

# The role of hen's egg-specific IgE, IgG and IgG4 in the diagnostic procedure of hen's egg allergy

B. Ahrens<sup>1</sup>, L. C. Lopes de Oliveira<sup>1</sup>, G. Schulz<sup>1</sup>, M. P. Borres<sup>2,3</sup>, B. Niggemann<sup>1,4</sup>, U. Wahn<sup>1</sup> & K. Beyer<sup>1</sup>

<sup>1</sup>Department of Pediatric Pneumology and Immunology, Charité, Universitätsmedizin, Berlin, Germany; <sup>2</sup>Phadia, Uppsala; <sup>3</sup>Department of Pediatrics, Sahlgrenska Academy at Göteborg University, Göteborg, Sweden; <sup>4</sup>German Red Cross Hospital Westend, Pediatric Allergology and Pneumology, Berlin, Germany

**To cite this article:** Ahrens B, Lopes de Oliveira LC, Schulz G, Borres MP, Niggemann B, Wahn U, Beyer K. The role of hen's egg-specific IgE, IgG and IgG4 in the diagnostic procedure of hen's egg allergy. *Allergy* 2010; DOI: 10.1111/j.1398-9995.2010.02429.x.

## Keywords

food allergy; hen's egg; IgG; IgG4; immunoglobulin; tolerance.

## Correspondence

Kirsten Beyer, Department of Pediatric Pneumology and Immunology, Charité Universitätsmedizin Berlin, Augustenburger Platz 1, 13353 Berlin, Germany.  
Tel.: +49 030 450 559054  
Fax: +49 030 450 559951  
E-mail: kirsten.beyer@charite.de

Accepted for publication 20 May 2010

DOI:10.1111/j.1398-9995.2010.02429.x

Edited by: Hans-Uwe Simon

## Abstract

**Background:** Hen's egg (HE) allergy is a common disease in childhood. HE-specific serum IgE has been correlated with the outcome of oral food challenge tests, and diagnostic decision points have been described as helpful but still not sufficient to reduce the requirement for oral food challenges. The aim of the study was to correlate HE-specific IgE, IgG and IgG4 levels with the outcome of double-blind, placebo-controlled food challenges (DBPCFC) in patients with suspected HE allergy to improve diagnostic procedures.

**Methods:** HE-specific IgE, IgG, and IgG4 levels were compared between 150 children with suspected HE allergy based on sensitization and/or patient's history who underwent DBPCFC. Sixty-six patients were HE-allergic (HE-sensitized with a positive DBPCFC), 48 HE-sensitized but tolerant (negative DBPCFC), and 36 patients were nonsensitized and tolerant (negative DBPCFC). Prior to food challenge HE-specific serum IgE, IgG, and IgG4 were measured with the Phadia CAP-system.

**Results:** HE-specific IgE was significantly higher in HE-allergic patients than in clinically tolerant ones. However, there was no difference in HE-specific IgG and IgG4 concentrations between the patient groups.

**Conclusion:** A proposed cut-off level of 12 kU/l IgE would identify children above this level correctly as HE-allergic. The level of HE-specific IgG or IgG4 in serum of children with suspected HE allergy does not add any additional information in the diagnostic procedure of HE allergy. For diagnostic purposes, specific IgG or IgG4 should not routinely be tested.

Hen's egg (HE) allergy is a common disease in infancy and childhood. Fortunately, the majority of children with HE allergy will become clinically tolerant by school age (1). Improved procedures have been developed for the diagnosis of HE allergy. In this regard, HE-specific IgE concentrations in the serum have been shown to correlate with the outcome of oral food challenge tests (2). However, the majority of children still need to undergo elaborate and costly oral food challenge tests to determine the clinical relevance of their

observed sensitization. Therefore, other parameters are in demand to develop better predictability.

In recent years, other classes of immunoglobulins, especially IgG antibodies, are discussed of being immunopathologically relevant in food allergy. Human IgG consists of four subclasses, IgG1-4, which are functionally diverse and differentially regulated. Whereas IgG1 is complement-fixing and associated with inflammatory systemic Th1-driven immune reactions, IgG4 is noncomplement-fixing, functionally monovalent (3) and has been associated with IgE and Th2 immune responses (4).

