

Position paper

Testing for IgG4 against foods is not recommended as a diagnostic tool: EAACI Task Force Report*

Serological tests for immunoglobulin G4 (IgG4) against foods are persistently promoted for the diagnosis of food-induced hypersensitivity. Since many patients believe that their symptoms are related to food ingestion without diagnostic confirmation of a causal relationship, tests for food-specific IgG4 represent a growing market. Testing for blood IgG4 against different foods is performed with large-scale screening for hundreds of food items by enzyme-linked immunosorbent assay-type and radioallergosorbent-type assays in young children, adolescents and adults. However, many serum samples show positive IgG4 results without corresponding clinical symptoms. These findings, combined with the lack of convincing evidence for histamine-releasing properties of IgG4 in humans, and lack of any controlled studies on the diagnostic value of IgG4 testing in food allergy, do not provide any basis for the hypothesis that food-specific IgG4 should be attributed with an effector role in food hypersensitivity. In contrast to the disputed beliefs, IgG4 against foods indicates that the organism has been repeatedly exposed to food components, recognized as foreign proteins by the immune system. Its presence should not be considered as a factor which induces hypersensitivity, but rather as an indicator for immunological tolerance, linked to the activity of regulatory T cells. In conclusion, 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. Therefore, testing of IgG4 to foods is considered as irrelevant for the laboratory work-up of food allergy or intolerance and should not be performed in case of food-related complaints.

**Steven O. Stapel¹, R. Asero²,
B. K. Ballmer-Weber³, E. F. Knol⁴,
S. Strobel⁵, S. Vieths⁶, J. Kleine-
Tebbe⁷**

¹Sanquin Diagnostic Services, Amsterdam, the Netherlands; ²Ambulatorio di Allergologia, Clinica San Carlo, Paderno Dugnano, Italy; ³Allergy Unit, Department of Dermatology, University Hospital Zürich, Zürich, Switzerland; ⁴Department of Dermatology/Allergology, University Medical Center Utrecht, Utrecht, the Netherlands; ⁵Peninsula College of Medicine and Dentistry, Institute of Clinical and Biological Sciences, Plymouth, UK; ⁶Paul-Ehrlich-Institut, Division of Allergology, Langen, Germany; ⁷Allergy and Asthma Center Westend, Berlin, Germany

Key words: food allergy; food intolerance; IgG; IgG4; *in vitro* tests.

Dr Steven O. Stapel
Sanquin; Laboratory of Allergy
Plesmanlaan 125
1066 CX Amsterdam
the Netherlands

*Coordinated by the EAACI Interest Group Allergy Diagnosis and EAACI Interest Group Food Allergy

Accepted for publication 12 February 2008

An *adverse food reaction* is a general term describing clinically abnormal responses to an ingested food that might occur secondary to nonallergic food hypersensitivity (*food intolerance*) or allergic food hypersensitivity (*food allergy*).

Food allergy is an immunologic reaction that involves in particular the immunoglobulin E (IgE) mechanism, of which anaphylaxis is the classic example.

Food intolerance, however, is a general term, describing an abnormal physiologic response to an ingested food or food additive. Diagnosis of food allergy aims to establish a reliable link between the clinical history of an adverse reaction to food as reported by the patient and the immunological basis of this reaction. In food allergy, an accurate diagnosis is extremely important, in particular to prevent patients from unnecessary and even potentially health threatening diets.

Measurement of food-specific IgE antibodies by *in vitro* assays or skin testing are the routine procedures to diagnose food allergy. These diagnostic tests, however, indicate the presence of food-specific IgE antibodies, but they do not establish the diagnosis of food allergy. The final proof of the clinical relevance of the reported history and the detected food-specific IgE is merely provided by a positive controlled food challenge (1).

When IgE-mediated food allergy cannot be established by regular diagnostic procedures, it is not uncommon that disappointed patients, who blame food components for their problems, seek further for confirmation of their personal suspicions elsewhere, looking for test results that are more in line with their expectations.

