody suppression. Based in particular on the results of this study, in which the immunization procedure in many aspects was similar to the clustered regimen applied in one of our patient groups (15, 48), but also on the other evidence summarized above, I would like to propose a model for an IgG antibody mediated suppression of IgE antibody suppression in allergic animals and patients:

Immunotherapy first results in a marked production of asIgG antibodies. If these are of a cytophilic subclass (in the human species subclass 1 or 3), they may form IgG antibody-allergen complexes, which may bind to and stimulate T suppressor cells. (T suppressor cells are presumed to carry Fc receptors for IgG (9)). The target of the activated T suppressor cells is T helper cells (82), which become inactivated.

The lack of T helper activity suppresses the ongoing IgE antibody production, probably by prevention of expansion of the B_e memory cell population (82). The reason why B_e cells are primarily affected by this regulatory process may be that these cells are more susceptible than B cells of other isotypes to both activating and suppressing influences (35, 82), but other explanations are possible (35, 82).

Several studies have documented that long-term immunotherapy with inhalant allergens results in blunting of the expected IgE antibody response on seasonal exposure and often an all-year suppression of the IgE antibody production as well (12, 35, 57). Also, in many patients undergoing venom immunotherapy, the IgE antibody level decreases steadily, and eventually the IgE antibody against the venom concerned may disappear completely (11, 25, 85). There is, thus, no doubt that immunotherapy generally has a suppressive effect on the ongoing IgE antibody production in allergic patients. However, the role of this decrease in the serum IgE antibody level in the symptom amelioration, and the mechanism(s) underlying the suppression of the IgE antibody production are subject to debate. Thus, as argument against a role for the decreased IgE antibody level in the clinical improvement, it has been stated that the level of serum IgE antibodies usually does not correlate with the degree of symptom amelioration (68). The role of the IgG antibody production in IgE antibody suppression has been questioned on the grounds that an inverse relationship between the rise in IgG antibody concentration and a decrease in IgE antibody level are not generally observed (12). Considering that the presumed decrease in T helper activity occurring during immunotherapy may decrease not only the concentration of IgE antibody, but also the affinity of the antibodies (83), it cannot be excluded that the changes in the IgE antibody production induced by immunotherapy may add significantly to the clinical improvement. In fact, the combined evidence from several recent studies of long-term immunotherapy with insect venoms suggests that some patients, especially perhaps the younger ones, actually become cured of their

disease as a result of suppression of the IgE antibody production (11, 25, 85). Also, the level of IgE antibody seems to be an important marker for the clinical sensitivity in the majority of patients allergic to honey bee venom (32). While the significance of the decrease in IgE antibody level for the clinical improvement during inhalant immunotherapy admittedly at present is questionable, it should be noted that some studies have found a good correlation between basophil responsiveness and IgE antibody level (34, 41).

Turning to the role of IgG antibodies in mediating the suppression of the IgE antibody synthesis, some studies have found an inverse relationship between the rise in IgG antibody level and the decrease in IgE antibody level (34, 42). Also, the decrease in IgG antibody production apparently always precedes the decrease in IgE antibody synthesis (12, 16, 34, 50). While this temporal relation may be taken as an indication of an IgE suppressive function of the IgG antibodies formed during immunotherapy, an inverse relationship between the levels of these two antibody classes is for two reasons not generally to be expected. First, as reviewed above, only IgG1 (and not IgG4) antibodies have the capacity for the cellular interactions necessary for initiation of the immunoregulation. Second, if the regulatory subclass acts through formation of (cell-bound) antibody-allergen complexes (as presumed in our model), the ratio between allergens and antibodies in the complexes becomes very important in determining the immunoregulatory potential of the antibodies (31, 75). Thus, not even the concentration of the regulatory subclass alone can be expected to show an inverse relationship to the IgE antibody level.

As evidence for a suppressive effect of IgG antibody-allergen complexes, Bernstein et al. found that combined passive (with IgG anti-ragweed) and active (with ragweed pollen) immunization resulted in a marked suppression of the seasonal rise in IgE antibodies as compared with the response in patients receiving active immunization alone (4).

