

## Immunoglobulin G4: an odd antibody

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### Clinical & Experimental Allergy

#### Summary

Despite its well-known association with IgE-mediated allergy, IgG4 antibodies still have several poorly understood characteristics. IgG4 is a very dynamic antibody: the antibody is involved in a continuous process of half-molecules (i.e. a heavy and attached light-chain) exchange. This process, also referred to as 'Fab-arm exchange', results usually in asymmetric antibodies with two different antigen-combining sites. While these antibodies are heterobivalent, they will behave as monovalent antibodies in most situations. Another aspect of IgG4, still poorly understood, is its tendency to mimic IgG rheumatoid factor (RF) activity by interacting with IgG on a solid support. In contrast to conventional RF, which binds via its variable domains, the activity of IgG4 is located in its constant domains. This is potentially a source of false positives in IgG4 antibody assay results. Because regulation of IgG4 production is dependent on help by T-helper type 2 (Th2) cells, the IgG4 response is largely restricted to non-microbial antigens. This Th2-dependency associates the IgG4 and IgE responses. Another typical feature in the immune regulation of IgG4 is its tendency to appear only after prolonged immunization. In the context of IgE-mediated allergy, the appearance of IgG4 antibodies is usually associated with a decrease in symptoms. This is likely to be due, at least in part, to an allergen-blocking effect at the mast cell level and/or at the level of the antigen-presenting cell (preventing IgE-facilitated activation of T cells). In addition, the favourable association reflects the enhanced production of IL-10 and other anti-inflammatory cytokines, which drive the production of IgG4. While in general, IgG4 is being associated with non-activating characteristics, in some situations IgG4 antibodies have an association with pathology. Two striking examples are pemphigoid diseases and sclerosing diseases such as autoimmune pancreatitis. The mechanistic basis for the association of IgG4 with these diseases is still enigmatic. However, the association with sclerosing diseases may reflect an excessive production of anti-inflammatory cytokines triggering an overwhelming expansion of IgG4-producing plasma cells. The bottom line for allergy diagnosis: IgG4 by itself is unlikely to be a cause of allergic symptoms. In general, the presence of allergen-specific IgG4 indicates that anti-inflammatory, tolerance-inducing mechanisms have been activated. The existence of the IgG4 subclass, its up-regulation by anti-inflammatory factors and its own anti-inflammatory characteristics may help the immune system to dampen inappropriate inflammatory reactions.

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#### Introduction

In the 1960s, it was discovered that the five classes of antibodies present in all mammals (IgM, IgG, IgA, IgD and IgE) could often be subdivided into subclasses. In contrast to the Ig classes, these subclasses developed relatively recently (after most of the evolutionary diversification of the mammalian species). As a consequence, the human subclasses do not have close structural homologues in, for example, the mouse. Nevertheless, there is often some

functional homology between subclasses among mice and man, although this is not reflected in their nomenclature. The human IgG subclasses have been assigned numbers according to their time of discovery. This numbering scheme corresponds to their abundance in plasma. IgG1 is the most abundant (> 50% of total IgG), IgG4 the least abundant (typically < 5%). In contrast, the number assignment to the rodent IgG subclasses initially reflected their electrophoretic mobility: rodent IgG1 is relatively fast (= acidic), while rodent IgG2 has a lower mobility.

Mouse IgG1 is not at all related to human IgG1, but there is some functional similarity [by way of electrophoretic mobility and T-helper type 2 (Th2) dependency] with human IgG4. Mouse IgG3 is functionally similar to human IgG2. Obviously, this is often quite confusing.