In respiratory and insect venom allergies, it had been shown that patients who became tolerant after allergen-specific immunotherapy were characterized by an increase in

## Abbreviations

DBPCFC, double-blind, placebo-controlled food challenge; HE, hen's egg; Ig, immunoglobulin.

allergen-specific IgG4 and a decrease in allergen-specific IgE (5). Similar results have been shown during oral immunotherapy in food-allergic patients (6, 7). Therefore, it is assumed that specific IgG4 plays a role in the induction of tolerance by allergen-specific immunotherapy. However, the role of IgG4 in natural tolerance development in food allergy is hardly understood. So far, mainly the role of IgG4 in cow's milk allergy has been studied, but obtained data are contradictory (8, 9).

The aim of the current study was to correlate in parallel HE-specific IgE, IgG, and IgG4 levels with the outcome of oral food challenge tests in patients with suspected HE allergy.

## Material and methods

Retrospectively, sera of 150 children (age 5 months to 14 years of age) with suspected HE allergy based on clinical history, who underwent double-blind, placebo-controlled oral food challenge tests (DBPCFC) for clinical purposes, were included into the study.

The study was approved by the ethical committee of the university hospital Charité (EA2/078/06). Sera from venous blood samples were taken prior to the food challenge and analyzed for HE (f2)-specific IgE, IgG, and IgG4 levels using the Phadia CAP-System FEIA<sup>®</sup> (Phadia-Diagnostics, Uppsala, Sweden). Measurements were performed on the Phadia 250 machine. A specific IgE titer of >0.35 kU/l was considered positive. Titers below were set to 0.01 kU/l.

Clinical reactivity to HE was investigated for clinical purpose by DBPCFC as described in detail previously (10). In brief, patients received placebo or verum on two different days. The clinical dietician performed the randomization and preparation of the challenges. A maximum of seven gradually increasing titration steps (half-logarithmic) were given at intervals of 30 min up to a maximum dose of 4.6 g of HE protein (raw HE white and yolk). The cumulative dose during DBPCFC was 6.2 g HE protein if no reaction was observed. Provocation was stopped if objective clinical symptoms such as urticaria, exacerbation of eczema (at least 10 point increase in SCORAD score), angioedema, wheezing, allergic rhinitis or conjunctivitis, vomiting, and/or diarrhea were observed or the highest dose was reached.

Data between groups were analyzed with the nonparametric Mann-Whitney *U*-test as the data were not normally distributed. Calculations were performed and graphs generated using GRAPHPAD PRISM (version 4; GraphPad Software Inc., San Diego, CA, USA). *P*-values <0.05 were considered significant.

## Results

Hen's egg-specific IgE, IgG, and IgG4 were measured using the Phadia CAP-System FEIA in 150 children with suspected HE allergy who underwent DBPCFC for clinical purpose. Sera from three groups of patients were selected: (A) 66 sera from HE-sensitized patients (median 22 months of age) with a positive food challenge test (sensitized and allergic), (B) 48 sera from HE-sensitized patients (median 29 months of age)

with a negative food challenge test (sensitized but tolerant) and (C) 36 sera from patients (median 30 months of age) with no sensitization and a negative food challenge test (nonsensitized and tolerant).

HE-specific IgE levels were significantly higher in HE-sensitized and allergic patients (group A) than in HE-sensitized but tolerant ones (group B) (Fig. 1,  $P < 0.0001$ ). In group C (nonsensitized and tolerant patients), no HE-specific IgE was detected. Using the proposed cut-off level of 12 kU/l HE-specific IgE by Sampson et al. (2), all children above this value would have been correctly identified as food allergic. Importantly, there was no significant difference regarding HE-specific IgG or IgG4 levels between sensitized and allergic, sensitized but tolerant and nonsensitized and tolerant children (Figs 2 and 3).

## Discussion

DBPCFC is still regarded as the gold standard test for the determination of clinically relevant food allergy although it

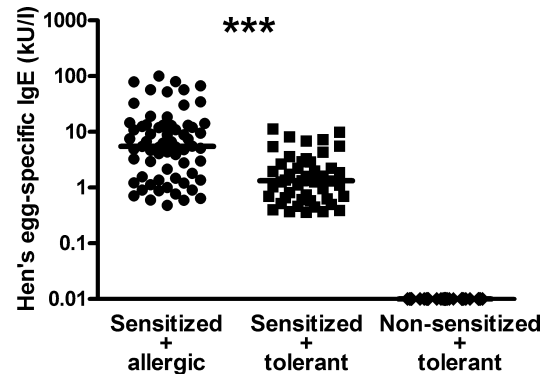


**Figure 1** Hen's egg (HE)-specific IgE in 150 children with suspected HE allergy because of clinical history. Sixty-six of these children were HE-sensitized and had a positive double-blind, placebo-controlled food challenge (DBPCFC) (allergic children), 48 were hen's sensitized but clinical tolerant and 36 were nonsensitized and clinical tolerant children. \*\*\* $P < 0.0001$ .