In support of the regulatory role of IgG1 antibodies, we have recently found that clus-

tered injections of yellow jacket venom given fortnightly resulted in a pronounced rise in IgG1 antibody concentration followed by a marked decrease in IgE antibody level (15). Thus, in this group the IgE antibody level had declined significantly (at the 10 % level) after only 6 months of treatment. In two other groups, in which the initial IgG1 and IgG4 antibody responses were closely parallel, the antibody level had risen significantly, or was unchanged, after 6 months. In the group showing the increase in IgE antibody concentration the IgG antibody response tended to be IgG4 dominated in the latter half of the observation period. While these observations taken together thus point to a causal relationship between the increase in IgG1 antibody production and the decrease in IgE antibody synthesis, they do not indicate whether it is possible to induce such immunoregulation in every patient. The three patient groups were similar as regards sex distribution, atopic predisposition and number of patients with high initial IgG4 antibody level, but differed in age and interrelationships between pretreatment levels in the three antibody classes (15). Due to these differences, a particular susceptibility to suppressive influences on the IgE antibody synthesis in the group experiencing the rapid decline in IgE antibody concentration cannot be ruled out, and further studies are needed to elucidate whether clustered injections of aqueous allergens given every other week (or at even longer intervals) in general induce this favourable antibody response. As persistent antigen stimulation apparently is necessary to obtain an IgG4 dominated response, I consider it likely that long intervals between the injections would favour an IgG1 dominated response.

Also, high dose immunization often promotes tolerance induction, and clustered injections might therefore be better than single injections for the purpose of suppressing the IgE antibody response.

The aforementioned observations do not elucidate the cellular mechanisms underlying suppression of IgE antibody synthesis during immunotherapy. However, as immunotherapy has been shown to result in formation of

allergen-specific T suppressor cells (69), the mechanisms underlying this phenomenon may closely resemble those suggested above for the animal model systems.

In summary, allergen-specific IgG1 antibodies formed during immunotherapy are considered to take part in suppression of the IgE antibody production. By virtue of their antigen neutralizing capacity they may also function as blocking antibodies during immunotherapy of patients allergic to parenteral allergens (see above). The deleterious effect of a too early or too pronounced IgG4 response may be explained by the capacity of these antibodies to prevent interaction between IgG1 antibodies and allergens, and, thus, to prevent formation of tolerogenic complexes.

Conclusions and perspectives

By use of monoclonal antibodies against human IgG subclass proteins it is now possible to quantitate allergen-specific IgG antibodies of each of the four subclasses without significant interference from antibodies of other subclasses or classes. We still do not know whether it is possible to obtain perfect equipotency over the whole dose-response range by use of the monoclonal reagents available today. As equipotency is easily obtained with antibodies of identical or nearly identical affinity, the producers should attempt to develop such antibodies for solid-phase assays. Furthermore, the assays for subclass antibody quantitation also need to be standardized in order to permit comparison of results from different studies. The solid-phase elution technique, or some modification hereof, appears most promising for this purpose.

The IgG antibody response during allergen-specific immunotherapy appears to be IgG1 and IgG4 restricted, but we have found low to moderate levels of IgG2 and IgG3 antibodies to some allergens. Conceivably, immunization with allergens containing pure carbohydrate antigens, or glycoprotein antigens with high carbohydrate content, may result in a marked IgG2 antibody response, but this has not yet been observed.