Commercial laboratories all over Europe are currently offering broad-scale IgG4 testing against foods to the public, claiming that these tests represent reliable tools for the diagnosis of food allergies. This idea is probably

based on observations from the early 1980s, indicating that antigen-specific IgG4 induces, like IgE, histamine release from basophils (2).

IgG4 and histamine release

After the discovery of histamine-releasing IgG antibodies in human serum in 1970, initially designated as 'Short-Term Sensitizing IgG' (3), the question arose as to which subclass these antibodies belong. The newly discovered IgG4 subclass was considered to be a serious candidate, which attributed IgG4 with an 'anaphylactic flavour'. In addition, the fact that IgG4-synthesis, like that of IgE, was later found to be influenced by T-helper2 cytokines, has helped to associate IgG4 with IgE-mediated allergy.

The capacity of IgG4 antibodies to release histamine from basophils has been the subject of long-standing debate, but firm evidence was not been obtained (4), and no recent observations have provided new insights on this matter as yet.

Until now, only one well-documented study on histamine release in which allergen-specific IgG4 is involved was published. However, in this study, a bivalent chimeric IgG4 antibody against the mite allergen Der p 2-induced histamine release from basophils by indirect crosslinking of rDer p 2-specific, basophil-immobilized, chimeric IgE (5). Importantly, IgG1 worked under these (artificial) conditions equally well, the IgG4 chimeric antibody used in this test was functionally *bivalent* [IgG4 in plasma is functionally monovalent (6)] and the release was dependent on the presence of IgE on the basophil membrane. These results therefore do not provide strong evidence for a supposed anaphylactic nature of the IgG4 subclass.

IgG testing in allergy

Testing for allergen-specific IgG, however, certainly has a role in allergy diagnosis. An example is testing for precipitating antibodies, mainly belonging to the IgG class, against Type III allergens.

In addition, in IgE-mediated allergic disease, however, there is renewed interest for determining specific IgG: IgG4, formed in the course of allergen-specific immunotherapy, not only shows blocking activity by inhibiting IgE-mediated facilitated antigen presentation (7), increasing evidence points to the conclusion that allergen-specific IgG4 responses are the result of the activities of regulatory T cells, being associated with the induction of immunological tolerance upon prolonged exposition to antigen (8). One of the main characteristics of Treg cells is the secretion of interleukin (IL)-10, which leads to the formation of an antigen-specific, non-complement-activating, IgG4 response (9, 10). In addi-

tion, in cow's milk allergy, it was demonstrated that tolerance was related to allergen-induced IL-10 production by T cells (11).

IgG responses against foods

Testing for IgG against foods may occasionally be indicated. An example is testing for IgG against wheat gliadin for the diagnosis of celiac disease, but the information obtained is of very poor clinical specificity and sensitivity, and this test should be applied only in case of IgA deficiency.

Measurement of IgG against foods is considered useful for antigen avoidance in irritable bowel syndrome by some investigators (12), but the evidence for this conclusion was strongly challenged on the basis of poor study design (13). Another paper reports a small, not significant increased concentration of IgG4 specific for whey proteins in delayed-type non-IgE-mediated cow's milk allergic patients (14).

In two studies in which the IgG response to foods was measured, the main goal was to investigate whether an IgG4 response against foods in early life could be predictive for the development of inhalant allergy during later life. IgG4 tests for several foods were carried out, using IgG4 radioallergosorbent test (RAST) for serum samples from unselected 12- to 16-year-old school children (15) and IgG enzyme-linked immunosorbent assay (ELISA) (16) for 1-year-old children with intermediate risk for atopy (17). It was striking that even in unselected children very pronounced IgG4 responses to foods were found (Fig. 1A). The same is true for food-specific IgG in intermediate risk children (Fig. 1B).