These subclasses were initially discovered as myeloma proteins and by careful analysis of sub-specificities in polyclonal class-specific antisera. A polyclonal antiserum to one Ig class does not cross-react with another Ig class, but an antibody to an Ig subclass will usually cross-react extensively with other subclasses (in the same class). However, in the early 1970s, specific reagents became available for the measurement of IgG subclasses. This resulted in the observation that types of antibody specificity were associated with particular subclasses. A striking example is that IgG antibodies to bacterial polysaccharides belong largely to the (human) IgG2 subclass. A similar association was discovered between IgG antibodies to allergens and the IgG4 subclass (in patients receiving allergen-specific immunotherapy). This was discovered independently by three groups: Devey [1] (Cambridge, UK), Stanworth (Birmingham, UK) and Van der Giessen [2] (Amsterdam, NL), who presented these results at the 2nd International Immunology Congress in Brighton (1974).

In this review we will focus on recent developments in the IgG4 field. We will discuss immunochemical and immune-regulatory peculiarities of IgG4 as well as its role in clinical conditions, with emphasis on IgE-mediated diseases.

## Structure and dynamics

### *Non-precipitating antibody: monovalent?*

At the time, few allergens had been well characterized, and even fewer were available in purified form. This made the measurement of allergen-specific IgG challenging. Extracts that were used in the IgE assay (Radio Allergo-Sorbent Test, RAST) were often found to result in high 'non-specific' responses, i.e. IgG antibodies directed to contaminating antigens present in the allergen extract, but unrelated to allergens. The specificity of the IgG antibody assay could be substantially increased by using an assay format in which the antibody was detected by its ability to cross-link a labelled fluid-phase antigen to a solid-phase antigen. However, we found that this cross-linking activity was often absent in sera with high levels of IgG4 (as measured by IgG4 RAST). Direct immunoprecipitation experiments and ultracentrifugation analyses (using  $^{125}\text{I}$ -labelled purified model allergens: cat serum albumin and bee venom phospholipase) confirmed that IgG4 antibodies were non-precipitating and behaved like monovalent antibodies [3].

### *Immunoglobulin G4 is not really monovalent, but heterobivalent*

King *et al.* [4] discovered an unusual feature of IgG4 that seemed to explain the monovalency of IgG4. Upon electrophoretic analysis in a non-reducing SDS gel a substantial part of IgG4 was found to lack interchain disulphide bonds and thus to be (under these denaturing conditions) a half-molecule of one heavy chain plus one light chain. This phenomenon was shown to be due to a single amino acid in the hinge, which differs between IgG1 and IgG4: a proline in IgG1 is replaced by a serine in IgG4. Mutating this serine into proline abolished the appearance of half-molecules on SDS electrophoresis [5]. However, no half-molecules were found upon size exclusion chromatography under native conditions. We addressed the structural basis for the apparent monovalency of IgG4 by producing recombinant monoclonal IgG4 antibodies to the mite allergen Der p 2 as a model allergen [6]. IgG1 antibody with the same specificity was used as a reference. In contrast to our expectation, in cross-linking assays the recombinant IgG4 antibody behaved as ordinary, bivalent IgG1 antibody. Yet, under denaturing conditions, half-molecules were present in our bivalent recombinant IgG4 as much as in (apparently monovalent) plasma-derived polyclonal IgG4 [7, 8]. We considered the possibility that the non-covalent interaction between the heavy chains was sufficiently strong to keep the two chains together, but would allow an exchange reaction among IgG4 molecules. This would fit with the observation that no half-molecules are found upon size exclusion chromatography under native conditions [7]. The exchange reaction would result in IgG4 combining two different specificities in a single molecule (see Fig. 1). We got preliminary supportive evidence from an analysis of