Our studies have indicated that too early

IgG4 response or a too pronounced IgG4 response in either the early or the late phase of the immunotherapy may predict a poor clinical result. As an early marker for the clinical outcome of the treatment would be extremely useful, future studies should attempt to establish the predictive value of the early IgG4 antibody response more firmly. In this context it must be kept in mind that the mechanisms underlying clinical improvement may be different for different types of allergens (inhalant allergens, parenteral allergens), and that one function of the antibodies (e.g. antigen neutralizing) may be responsible for the protection achieved during ongoing immunotherapy, whereas another function (e.g. immunoregulation) may ensure long-lasting clinical improvement. The possibility that different subgroups of patients (e.g. atopics versus non-atopics, high responders versus low responders) gain symptom amelioration by different mechanisms should also be taken into consideration in future studies. Thus, we have used a relatively simple statistical analysis to reach the aforementioned conclusions about the relationships between the IgG subclass antibody response and the clinical outcome, and between the subclass response and the IgE antibody production. It appears likely that a more advanced statistical analysis of the results from a greater number of patients may uncover important new relationships and help to identify subgroups of patients who may need special kinds of immunotherapy. An obvious subject for this kind of investigation is the relationship between the IgG1 antibody response and the IgE antibody production in patients of different age, with different atopic predisposition, and with different predisposition to mount an early IgG4 dominated antibody response.

Although such studies should primarily attempt to establish the value of IgG subclass antibody quantitation as marker for the clinical outcome, they may also help to elucidate the mechanisms underlying symptom amelioration more clearly. However, it must be recognized that studies of this kind are unable to give any definitive evidence concerning the mechanisms underlying clinical improvement of allergic

patients undergoing immunotherapy.

Today, when it is possible to measure human IgE antibody production in cell cultures (71) and to isolate IgG antibodies of each subclass by simple and mild methods (6), it should be possible to test some of the aforementioned hypotheses about the suppressive effect of IgG1 antibodies (or IgG1 antibody-allergen complexes) on the IgE synthesis and the importance of the balance of the IgG1 and IgG4 antibody production in the early phase of immunotherapy. The target cells for regulatory antibodies or complexes should also be identifiable by *in vitro* experiments (53).

In conclusion, I would like to emphasize that the study of the IgG subclass antibody response during allergen-specific immunotherapy is in its infancy, and that the actual results are few and their interpretations open to question. I hope that this review will stimulate further research in the field.