An important conclusion from these investigations was that the IgG tests were *only* useful to provide information in epidemiological studies, meaning that they gave *no* relevant information on an *individual* basis: it is obvious that treatment, such as dietary intervention based on the presence of food-specific IgG(4) for these test groups, would have resulted in massive overtreatment. Another observation from both the investigations was that there were no indications that food-specific IgG(4), which appeared to be common in the young population, was associated with food-allergic complaints, even at the population level. Furthermore, these studies established a positive association between IgG and the development of *inhalant* allergy at a later age.

The explanation for the presence of increased levels of food-specific IgG(4) in serum is probably that the immune system of some individuals tends to react more actively to (harmless) antigens than that of others. Such hyperreactivity of the immune system may lead in certain cases to IgE-mediated allergic disease, but might in young children also result in a food-specific IgG response, for instance by (temporarily) increased permeability of the immature gut.

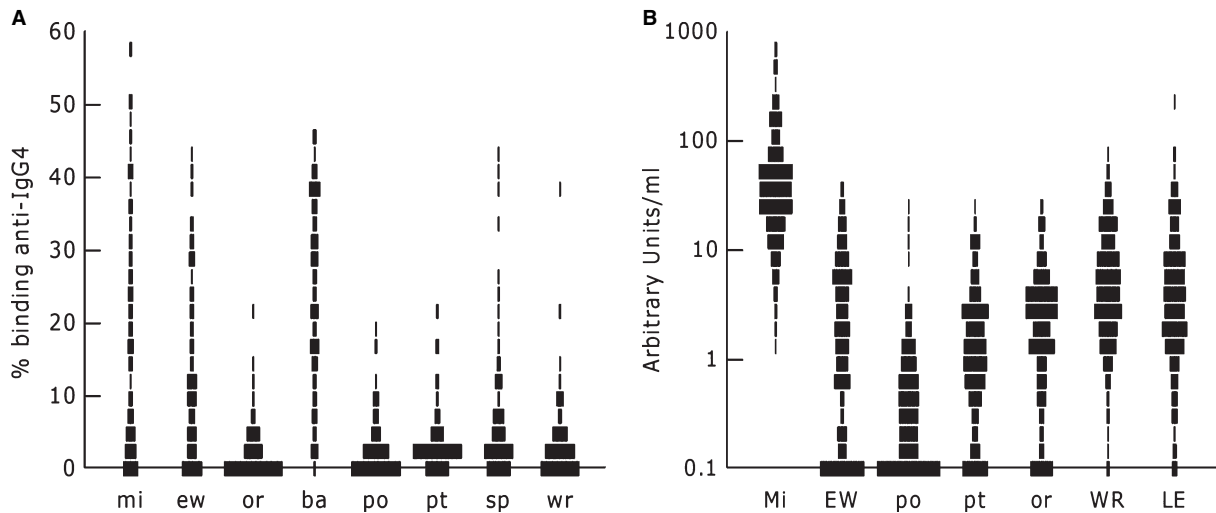


Figure 1. (A) IgG4 RAST for cow's milk (mi), egg white (ew), orange (or), banana (ba), pork (po), potato (pt), soy/peanut (sp, mixture) and wheat/rice (wr, mixture), as observed for 100 unselected 12- to 16-year-old school children (15). (B) IgG ELISA for cow's milk, egg white, orange, pork, potato, soy/peanut (mixture) and wheat/rice (mixture), as observed for 264 intermediate risk 1-year-old children (16).