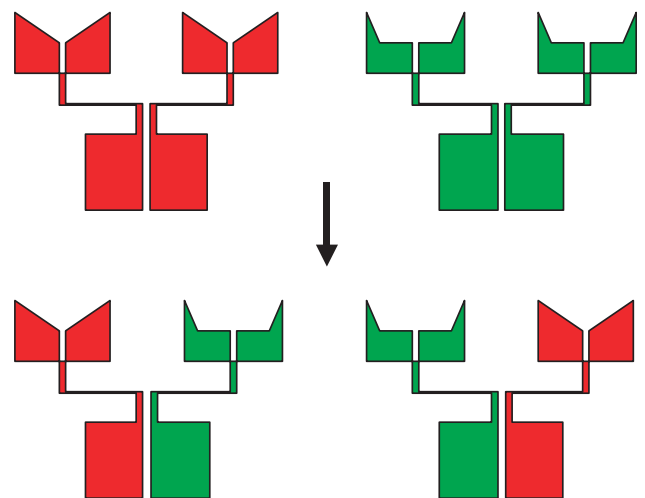


Fig. 1. Schematic representation of the generation of bispecific IgG4 antibodies by the exchange of half-molecules ('Fab-arm exchange'). Taken from Aalberse and Schuurman [8] with permission of the publishers.

sera from patients who received immunotherapy with two non-cross-reactive allergens (e.g. mite and cat). In such sera we found IgG4 antibodies that were able to cross-link these two allergens, supporting the existence of bi-specific IgG4 antibodies [7]. The exchange was very prominent upon injection of a mixture of two IgG4 antibodies into mice [9]. Whereas, simply mixing two IgG4 antibodies *in vitro* did not result in a measurable exchange of half-molecules, we established that the presence of a low concentration of reduced glutathione allowed this Fab-arm exchange reaction to proceed *in vitro*. To our surprise, mutating the proline in the IgG1 hinge to its IgG4 variant did not induce exchangeability: the CH3 domain was found to be critical too. Only if the CH3 domain in IgG1 is replaced by the IgG4 equivalent, the mutated IgG1 can exchange half-molecules. It has been suggested, [10] that this exchange process is irrelevant *in vivo*, because we showed that (as expected) irrelevant IgG4 inhibited the generation of bi-specific activity. It should be clear, however, that all IgG4 will participate in the exchange reaction and that the process is continuously ongoing. Because of the Fab-arm exchange reaction all IgG4 is transformed from symmetric, homo-bivalent cross-linking antibodies to asymmetric, hetero-bivalent antibodies that in most situations will behave as a monovalent, non-cross-linking antibody.

Generation of potentially significant levels of bi-specific antibody occurs in exceptional situations, as we have shown in our initial studies in selected patients after allergen-specific immunotherapy [7]. Theoretically, such bi-specificity might have undesirable consequences. If, for example, a patient with a pre-existing high IgG4 antibody titre to some pathogen receives a therapeutic treatment with a high dose of a recombinant IgG4 antibody to a cell surface antigen, the ensuing bi-specificity might result in targeting the pathogen to the cell in question. In most situations, the main effect of this Fab-arm exchange is the generation of monovalent, non-cross-linking (and therefore anti-inflammatory) antibodies rather than the generation of bi-specific (potentially pro-inflammatory) antibodies. Based on preliminary comparisons between recombinant (i.e. homo-bivalent) IgG4 and plasma-derived (hetero-bivalent) IgG4, it is unlikely that the interaction between IgG4 and either Fc gamma receptors or C1q is different if the antibody is homo-bivalent or hetero-bivalent.

### Interactions

Apart from the above-mentioned unusually weak interaction with its intermolecular heavy-chain partner, its interaction with the classical Fc gamma receptors as well as with C1q is weaker than for other subclasses.

There is a peculiar other side of IgG4: its tendency to interact with other Igs. This has been studied in most

detail in relation to IgG rheumatoid factor [11]. IgG4 was found to possess an intrinsic affinity for IgG coated to a solid phase. Surprisingly, this binding activity was not located in its variable domains, but in its constant domain. This Fc-binding activity is most easily demonstrated using IgG1 as the binding target. However, using labelled IgG4 it can be shown that IgG4 will also bind to coated IgG4. To further complicate the situation, IgG4 with irrelevant specificity was found to bind to IgG4 antibody (but not to the homologous IgG1 antibody) bound to its (coated) antigen (Rispen et al., manuscript in preparation). Obviously, this is a potential source of artifacts in analytical assays, not only for the measurement of bi-specific IgG4, but also for the measurement of IgG4 antibody in general. In the case of the measurement of bi-specificity of IgG4, this 'non-specific' binding was blocked by adding pooled Ig to the incubation buffer.