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REFERENCES

1. Aalberse, R. C., Gaag, R. van der & Leuwen, J. van: Serologic aspects of IgG4 antibodies. I. Prolonged immunization results in an IgG4-restricted response. *J. Immunology* 130, 722-726, 1983.
2. Aalberse, R. C. & Zee, J. S. van der: Immunoassays in allergic diseases with special reference to IgG antibodies. In Errigo, E. (ed.): Proceedings of XII Congress of the European Academy of Allergology and Clinical Immunology, pp. 73-80. O.I.C. Medical Press, Florence, 1983.
3. Aas, K.: Adequate clinical trials of immunotherapy. *Allergy* 47, 1-14, 1982.
4. Bernstein, I. L., Michael, J. G., Malkiel, S., Sweet, L. C. & Brackett, R. G.: Immunoregulatory function of specific IgG. II. Clinical evaluation of combined active and passive immunotherapy. *Int. Arch. Allergy Appl. Immunol.* 58, 30-37, 1979.
5. Bird, P., Lowe, J., Jefferis, R., Lange, G. de, Loghem, E. van & Ling, N. R.: Monoclonal antibodies to an immunoglobulin allotype marker G1m(f). *Vox. Sang* 47, 366-372, 1984.
6. Bird, P., Lowe, J., Stokes, R. P., Bird, A. G., Ling, N. R. & Jefferis, R.: The separation of human serum IgG into subclass fractions by immunoaffinity chromatography and assessment of specific antibody activity. *J. Immunol. Methods* 71, 97-105, 1984.
7. Borel, Y.: Isologous IgG-induced immunologic tolerance to haptens: A model for self versus non-self recognition. *Transplant. Rev.* 31, 3-21, 1976.
8. Butler, J. E.: The amplified ELISA: Principles of and applications for the comparative quantitation of class and subclass antibodies and the distribution of antibodies and antigens in biochemical separates. *Methods Enzymol* 73, 482-525, 1981.
9. Canonica, G. W., Mingari, M. C., Melioli, G., Colombatti, M. & Moretta, L.: Imbalances of T cell subpopulations in patients with atopic diseases and effect of specific immunotherapy. *J. Immunology* 123, 2669-2672, 1979.
10. Chapman, M. D., Platts-Mills, T. A. E., Gabriel, M., et al.: Antibody response following prolonged hyposensitization with Dermatophagoides pteronyssinus extract. *Int. Arch. Allergy Appl. Immunol.* 61, 431-440, 1980.
11. Clayton, W. F., Reisman, R. E., Georgitis, J. W., Wypych, J. I. & Arbesman, C. E.: Effect of prolonged immunotherapy on serum venom-specific IgE and IgG. *Clin. Allergy* 13, 301-307, 1983.
12. Creticos, P. S., Metre, T. E. van, Mardiney, M. R., Rosenberg, G. L., Norman, P. S. & Adkinson, N. F.: Dose response of IgE and IgG antibodies during ragweed immunotherapy. *J. Allergy Clin. Immunol.* 73, 94-104, 1984.
13. Deuschl, H., Johansson, S. G. O. & Fagerberg, E.: IgE, IgG and IgA antibodies in serum and nasal secretion during parenteral hyposensitization. *Clin. Allergy* 7, 315-324, 1977.
14. Devey, M. E., Wilson, D. V. & Wheeler, A. W.: The IgG subclasses of antibodies to grass pollen allergens produced in hay fever during hyposensitization. *Clin. Allergy* 6, 227-236, 1976.
15. Djurup, R., Malling, H.-J., Søndergaard, I. & Weeke, B.: The IgE and IgG subclass antibody response in yellow jacket sting-allergic patients undergoing different regimens of venom immunotherapy. *J. Allergy Clin. Immunol.* (In press).
16. Djurup, R. & Østerballe, O.: IgG subclass antibody response in grass pollen-allergic patients undergoing specific immunotherapy. *Allergy* 39, 433-441, 1984.
17. Djurup, R., Søndergaard, I., Magnusson, C. G. M. & Weeke, B.: A three-layer immunoradiometric assay for determination of IgG subclass antibodies in human sera ("IgG subclass RAST"). Validation of the subclass specificity, and establishment of equipotency. *Allergy* 39, 51-63, 1983.
18. Etievant, M., Leluc, B., Bouclier, R. & Henocq, E.: Immuno-enzymatic study of IgG subclasses specific for allergen in house dust immediate hypersensitivity. *Ann. Allergy* 43, 169-173, 1979.
19. Fagan, D. L., Slaughter, C. A., Capra, J. D. & Sullivan, T. J.: Monoclonal antibodies to immunoglobulin G₄ induce histamine release from human basophils *in vitro*. *J. Allergy Clin. Immunol.* 70, 399-404, 1982.