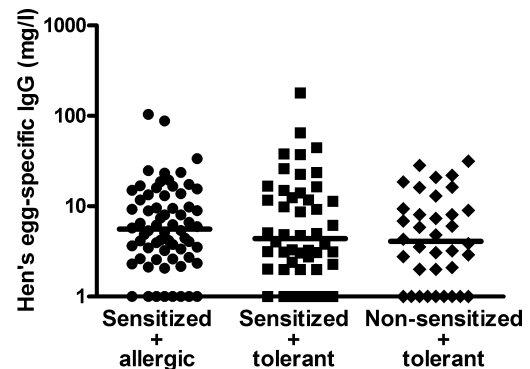


**Figure 2** Hen's egg (HE)-specific IgG in 66 HE-sensitized and allergic children, 48 hen's sensitized but tolerant and 36 nonsensitized and tolerant children.

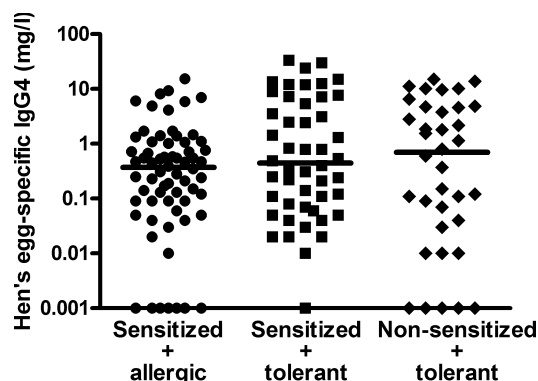


**Figure 3** Hen's egg (HE)-specific IgG4 in 66 HE-sensitized and allergic children, 48 hen's sensitized but tolerant and 36 nonsensitized and tolerant children.

is a complex and time-consuming procedure. Using diagnostic decision points of HE-specific IgE concentrations is helpful to reduce the requirement for oral food challenge tests (2, 10). However, the vast majority of patients have HE-specific IgE levels below the cut-off level and need to undergo oral food challenge test to determine the individual clinical relevance. Therefore, we intended to delineate the role of HE-specific IgG and IgG4. As expected and in former studies described, HE-specific IgE concentrations in the serum have been significantly higher in HE-sensitized patients with clinically relevant allergy, than in HE-sensitized, but nonallergic ones. Using the proposed cut-off level of 12 kU/l HE-specific IgE by Sampson et al. (2), all children above these value are correctly identified as food allergic. Children below this cut-off level would need to undergo oral food challenge test to determine their clinical reactivity. We were able to show that neither IgG nor IgG4 adds additional value to IgE measurement in the diagnostic procedure of HE allergy.

It is currently discussed that IgG production is a normal physiological response to ingested dietary antigens, which can also be found in a large proportion of healthy subjects and is not considered as a marker of allergy (11–15). Interestingly, in all patient groups, we were able to detect HE-specific IgG although all patients were on a hen's egg-free diet indicating that this antibody response is persisting. It has been shown by others and us that food-allergic patients produce significantly more food-specific antibodies than controls without despite the fact that the patients are on an allergen-free diet. This has been discussed as a polyisotypic response. We would

like to point out that there is no difference between patients with clinically relevant HE allergy and sensitized but clinical tolerant ones emphasizing that measurement of HE-specific IgG is not helpful as a diagnostic tool in suspected HE allergy. For this observation, it is important to mention that there was no significant difference in the age distribution of the children in the three groups as IgG and IgG4 are known to be age dependent.

There is evidence from oral immunotherapy trials that elevated food-specific IgG4 levels in sensitized individuals may indicate acquisition of clinical tolerance in cases of resolving IgE-mediated allergy (7, 8). These correlations between IgG4 antibodies and tolerance were first described after specific immunotherapy for instance in subcutaneous venom immunotherapy (5). Protective or blocking functions for this subclass are assumed (16).

Ruiter et al. (9) came to quite similar results regarding natural tolerance development in cow's milk allergy, showing that IgG4 levels were the highest in atopic subjects who were tolerant to cow's milk, whereas Shek et al. (8) reported an increased IgG4 response in cow's milk allergic subjects in comparison with atopic patients without suspected food allergy. In contrast, in the current study, no differences in HE-specific IgG4 levels have been observed between tolerant and allergic children. Interestingly, Tay et al. (14) observed distinct patterns of IgG and IgG4 responses in various food allergens. It appears that the role of IgG4 in tolerance development is still not completely understood. From the current study, however, measurement of HE-specific IgG4 appears not to be helpful in the diagnostic procedure of suspected HE allergy.