### The regulation of immunoglobulin G4 production

The requirements for the class switch to IgG4 are similar to those for IgE. Both depend on IL-4/IL-13 [12–15]. Both are therefore considered to be part of the Th2 immune response. In relation to allergen-specific immunotherapy it is sometimes suggested that a switch occurs from IgE production to IgG4 production. While a B cell can switch sequentially, such a sequential switch can transform an IgG4-producing B cell into an IgE-producing B cell, but not the other way around as a consequence of the sequence order in which the genes for the isotypes are arranged on the chromosome [13–15].

One of the effects of this common dependency on Th2 cells is that antigens that induce IgE responses (i.e. allergens and nematode antigens) are also good inducers of IgG4 responses. There must be some regulatory differences before the class switch, because the occurrence of IgG4 antibodies without IgE antibodies is not uncommon (the 'modified Th2 response', see below). One type of regulation is particularly important: the effects of IL-10 and related cytokines. IL-10 interferes with the class switch [16], which affects both IgE and IgG4 production [17]. In addition, IL-10 is presumably needed to drive the differentiation of IgG4-switched B cells to IgG4-secreting plasma cells [18]. In addition to IL-10, also IL-21 has been found to increase IgG4 production *in vitro* [19, 20]. For a recent review of IL-21 and its pleiotropic effects on IgG and IgE production, see [21].

### The modified T-helper type 2 response

This term 'modified Th2 response' was first used in relation to the antibody response to cat allergen [22]. It refers to subjects with IgG4 antibodies, but without demonstrable IgE antibodies. As the presence of IgG4 antibodies indicates a Th2 response, the absence of IgE

antibodies is considered to be unexpected; however, this situation is in fact quite common. It is, for example, found in most beekeepers. Also occupational exposure to protein antigens, e.g. to rodent allergens in the animal house, or mammalian serum albumin in animal blood processing industry [23] usually results in this phenotype [24, 25]. Therefore, the modified Th2 response seems to be the typical, 'healthy' response to an innocuous antigen [26, 27]. It is intriguing why this type of response is not found in all situations where allergen exposure does not result in IgE production. Well-established examples of the latter type of response are grass pollen: it is strikingly rare to find IgG4 antibodies to pollen or mite allergens in the absence of IgE antibodies [28] and house dust mites [29, 30].

Remarkably, this is true not only for IgG4, but also for IgG1. The presence of high-affinity IgG antibodies (IgG1 and/or IgG4) to pollen- or mite allergens is much more common in subjects with allergen-specific IgE than in IgE-negative subjects. This difference is more marked for some allergens than for others, which suggests that not all allergens are equal. This difference among allergens becomes visible by the reactivity of these allergens in non-allergic subjects. Some allergens do not induce an IgG antibody response at all ('classical Th2 allergens', possibly synonymous to classical atopic allergens), whereas others ('modified Th2 allergens') induce an IgG (IgG1 and/or IgG4) response without IgE [31].

#### *Is (human) immunoglobulin G1 indicative of T-helper 1?*

Before discussing this, it may be important to recall an awkward nomenclature issue: in rodents, the prototypic Th2 IgG isotype is called IgG1.

Human IgG1 antibody responses are prominent in anti-microbial responses, which are usually driven by Th1 cells. This information has been used to argue that finding IgG1 antibodies implies the activation of a Th1 immune response. It is important to note that IgE antibodies are actually more often found in combination with IgG1 antibodies than with IgG4 antibodies; the association between IgE and IgG4 becomes strong only after allergen-specific immunotherapy (sIT). Assuming that IgE is largely produced in situations of strong Th2 polarization (which suppresses Th1 responses), it would be unexpected if the IgE-associated IgG1 production would fully depend on Th1 activation. This is supported by *in vitro* data showing that not only Th1 cytokines, but also Th2 cytokines can drive (human) IgG1 responses [32–34].