20. Fischer, T. J., Entis, G. N., Winant, J. G., Jr. & Bernstein, I. L.: Basic principles of therapy for allergic diseases. In Lawlor, G. J., Jr. & Fischer, T. J. (eds.): *Manual of allergy and immunology*, pp. 45-93. Little, Brown & Co., Boston, 1981.
21. Flanagan, J. G. & Rabbitts, T. H.: Arrangement of human immunoglobulin heavy chain constant region genes implies evolutionary duplication of a segment containing γ , ϵ and α genes. *Nature* **300**, 709-713, 1982.
22. Freijd, A., Hammarström, L., Persson, M. A. A. & Smith, C. I. E.: Plasma anti-pneumococcal antibody activity of the IgG class and subclasses in otitis prone children. *Clin. Exp. Immunol.* **56**, 233-238, 1984.
23. Giessen, M. van der, Homan, W. L., Kernebeek, G. van, Aalberse, R. C. & Dieges, P. H.: Subclass typing of IgG antibodies formed by grass pollen-allergic patients during immunotherapy. *Int. Arch. Allergy Appl. Immunol.* **50**, 625-640, 1976.
24. Golden, D. B. K., Meyers, D. A., Kagey-Sobotka, A., Valentine, M. D. & Lichtenstein, L. M.: Clinical relevance of the venom-specific immunoglobulin G antibody level during immunotherapy. *J. Allergy Clin. Immunol.* **69**, 489-493, 1982.
25. Graft, D. F., Schuberth, K. C., Kagey-Sobotka, A., et al.: The development of negative skin tests in children treated with venom immunotherapy. *J. Allergy Clin. Immunol.* **73**, 61-68, 1984.
26. Gray, B. M.: ELISA methodology for polysaccharide antigens: Protein coupling of polysaccharides for adsorption to plastic tubes. *J. Immunol. Methods* **28**, 187-192, 1979.
27. Haaijman, J. J., Deen, C., Kröse, C. J. M., Zijlstra, J. J., Coolen, J. & Radl, J.: Monoclonal antibodies in immunocytology: A jungle full of pitfalls. *Immunology Today* **5**, 56-58, 1984.
28. Hamilton, R. G. & Adkinson, N. F., Jr.: Quantitation of antigen-specific IgG in human serum: Standardization by a Staph A solid-phase radioimmunoassay elution technique. *J. Immunology* **124**, 2966-2971, 1980.
29. Hamilton, R. G. & Adkinson, N. F., Jr.: Quantitation of antigen-specific immunoglobulin G in human serum. II. Comparison of radioimmunoprecipitation and solid-phase radioimmunoassay techniques for measurement of immunoglobulin G specific for a complex allergen mixture (yellow jacket venom). *J. Allergy Clin. Immunol.* **67**, 14-21, 1981.
30. Hamilton, R. G., Rendell, M. & Adkinson, N. F., Jr.: Serologic analysis of human IgG and IgE anti-insulin antibodies by solid-phase radioimmunoassays. *J. Lab. Clin. Med.* **96**, 1022-1036, 1980.
31. Henney, C. S. & Ishizaka, K.: Studies on the immunogenicity of antigen-antibody precipitates. II. The suppressive effect of anti-carrier and anti-hapten antibodies on the immunogenicity of dinitrophenylated human γ G globulin. *J. Immunology* **104**, 1540-1549, 1970.
32. Hoffman, D. R., Gillman, S. A., Cummins, L. H., Kozak, P. P., Jr. & Oswald, A.: Correlation of IgG and IgE antibody level to honey bee venom allergens with protection to sting challenge. *Ann. Allergy* **46**, 17-23, 1981.
33. Irons, J. S., Pruzansky, J. J. & Patterson, R.: Immunotherapy: Mechanisms of action suggested by measurements of immunologic and cellular parameters. *J. Allergy Clin. Immunol.* **56**, 64-77, 1975.
34. Irons, J. S., Pruzansky, J. J., Patterson, R. & Zeiss, C. R.: Studies of perennial ragweed immunotherapy. Associated changes in cellular responsiveness, total serum antigen-binding capacity, and specific IgE antibody concentration. *J. Allergy Clin. Immunol.* **59**, 190-199, 1977.
35. Ishizaka, K. & Ishizaka, T.: Mechanisms of reaginic hypersensitivity and IgE antibody response. *Immunol. Rev.* **41**, 109-148, 1978.
36. Iskander, R., Das, P. K. & Aalberse, R. C.: IgG4 antibodies in Egyptian patients with schistosomiasis. *Int. Arch. Allergy Appl. Immunol.* **66**, 200-207, 1981.
37. Kay, A. B. & Walsh, G. M.: Chemotactic factor-induced enhancement of the binding of human immunoglobulin classes and subclasses to neutrophils and eosinophils. *Clin. Exp. Immunol.* **57**, 729-734, 1984.
38. Kemeny, D. M., MacKenzie-Mills, M., Harries, M. G., Youtlen, L. J. F. & Lessof, M. H.: Antibodies to purified bee venom proteins and peptides. II. A detailed study of changes in IgE and IgG antibodies to individual bee venom antigens. *J. Allergy Clin. Immunol.* **72**, 376-385, 1983.
39. Lee, W. Y. & Sehon, A. H.: Suppression of reaginic antibodies. *Immunol. Rev.* **41**, 200-247, 1978.
40. Leinikki, P. O., Shekarchi, I., Dorsett, P. & Sever, J. L.: Enzyme-linked immunosorbent assay determination of specific rubella antibody levels in micrograms of immunoglobulin G per milliliter serum in clinical samples. *J. Clin. Microbiol.* **8**, 419-423, 1978.
41. Levy, D. A., Lichtenstein, L. M., Goldstein, E. O. & Ishizaka, K.: Immunologic and cellular changes accompanying the therapy of pollen allergy. *J. Clin. Invest.* **50**, 360-369, 1971.
42. Lichtenstein, L. M., Ishizaka, K., Norman, P. S., Sobotka, A. K. & Hill, B. M.: IgE antibody measurements in ragweed hay fever. Relationship to clinical severity and the results of immunotherapy. *J. Clin. Invest.* **52**, 472-482, 1973.
43. Lichtenstein, L. M., Norman, P. S. & Winkenwerder, W. L.: A single year of immunotherapy for ragweed hay fever. Immunologic and clinical studies. *Ann. Intern. Med.* **75**, 663-671, 1971.
44. Lieberman, P. & Patterson, R.: Immunotherapy for atopic diseases. *Adv. Intern. Med.* **19**, 391-411, 1974.
45. Lowe, J., Bird, P., Hardie, D., Jefferis, R. & Ling, N. R.: Monoclonal antibodies (McAbs) to determinants on human gamma chains: Properties of antibodies showing subclass restriction or subclass specificity. *Immunology* **47**, 329-336, 1982.
46. Mäkelä, O. & Péterfy, F.: Standard sera in solid-phase immunoassays. *Eur. J. Immunol.* **13**, 815-819, 1983.
47. Magnusson, C. G. M. & Masson, P. L.: Particle