In summary, the current data point out that neither IgG nor IgG4 adds additional value to IgE measurement in the diagnostic procedure of HE allergy. This seems to stand in contrast to immunotherapy trials where increase in specific IgG4 levels indicates acquisition of tolerance. Therefore, for diagnostic purposes in children with suspected HE allergy-specific IgG or IgG4 should not routinely be tested. Unfortunately, the majority of children with suspected HE allergy still need to undergo oral food challenge tests. Identification of better diagnostic tools is still required.

## Acknowledgments

This study was funded in part by institutional funds and in part by Phadia. We thank our dieticians Christiane Binder, Mandy Ziegert and Kerstin Dobberstein for diligently performing the oral food challenges.

## References

1. Savage JH, Matsui EC, Skripak JM, Wood RA. The natural history of egg allergy. *J Allergy Clin Immunol* 2007;**120**:1413–1417.
2. Sampson HA. Utility of food-specific IgE concentrations in predicting symptomatic food allergy. *J Allergy Clin Immunol* 2001;**107**:891–896.
3. Aalberse RC, Schuurman J. IgG4 breaking the rules. *Immunology* 2002;**105**:9–19.
4. Vercelli D. One cytokine, two isotypes: a trojan horse, pandora's box, and an evolving paradigm. *Am J Respir Crit Care Med* 2000;**162**(3 Pt 2):S86–S90.
5. Wyss M, Scheitlin T, Stadler BM, Wuthrich B. Immunotherapy with aluminum hydroxide adsorbed insect venom extracts (Alutard SQ): immunologic and clinical results of a

- prospective study over 3 years. *Allergy* 1993; **48**:81–86.
6. Buchanan AD, Green TD, Jones SM, Scurlock AM, Christie L, Althage KA et al. Egg oral immunotherapy in nonanaphylactic children with egg allergy. *J Allergy Clin Immunol* 2007; **119**:199–205.
  7. Jonas SM, Pons L, Roberts JL, Scurlock AM, Perry TT, Kulis M et al. Clinical efficacy and immune regulation with peanut oral immunotherapy. *J Allergy Clin Immunol* 2009; **124**:293–300.
  8. Shek LP, Bardina L, Castro R, Sampson HA, Beyer K. Humoral and cellular responses to cow milk proteins in patients with milk-induced IgE-mediated and non-IgE-mediated disorders. *Allergy* 2005; **60**:912–919.
  9. Ruiter B, Knol EF, van Neerven RJ, Garssen J, Bruijnzeel-Koomen CA, Knulst AC et al. 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.
  10. Celik-Bilgili S, Mehl A, Verstege A, Staden U, Nocon M, Beyer K et al. The predictive value of specific immunoglobulin E levels in serum for the outcome of oral food challenges. *Clin Exp Allergy* 2005; **35**:268–273.
  11. Husby S. Dietary antigens: uptake and humoral immunity in man. *APMIS* 1988; **1**(Suppl.):1–40. Review.
  12. Rowntree S, Platts-Mills TA, Cogswell JJ, Mitchell EB. A subclass IgG4-specific antigen-binding radioimmunoassay (RIA): comparison between IgG and IgG4 antibodies to food and inhaled antigens in adult atopic dermatitis after desensitization treatment and during development of antibody responses in children. *J Allergy Clin Immunol* 1987; **80**: 622–630.
  13. Germano P, Pezzini A, Boccagni P, Zanoni G, Tridente G. Specific humoral response to cows' milk proteins and ovalbumin in children with atopic dermatitis. *Int J Clin Lab Res* 1993; **23**:206–211.
  14. Tay SS, Clark AT, Deighton J, King Y, Ewan PW. Patterns of immunoglobulin G responses to egg and peanut allergens are distinct: ovalbumin-specific immunoglobulin responses are ubiquitous, but peanut-specific immunoglobulin responses are up-regulated in peanut allergy. *Clin Exp Allergy* 2007; **37**:1512–1518.
  15. Stapel SO, Asero R, Ballmer-Weber BK, Knol EF, Strobels S, Vieths S et al. Testing for IgG4 against foods is not recommended as a diagnostic tool: EAACI Task Force Report. *Allergy* 2008; **63**:793–796.
  16. Wachholz PA, Durham SR. Mechanisms of immunotherapy: IgG revisited. *Curr Opin Allergy Clin Immunol* 2004; **4**:313–318. Review.