#### *Chronic exposure*

A noticeable aspect of the IgG4 response is its slow manifestation of IgG4 antibodies. It usually takes many months of repeated antigen exposure before IgG4

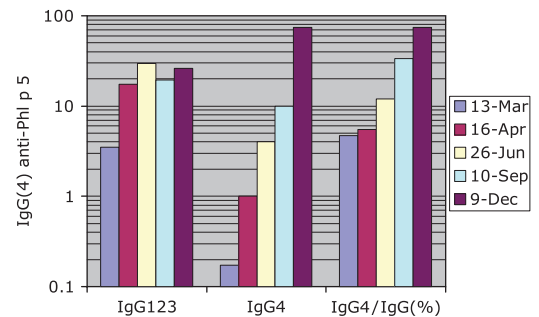


Fig. 2. Analysis of the IgG antibody levels in five serum samples from one subject taken at the indicated time-points during subcutaneous immunotherapy with grass pollen extract (*P. pratense*). The sera were provided by Dr S. Durham. IgG (total) and IgG4 levels were measured using Sepharose-coupled protein G and Sepharose-coupled anti-IgG4. Antibodies were detected with  $^{125}\text{I}$ -labelled purified Phl p 5 allergen. The data were converted to arbitrary units. The IgG123 value was calculated as the difference between the units for IgG (total) and IgG4. Note the slow increase not only in the absolute IgG4 antibody level, but also in the level relative to the IgG (total) antibody level.

responses become prominent. This is illustrated by the sequential analysis of sera from novice bee-keepers [35] and analysis of sequential samples from patients that received subcutaneous sIT shows the same pattern. It is likely that production of sufficient IL-10 (and/or IL-21?) is a rate-limiting step.

Data to show the relatively slow production of allergen-specific IgG4 are being illustrated in Fig. 2. For the assays serum is being incubated with an IgG-binding solid phase. After washing,  $^{125}\text{I}$ -labelled-purified allergen is added and binding of radioactivity is measured. For total IgG binding we use Sepharose-coupled protein-A (which binds all IgG subclasses but IgG3, which does not contribute noticeably) or protein-G (which binds all IgG subclasses). For IgG4, Sepharose-coupled monoclonal anti-IgG4 is used. In this protocol, the IgG4 reactivity can be expressed as percentage of IgG (total). In Fig. 2 the allergen-specific IgG results are shown from a patient receiving sIT. This patient shows the characteristic pattern: not only the absolute level of specific IgG4 increases, but also the relative contribution of IgG4 antibody to total IgG antibody, in this case from 5% to 75%.

#### *Immunoglobulin G4 responses in infancy*

The total IgG4 levels are low in infancy. Total IgG4 is similar in its kinetics to IgG2 and IgA. This presumably reflects a dependency on the maturity of accessory cells (macrophages, dendritic cells, etc.), which are important producers of IL-10. Moreover, some of the IL-10 effects are mediated via such accessory cells [16].

This slow kinetics of IgG4-expressing cells is also reflected in IgG4-specific antibody levels. The IgG4/IgG1 ratio of antibodies to common foods is lower in infancy

