# Significance of ovomucoid- and ovalbumin-specific IgE/IgG$_4$ ratios in egg allergy

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**Background: The role of specific IgG$_4$ antibodies in natural tolerance acquisition remains a matter of debate; the specific IgE/IgG$_4$ ratio might add value to the measurement of absolute amounts of IgE for assessing the ongoing status of egg reactivity.**
**Objective: We sought to determine the significance of IgG$_4$ antibodies to ovalbumin (OVA) and ovomucoid (OVM) in egg-allergic children.**
**Methods: One hundred seven egg-allergic children (mean age 6.9 years; range 1.6-18.6 years) were challenged to baked egg. The outcomes of the challenges were related to the level of specific IgE and IgG$_4$ to OVM and OVA, component IgE/IgG$_4$ ratios, and mediator release in a functional assay based on the rat basophil leukemia cell line.**
**Results: Baked egg–reactive children had significantly higher OVA and OVM ratios of IgE/IgG$_4$ and mediator release in the rat basophil leukemia–based assay than did tolerant children ($P < .05$ for both). The OVA- and OVM-specific IgE/IgG$_4$ ratios and mediator release were correlated. In the receiver operating characteristic analysis, the areas under the curve for a logistic regression model including specific IgE and IgG$_4$ to OVA and OVM were significantly greater compared with the areas under the curve for egg white–specific IgE and OVM-specific IgE.**
**Conclusions: The balance between IgE and IgG$_4$ to OVA and OVM has functional consequences. A model that includes the interactions between IgE and IgG$_4$ to OVA and OVM accurately predicts reactivity to baked egg and warrants further investigation. (J Allergy Clin Immunol 2012;129:739-47.)**

***Key words:*** *Egg, egg white, ovalbumin, ovomucoid, egg allergy, food allergy, children, hypersensitivity, IgE, IgG$_4$, IgE/IgG$_4$ ratio*

Hen's egg white allergy affects approximately 1.6% of children.[1-4] In a large (n = 2848), population-based study from Australia, the prevalence of challenge-proven, uncooked-egg allergy in 12-month-old infants was 8.9% (95% CI, 7.8-10.0).[5] Egg allergy prevalence is considerably higher, up to 30%, in children with atopic dermatitis or other food allergies.[6,7] Although egg allergy usually resolves with age, it can persist beyond the age of 5 years.[8,9] The majority (>70%) of children reacting to regular egg (lightly heated such as French toast or scrambled egg) can tolerate ingestion of baked egg.[5,10-15] Consumption of baked egg (muffin and waffle) by children allergic to regular egg (French toast, scrambled egg) was associated with immunologic changes that suggested accelerated development of tolerance to regular egg.[14]

Although our knowledge of the pathophysiology of food allergy has improved, mechanisms of tolerance development remain poorly understood.[16,17] In type I food hypersensitivity, food-specific IgE antibodies are implicated in the pathophysiology. Emerging data suggest a role for the IgG$_4$ immunoglobulin class in tolerance development. Naturally developing tolerance to cow's milk was associated with increased regulatory T cells and increased casein-IgG$_4$ levels.[18-20] In oral immunotherapy trials, increasing food-specific IgG$_4$ levels in sensitized individuals accompanied acquisition of clinical tolerance.[21,22] Protective or blocking functions for IgG$_4$ subclass antibodies have been proposed[23,24]; in peanut, egg, and milk allergy, IgG$_4$ antibodies generally overlap with IgE antibodies in respect to sequential epitope specificity.[25-28]

We sought to determine the functional significance of IgG$_4$ antibodies to ovalbumin (OVA) and ovomucoid (OVM) in children with IgE-mediated egg allergy. We hypothesized that high IgE/IgG$_4$ ratios to OVA and OVM are associated with a higher likelihood of reactivity to baked egg in egg-allergic children. We evaluated the performance of a logistic regression model that includes both specific IgE and IgG$_4$ to OVA and OVM for predictions of reactivity to baked egg.

## METHODS

### Study population

We analyzed data from a study on tolerance to baked egg.[14] Tolerance to baked egg was determined by oral food challenge (OFC). The study was

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*Abbreviations used*
EW: Egg white
OFC: Oral food challenge
OVA: Ovalbumin
OVM: Ovomucoid
RBL: Rat basophil leukemia

approved by the Mount Sinai Institutional Review Board, and informed consent was obtained before enrollment.