- counting immunoassay of immunoglobulin E antibodies after their elution from allergosorbents by pepsin: an alternative to the radioallergosorbent test. *J. Allergy Clin. Immunol.* 70, 326-336, 1982.
48. Malling, H.-J., Djurup, R., Søndergaard, I. & Weeke, B.: Clustered immunotherapy with yellow jacket venom. Evaluation of influence of time interval on in vivo and in vitro parameters. *Allergy* 40, 373-383, 1985.
 49. Marcinkiewicz, J., Bereta, M., Malinowski, J. & Ptak, W.: The induction of oxazolone-specific T suppressor afferent cells in mice by hapten-modified isologous IgG. *Eur. J. Immunol.* 14, 759-762, 1984.
 50. Melam, H., Pruzansky, J., Patterson, R. & Singer, S.: Clinical and immunologic studies of ragweed immunotherapy. *J. Allergy* 47, 262-272, 1971.
 51. Merrett, J., Barnetsson, R. StC., Burr, M. L. & Merrett, T. G.: Total and specific IgG4 antibody levels in atopic eczema. *Clin. Exp. Immunol.* 56, 645-652, 1984.
 52. Metre, T. E. van, Adkinson, N. F., Jr., Amodio, F. J., et al.: A comparison of immunotherapy schedules for injection treatment of ragweed pollen hay fever. *J. Allergy Clin. Immunol.* 69, 181-193, 1982.
 53. Morimoto, C., Reinherz, E. L., Todd, R. F., Distaso, J. A. & Schlossman, S. F.: Generation of antigen-specific suppressor cells in vitro in man. *J. Immunology* 131, 1209-1213, 1983.
 54. Natvig, J. B. & Kunkel, H. G.: Human immunoglobulins: Classes, subclasses, genetic variants, and idiotypes. *Adv. Immunol.* 16, 1-59, 1973.
 55. Norman, P. S.: An overview of immunotherapy: Implications for the future. *J. Allergy Clin. Immunol.* 65, 87-96, 1980.
 56. Norman, P. S., Winkelwerder, W. L. & Lichtenstein, L. M.: Trials of alum-precipitated pollen extracts in the treatment of hay fever. *J. Allergy Clin. Immunol.* 50, 31-44, 1972.
 57. Østerballe, O.: Specific immunotherapy with purified grass pollen extracts. Thesis. University of Copenhagen, 1983.
 58. Parish, W. E.: The clinical relevance of heat-stable short-term sensitizing anaphylactic IgG antibodies (IgG S-TS) and of related activities of IgG₄ and IgG₂. *Br. J. Dermatol.* 105, 223-231, 1981.
 59. Parker, C. W.: Asthma and rhinitis. In Parker, C. W. (ed.): *Clinical immunology*, pp. 1372-1438. Saunders, Philadelphia, 1980.
 60. Parkes, A. B., McLachlan, S. M., Bird, P. & Smith, B. R.: The distribution of microsomal and thyroglobulin antibody activity among the IgG subclasses. *Clin. Exp. Immunol.* 57, 239-243, 1984.
 61. Patterson, R.: Clinical efficacy of allergen immunotherapy. *J. Allergy Clin. Immunol.* 64, 155-158, 1979.
 62. Patterson, R., Lieberman, P., Irons, J. S., et al.: Immunotherapy. In Middleton, E., Jr., Reed, C. E. & Ellis, E. F. (eds.): *Allergy: Principles and practice*, pp. 877-898. C. V. Mosby Co., St. Louis, 1978.
 63. Paull, B. R., Jacob, G. L., Yunginger, J. W. & Gleich, G.: Comparison of binding of IgE and IgG antibodies to honeybee venom phospholipase-A. *J. Immunology* 120, 1917-1923, 1978.