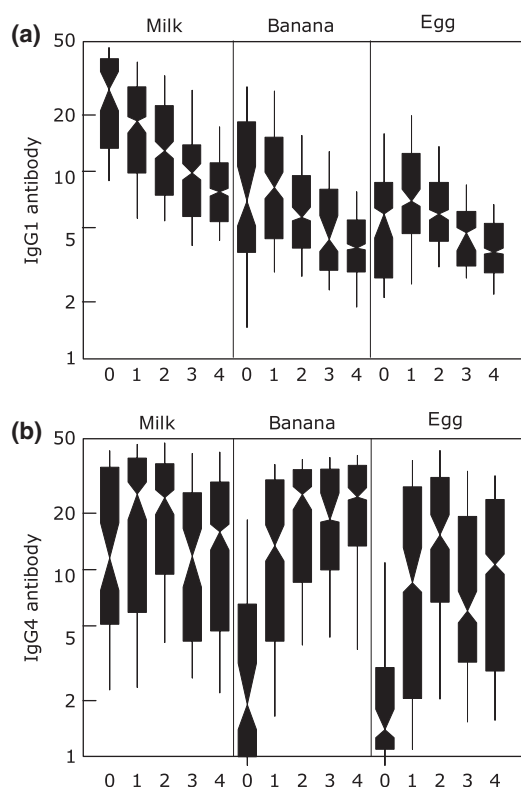


Fig. 3. IgG1 (a) and IgG4 (b) antibodies to milk, banana and egg in non-selected children of five age groups. Age group 0: 3–13 months,  $N=21$ ; group 1: 13–36 months,  $N=44$ ; group 2: 37–60 months,  $N=47$ ; group 3: 61–96 months,  $N=53$ ; group 4: 97–168 months,  $N=48$ . The results are shown as box plots with 10, 25, 50, 75 and 90 percentiles, with a standard error for the 50 percentile indicated by the wedged shape. Data from Aalberse [31].

than in adolescence. This shift in IgG4/IgG1 antibody may be related to the chronic stimulation requirement for IgG4 production, as discussed before. This shift to IgG4 is, however, only partially due to an earlier appearance of IgG1 antibodies, it also reflects an earlier decline of IgG1 antibodies [36] as shown in Fig. 3.

### Clinic

IgG4 is associated with several clinical conditions. Some of these associations suggest a protective effect, such as in allergen-specific immunotherapy, tolerance induction after food avoidance [37] and protection from allergic effects during parasitosis [38, 39]. In a few situations, the IgG4 is associated with a pathogenic effect, such as pemphigoid diseases, the sclerosis-associated hyper-IgG4 syndrome and IgE-negative allergies, which will be discussed below. In most situations, IgG4 antibodies are presumably innocent bystanders, appearing as an outcome of chronic exposure to non-microbial antigens. A practical application of IgG4 antibody assays is bio-monitoring of occupational exposure, e.g. in animal house workers

[24, 25]. Furthermore, IgG4 antibodies are also prominent in the response to therapeutic proteins such as clotting factor VIII [40–42] and therapeutic antibodies such as infliximab (Remicade) [43]. In these situations the IgG4 responses are undesirable, because they decrease the therapeutic efficacy of the treatment.

### Association of immunoglobulin G4 with immunoglobulin E-related allergy

The association between IgG4 and IgE-related allergy should not come as a surprise. As already mentioned before, both IgE and IgG4 require help by Th2 cells. So, all IgE-inducing allergens are also efficient IgG4 inducers. The reverse may also be true, but there seems to be at least one notable exception: BanLec1, a lectin from banana, is one of the most potent IgG4-inducing proteins [44], but is a minor allergen at best. We considered the possibility that this IgG4 binding activity was due to binding of the lectin to sugars on IgG4 rather than to a true antigen–antibody interaction, but the binding persisted even if the sugar-binding activity of the lectin was blocked with the appropriate sugar.

The possible involvement of IgG4 antibodies as a pathogenic factor in IgE-independent food-related disorders is still a not fully resolved issue. In a recent EAACI Task Force Report it is concluded that ‘food-specific IgG4 does not indicate (imminent) food allergy or intolerance, but rather a physiological response of the immune system after exposition to food components’ [45]. A major problem is the paucity of well-designed clinical studies. Another problem is the wide range of antibody levels in apparently healthy subjects.