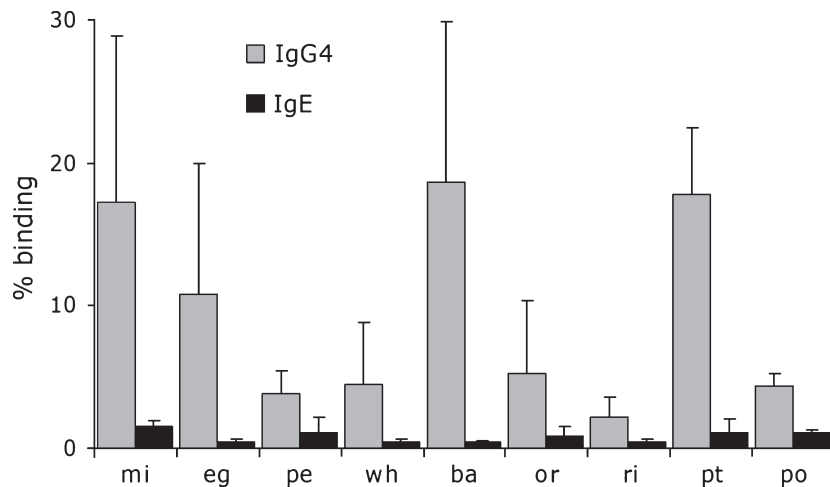


Figure 2. IgG4/IgE RAST for cow's milk (mi), hen's egg (eg), peanut (pe), wheat (wh), banana (ba), orange (or), rice (ri), potato (pt) and pork (po), as found for 13 healthy laboratory workers. Results are expressed as average binding (^{125}I -anti-IgG4/ ^{125}I -anti-IgE) percentages.

Since there are few data available on IgG4 responses to foods in adults, we tested 13 healthy laboratory workers for IgG4 and IgE to foods by RAST-type assays, and found the following results (Fig. 2).

Positive results for IgG4 against different foods were found in all samples, and did not significantly coincide with positive IgE tests. The observed increased IgG4 results are in none of the subjects tested related to clinical problems by intake of foods which were positive in the IgG4 RAST.

Positive IgG4 tests to foods therefore do not indicate the presence of food allergy, but are probably reflecting prolonged exposure to food components.

In case of a persistent exposure of the immune system to food proteins, e.g. as a result of local increased permeability of the gut, it is likely that the immune system will eventually react with an antigen-specific IgG4 response, as is the case in many situations where the immune system is challenged by ongoing antigen exposition. Well-known examples of such IgG4-dominated responses are found in frequently stung beekeepers, where more than 90% of venom-specific IgG belongs to the IgG4 subclass (18), and in (clinically successful) immunotherapy, where a gradual rise in the allergen-specific IgG4 : IgG1 ratio can be observed. Thus, the presence of IgG4 antibodies against food antigens should probably

be interpreted as a result of antigen exposure, rather than of disease, and their presence will, if anything, presumably rather be beneficial than detrimental to the individual. This is in accordance with the recent observation that the induction of tolerance in cow's milk allergic children was associated with an increased concentration of cow's milk-specific IgG4 antibodies (19). In addition, testing for food-specific IgG4 in atopic dermatitis patients does not indicate that raised levels should be associated with clinical complaints (20).

Conclusion

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. Therefore, testing of IgG4 to foods is considered as irrelevant for the laboratory work-up of food allergy or intolerance and should not be performed in case of food-related complaints (21).