 64. Platts-Mills, T. A. E.: Local production of IgG, IgA, and IgE antibodies in grass pollen hay fever. *J. Immunology* 122, 2218-2225, 1979.
 65. Platts-Mills, T. A. E., Mauer, R. K. von, Ishizaka, K., Norman, P. S. & Lichtenstein, L. M.: IgA and IgG anti-ragweed antibodies in nasal secretions. Quantitative measurements of antibodies and correlation with inhibition of histamine release. *J. Clin. Invest.* 57, 1041-1050, 1976.
 66. Platts-Mills, T. A. E., Snajdr, M. J., Ishizaka, K. & Frankland, A. W.: Measurements of IgE antibody by an antigen-binding assay: Correlation with PK activity and IgG and IgA antibodies to allergens. *J. Immunology* 120, 1201-1210, 1978.
 67. Robboy, S. J., Lewis, E. J., Schur, P. H. & Colman, R. W.: Circulating anticoagulants to factor VIII. Immunochemical studies and clinical response to factor VIII concentrates. *Am. J. Med.* 49, 742-752, 1970.
 68. Rocklin, R. E.: Clinical and immunological aspects of allergen-specific immunotherapy in patients with seasonal allergic rhinitis and/or allergic asthma. *J. Allergy Clin. Immunol.* 72, 323-334, 1983.
 69. Rocklin, R. E., Sheffer, A. L., Greinder, D. K. & Melmon, K. L.: Generation of antigen-specific suppressor cells during allergy desensitization. *N. Engl. J. Med.* 302, 1213-1219, 1980.
 70. Rubin, R. L., Hardtke, M. A. & Carr, R. I.: The effect of high antigen density on solid-phase radioimmunoassays for antibody regardless of immunoglobulin class. *J. Immunol. Methods* 33, 277-292, 1980.
 71. Sarfati, M., Rubio-Trujillo, M., Wong, K., Rector, E., Schon, A. H. & Delespesse, G.: In vitro synthesis of IgE by human lymphocytes. I. The spontaneous secretion of IgE by B lymphocytes from allergic individuals: A model to investigate the regulation of human IgE synthesis. *Immunology* 53, 187-196, 1984.
 72. Schellenberg, R. R. & Adkinson, N. F., Jr.: Measurement of absolute amounts of antigen-specific human IgE by a radioallergosorbent test (RAST) elution technique. *J. Immunology* 115, 1577-1583, 1975.
 73. Settignano, G. A. & Chafee, F. H.: Natural history of allergy to Hymenoptera. *Clin. Allergy* 9, 385-390, 1979.
 74. Shimizu, A. & Honjo, T.: Immunoglobulin class switching. *Cell* 36, 801-803, 1984.
 75. Sinclair, N. R. StC.: Immunoregulation by antibody and antigen-antibody complexes. *Transplant. Proc.* 10, 349-353, 1978.
 76. Skvaril, F. & Joller-Jemelka, H.: IgG subclasses of anti-HBs antibodies in vaccinated and nonvaccinated individuals and in anti-HBs immunoglobulin preparations. *Int. Arch. Allergy Appl. Immunol.* 73, 330-337, 1984.
 77. Søndergaard, I., Djurup, R. & Weeke, B.: Characterization of IgG subclass antibodies to individual allergens by crossed radioimmuno-electrophoresis. *Allergy* 39, 622-629, 1984.
 78. Spiegelberg, H. L.: Biological activities of immuno-