The relation between allergen-specific IgG4 and beneficial effects of sIT are still a matter of some dispute. While it is well accepted that most patients who have been successfully treated by conventional sIT will have high levels of allergen-specific IgG4, there are exceptions to the association. More importantly, an association obviously does not prove causality. Symptom relief could be induced by protective cytokines, such as IL-10, which is known to stimulate the production of IgG4 antibodies [46]. The Durham group showed that the increased IL-10 production preceded both the clinical improvement and the IgG4 antibody production. Furthermore, data from the same group suggest that the clinical benefit of the treatment upon treatment withdrawal is maintained better than the allergen-specific IgG4 level. Statistical evaluations are hampered by the low number of treatment failures without confounding additional sensitivities, as well as the lack of accurate information on the complex dose–response relation between IgG4 levels, sensitization levels and symptoms. It would, obviously, be interesting to have a passive immunization option with human IgG4 antibodies as proof of principle. The only such study we

know of is by Lessof *et al.* [47] in the bee venom system, in which subjects with venom allergy were treated with Igs derived from pooled beekeeper plasma (presumably rich in IgG4 antibodies) and subsequently sting challenged. This study showed protection however. Unfortunately, no IgG4 antibody levels were measured to correlate IgG4 to the protective effect.

The measurement of allergen-specific IgG4 during follow-up of sIT is useful as a marker of the adequacy of the allergen vaccination regime on a per-patient basis. If the vaccination results in a substantial rise (typically 10–100-fold) in allergen-specific IgG4, it does not make sense to increase the dose and if no substantial increase is found, a change in vaccination protocol might be warranted. This holds for conventional sIT and is not meant to imply that other forms of treatment that do not increase allergen-specific IgG4 are necessarily inefficient. A more informative parameter is the measurement of the ratio of IgG4 to total IgG. As was mentioned before, IgG4 antibodies are produced largely after prolonged immunization. The early antibody response during immunotherapy is predominantly IgG1. This shift from IgG1 to IgG4 can be expressed as IgG4/IgG<sub>total</sub> ratio. An increase in this ratio from <20% to >80% is typical for successful conventional sIT [46, 48].

The measurement of IgG antibodies to allergens is not without pitfalls. This is particularly true for the isotypes that are not so markedly dependent on IL-4. For these non-IgG4 isotypes it is important to appreciate that their production is largely driven by microbial antigens, with much less stringent T cell control. This often results in antibodies with lower affinity and specificity. This strongly argues against the use of whole allergen extracts for allergen-specific IgG assays, particularly because sIT is also performed (for the time being, at least) with whole allergen extracts rather than with purified allergens. To ensure that the results of the IgG assays reflect levels of IgG-specific for allergens rather than for contaminating antigens in the allergen extract, the use of purified allergens is essential. To minimize the contribution of low-affinity antibodies to the assay result, we prefer to use purified <sup>125</sup>I-labelled allergens for the IgG assay rather than allergen extracts coupled to a solid phase, as specified before [46, 48].

#### Immunoglobulin G4-associated non-allergic diseases

IgG4 is generally considered to be a benign, non-pathogenic antibody. It is inefficient in activating potentially dangerous effector systems due to its low affinity for C1q and the classical Fcγ-receptors. Furthermore, its effective monovalency prevents the formation of large immune complexes, which might otherwise become problematic under conditions of chronic antigenic stimulation. In a mouse model of myasthenia gravis IgG4 was protective

due to its acquired monovalency (as a result of Fab-arm exchange) by competition with a pathogenic IgG1 antibody [9]. However, IgG4 has been found to be closely associated with pathogenicity in two groups of diseases (both without an obvious relation to allergy). In one, IgG4 antibody has actually been demonstrated to transfer disease. In the other, it is more likely that IgG4 is a consequence rather than the cause of the disease.

#### *Pemphigus* [49]

IgG4 auto-antibodies from patients with pemphigus and mucous membrane pemphigoid cause intra-epithelial blisters just by binding to their target antigen in the desmosome, resulting in blister induction independent of complement- and leucocyte activation [50]. IgG4 antibodies purified from patients with an endemic form of pemphigus foliaceus have been shown to be pathogenic in mice [51]. A monoclonal IgG4 antibody cloned from a patient with pemphigus-induced acantholysis when injected into neonatal mice [52].