References

1. Bindslev-Jensen C, Ballmer-Weber BK, Bengtsson U, Blanco C, Ebner C, Hourihane J, et al. Standardization of food challenges in patients with immediate reactions to foods – position paper from the European Academy of Allergy and Clinical Immunology. *Allergy* 2004;**59**:690–697.
2. Fagan DL, Slaughter CA, Capra JD, Sullivan TJ. Monoclonal antibodies to immunoglobulin G4 induce histamine release from human basophils *in vitro*. *J Allergy Clin Immunol* 1982;**70**:399–404.
3. Parish WE. Short-term anaphylactic IgG antibodies in human sera. *Lancet* 1970;**2**:591–592.
4. Van der Zee JS, Aalberse RC. The role of IgG in allergy. In: Lessof MH, Lee TH, Kemeny DM, editors. *Allergy: an international textbook*. London: Wiley, 1987:49–67.
5. Schuurman J, Perdok GJ, Mueller GA, Aalberse RC. Complementation of Der p 2-induced histamine release from human basophils sensitized with monoclonal IgE: not only by IgE, but also by IgG antibodies directed to a nonoverlapping epitope of Der p 2. *J Allergy Clin Immunol* 1998;**101**:404–409.
6. Van der Zee JS, van Swieten P, Aalberse RC. Serologic aspects of IgG4 antibodies II. IgG4 antibodies form small, nonprecipitating immune complexes due to functional monovalency. *J Immunol* 1986;**137**:3566–3571.
7. Neerven RJ, Knol EF, Eijraes A, Wurzen PA. IgE-mediated allergen presentation and blocking antibodies: regulation of T-cell activation in allergy. *Int Arch Allergy Immunol* 2006;**141**:119–129.
8. Satoguina J, Weyand E, Larbi J, Hoerauf A. T regulatory-1 cells induce IgG4 production by B cells: role of IL-10. *J Immunol* 2005;**174**:4718–4726.
9. Jutel M, Akdis M, Blaser K, Akdis CA. Mechanisms of allergen specific immunotherapy T cell tolerance and more. *Allergy* 2006;**61**:796–807.
10. Nouri-Aria KT, Wachholz PA, Francis JN, Jacobson MR, Walker SM, Wilcock LK, et al. Grass pollen immunotherapy induces mucosal and peripheral IL-10 responses and blocking IgG activity. *J Immunol* 2004;**172**:3252–3259.
11. Tiemessen MM, Van Ieperen-Van Dijk AG, Bruijnzeel-Koomen CA, Garssen J, Knol EF, Van Hoffen E. Cow's milk-specific T-cell reactivity of children with and without persistent cow's milk allergy: key role for IL-10. *J Allergy Clin Immunol* 2004;**113**:932–939.
12. Atkinson W, Sheldon A, Shaath N, Whorwell PJ. Food elimination based on IgG antibodies in irritable bowel syndrome: a randomised controlled trial. *Gut* 2004;**53**:1459–1464.
13. Hunter JO. Food elimination in IBS: the case for IgG testing remains doubtful. *Gut* 2005;**54**:1203.
14. Sletten GB, Halvorsen R, Egaas E, Halstensen TS. Changes in humoral responses to β -lactoglobulin in tolerant patients suggest a particular role for IgG4 in delayed, non-IgE-mediated cow's milk allergy. *Pediatr Allergy Immunol* 2006;**17**:435–443.
15. Calkhoven PG, Aalbers M, Koshte VL, Schilte PP, Yntema JL, Griffioen RW, et al. Relationship between IgG1 and IgG4 antibodies to foods and the development of IgE antibodies to inhalant allergens. II. Increased levels of IgG antibodies to foods in children who subsequently develop IgE antibodies to inhalant allergens. *Clin Exp Allergy* 1991;**21**:99–107.
16. Eysink PED, de Jonge MH, Bindels PJE, Scharp-van der Linden VTM, de Groot CJ, Stapel SO, et al. Relation between IgG antibodies to foods and IgE antibodies to milk, egg, cat, dog and/or mite in a cross-sectional study. *Clin Exp Allergy* 1999;**29**:604–610.
17. De Jong MH, Scharp-van der Linden VTM, Aalberse RC, Oosting J, Tijssen JGP, de Groot CJ. Randomised controlled trial of brief neonatal exposure to cow's milk on the development of atopy. *Arch Dis Child* 1998;**79**:126–130.
18. Aalberse RC, van der Gaag R, van Leeuwen J. Serologic aspects of IgG4 antibodies. *J Immunol* 1983;**130**:722–726.
19. Ruiter B, Knol EF, Garssen J, Knulst AC, Van Hoffen E. Maintenance of tolerance to cow's milk in atopic individuals is characterized by high levels of specific immunoglobulin G4. *Clin Exp Allergy* 2007;**37**:1103–1110.
20. Noh G, Ahn HS, Cho NY, Lee S, Oh JW. The clinical significance of food specific IgE/IgG4 in food specific atopic dermatitis. *Pediatr Allergy Immunol* 2007;**18**:63–70.
21. Wüthrich B. Specific IgG antibodies as markers of adverse reactions to food. *Contra! Monogr Allergy* 1996;**32**:226–227.