- globulins of different classes and subclasses. *Adv. Immunol.* 19, 259-294, 1974.
79. Stanworth, D. R.: IgG4 antibodies in atopics and their families. In Kerr, J. W. & Ganderton, M. A. (eds.): XI International congress of allergology and clinical immunology. Proceedings of invited symposia, pp. 337-340. Macmillan Press, Ltd., London, 1983.
 80. Strannegård, Ö. & Belin, L.: Suppression of reagin synthesis in rabbits by passively administered antibody. *Immunology* 18, 775-785, 1970.
 81. Tada, T. & Okumura, K.: Regulation of homocytotropic antibody formation in the rat. I. Feed-back regulation by passively administered antibody. *J. Immunology* 106, 1002-1011, 1971.
 82. Takatsu, K. & Ishizaka, K.: Reaginic antibody formation in the mouse. VIII. Depression of the ongoing IgE antibody formation by suppressor T cells. *J. Immunology* 117, 1211-1218, 1976.
 83. Teale, J. M. & Klinman, N. R.: Control of the production of different classes of antibody. In Paul, W. E. (ed.): *Fundamental immunology*, pp. 519-535. Raven Press, New York, 1984.
 84. Thompson, P. M., McLachlan, S. M., Parkes, A., Clark, F., Howel, D. & Smith, B. R.: The IgG subclass distribution of thyroglobulin synthesized in culture. *Scand. J. Immunol.* 18, 123-129, 1983.
 85. Thurnheer, U., Müller, U., Stoller, R., Lanner, A. & Hoigné, R.: Venom immunotherapy in Hymenoptera sting allergy. *Allergy* 38, 465-475, 1983.
 86. Toorenenbergen, A. W. van & Aalberse, R. C.: IgG4 and release of histamine from human peripheral blood leukocytes. *Int. Arch. Allergy Appl. Immunol.* 67, 117-122, 1982.
 87. Urbanek, R., Forster, J., Karitzky, D. & Ziupa, J.: The prognostic significance of specific IgG antibodies in insect sting allergy. *Eur. J. Pediatr.* 136, 31-34, 1981.
 88. Urbanek, R., Forster, J., Ziupa, J. & Karitzky, D.: Immunologic studies on bee-keepers: Specific IgG and subclass typing against bee venom and bee venom components. *Klin. Wochenschr.* 58, 1257-1260, 1980.
 89. Voisin, G. A.: Role of antibody classes in the regulatory facilitation reaction. *Immunol. Rev.* 49, 3-59, 1980.
 90. Wahlgren, M., Berzins, K., Perlmann, P. & Persson, M.: Characterization of the humoral response in *Plasmodium falciparum* malaria. II. IgG subclass levels of anti-*P. falciparum* antibodies in different sera. *Clin. Exp. Immunol.* 54, 135-142, 1983.
 91. Zanussi, C. & Pastorello, E.: Hyposensitization. A contribution to the study of mechanisms and efficacy of specific immunotherapy in allergic patients. In Errigo, E. (ed.): *Proceedings of XII Congress of the European Academy of Allergology and Clinical Immunology*, pp. 197-203. O.I.C. Medical Press, Florence, 1983.
 92. Zollinger, W. D. & Boslego, J. W.: A general approach to standardization of the solid-phase radioimmunoassay for quantitation of class-specific antibodies. *J. Immunol. Methods* 46, 129-140, 1981.

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