Also in sub-epidermal blistering diseases such as bullous pemphigoid, purified IgG4 auto-antibodies were shown to induce dermal-epidermal separation in an *ex vivo* skin model [53]. In this model, which is leucocyte dependent, IgG1 is more active than IgG4.

#### *Sclerosing autoimmune pancreatitis* [54]

A condition known as retroperitoneal fibrosis is characterized by multifocal fibrosclerosis. A well-studied example is autoimmune pancreatitis (see Neild *et al.* [54] for examples of related diseases in other organs). This is usually accompanied by infiltrates of plasma cells, of which often a large fraction produces IgG4. This expansion of IgG4-secreting plasma cells can be so intense that a marked increase (often more than 10-fold) in plasma total IgG4 is found. This may result in a tissue mass that can be mistaken for a tumour. This condition has to be considered if a pancreas tumour is suspected and a characteristic feature is its sensitivity to corticosteroid treatment.

The pathogenesis of this condition is still a matter of debate. However, in view of the IgG4-promoting effects of anti-inflammatory cytokines, it is tempting to assume that some initial trigger activates a fibrotic process, including the enhanced production of TGF and IL-10. These cytokines subsequently generate a local environment that attracts IgG4-committed B cells and promotes the generation and maintenance of IgG4-producing plasma cells.

#### Conclusion

IgG4 antibodies are odd, both in the sense of appearing one handed, but also in the sense of having peculiar

properties and appearances. In the context of allergy, IgG4 antibodies are puzzling because of their ambiguous association with allergic symptoms. The association with symptoms largely reflects the connection with IgE: antigens that induce IgE antibodies are good inducers of IgG4 antibodies. Even though there are IgG4-positive 'allergic' patients with little or no specific IgE, there is no solid evidence that their symptoms are caused by these IgG4 antibodies. Moreover, there is no plausible mechanism that would lead to activation of mast cells or basophils by the interaction of allergen-specific IgG4 and its allergen. Attempts to sensitize human basophils with allergen-specific IgG4 for antigen-induced histamine release failed [55–57] and it is important to stress that high levels of IgG4 antibodies (e.g. to foods or insect venom) are found in many symptom-free subjects.

The association of IgG4 with lack of symptoms has at least two (mutually non-exclusive) explanations: (1) blocking antibody and (2) marker of tolerance induction. The *blocking antibody* feature is likely to work in two ways. The classical mechanism is competition for allergen between the IgG4 antibody and mast cell-bound IgE antibody, possibly in conjunction with activation of a negative-signalling pathway via Fc $\gamma$ RIIb receptor [58] (but, as expected from its low-affinity for this receptor, our results with chimeric IgG4 antibodies do not support such a negative-signalling contribution [59]). The other mechanism is related to the process known as IgE-facilitated allergen presentation [46, 60–62]. In the presence of antigen-presenting cells (APCs) with an IgE receptor, IgE antibody has been found to substantially lower the allergen dose needed to activate T cells via the APC. In the presence of IgG4 antibody, this IgE-facilitation is prevented (again because of competition between IgE and IgG4). This results in a diminished sensitivity of T cells and thus in a suppression of the late-phase reaction. It goes without saying that IgG4 must be able to compete with IgE in order to be protective.

Besides, IgG4 is a marker of tolerance induction. It is clearly not a perfect marker, because the levels in symptomatic patients are often higher than in symptom-free patients. However, in contrast to measurements at a single point in time, IgG4 antibody measurements may be particularly valuable in follow-up studies, where a substantial increase in IgG4 antibody [and particularly in the IgG4/IgG(total) antibody ratio] as a much more robust indicator of the activation of tolerance-inducing mechanisms.

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