## Antibody measurements

A serum sample was collected from each subject to measure egg white (EW)-, OVM-, and OVA-specific IgE and OVM- and OVA-specific $IgG_4$ antibody concentrations with the UniCAP system (Phadia US, Portage, Mich). For specific IgE, the lower limit of detection was 0.35 $kU_A/L$ and the upper limit of detection was 100 $kU_A/L$. For food-specific $IgG_4$, the lower limit of detection was 0.02 $mg_A/L$ and the upper limit of detection was 35 $mg_A/L$. For analysis, values below the lower limit of detection for specific $IgG_4$ and IgE were replaced by half the lower limit of detection,[29] that is, 0.01 $mg_A/L$ for $IgG_4$ and 0.175 $kU_A/L$ for IgE.

## Mediator release assay

Sera were obtained from baked egg–reactive children (n = 10), baked egg–tolerant/regular egg–reactive children (n = 9), and children with a history of egg allergy who tolerated baked egg and regular egg during an OFC (n = 8). Rat basophil leukemia (RBL) cells transfected with human Fcε receptor I (confirmed expression of alpha chain; kind gift from Dr Stefan Vieths and Dr Lothar Vogel) are an accepted *in vitro* model for studying IgE-mediated reactions. [30] RBL cells were passively sensitized with human serum overnight and stimulated with a range of 10-fold serial dilutions of unheated and heated (>98.5°C [203°F] for 30 minutes) EW powder, purified OVA grade V, and OVM grade VII (Sigma, St Louis, Mo), starting concentration of 32 μg/mL. The assay was performed as previously published.[31] N-Hexosaminidase was measured in the supernatant as a marker of RBL-cell mediator release. Results were expressed as a percentage of the cell release minus the spontaneous release that was divided by the total release.

## Statistical analysis

Analyses were performed by using SAS/STAT version 9.2 (SAS Institute, Inc, Cary, NC). Comparison of data between baked egg–reactive and baked egg–tolerant patients was performed with the Mann-Whitney *U* test. A probability level of less than 5% was considered as significant. The Spearman rank order correlation coefficient ($r_s$) was used to measure the strength of the relationship between specific IgE and specific $IgG_4$. A Wilcoxon rank-sum test was used to compare peak mediator release in baked egg–reactive and baked egg–tolerant subjects. The linear relationship between peak mediator release and specific IgE/$IgG_4$ ratios to OVA and OVM was assessed with a mixed model, which accounted for the correlation between repeated measures made on each subject.

A logistic regression model was used to estimate the probability of baked egg allergy among different combinations of specific IgE and $IgG_4$ to OVA and OVM by using the following formula: 1/(1+exp(−2.33 + 0.26(specific IgE to OVA) + 0.19(specific IgE to OVM) − 0.18(specific $IgG_4$ to OVA) + 0.99(specific $IgG_4$ to OVM) − 0.12(specific IgE to OVA × specific $IgG_4$ to OVA) − 0.34(specific IgE to OVM × specific $IgG_4$ to OVM))). Receiver operating characteristic curves were generated for specific IgE to EW, OVM, and OVA as well as for the logistic regression model. The areas under the curves were estimated and compared by using PROC LOGISTIC in SAS. Youden's index, the value that maximizes the true-positive rate while minimizing the false-positive rate,[32] was used to estimate the optimal cutpoint (dichotomizing the predicted probabilities of response) for each curve. Using the dichotomized version of these predicted probabilities of baked egg allergy, screening diagnostic statistics including sensitivity, specificity, positive predictive value, and negative predictive value were computed along with 95% CIs.

# RESULTS

## Baseline clinical characteristics

One hundred seventeen children, mean age of 6.9 years (range, 1.6-18.6 years), were enrolled; 10 were excluded from the analysis because of missing specific IgE and $IgG_4$ data (Table I). Twenty-five subjects (group A) reacted to baked egg when challenged. They were considered allergic to both baked and regular egg. Of the 82 subjects who tolerated baked egg, 35 reacted to regular egg during the OFC. Twenty-one subjects were not challenged to regular egg, because of EW-specific IgE or skin prick test values greater than the highly predictive levels of reactivity or because of a recent (within previous 6 months) convincing clinical reaction to regular egg. Four subjects refused the regular egg challenge. These 60 patients were grouped together (group B). The remaining 22 subjects were tolerant to both baked and regular egg (group C).

## Baseline immunologic parameters in relation to the outcome of the baked egg OFC

The levels of specific IgE to EW, OVM, and OVA measured at baseline during strict avoidance of dietary egg were significantly higher in baked egg–reactive subjects (group A) than in baked egg–tolerant subjects (groups B and C) ($P < .05$) (Table I). There was a strong correlation between IgE antibody levels to OVA and to EW ($r_s = 0.95$; $P < .001$), between specific IgE to OVM and to EW ($r_s = 0.8$; $P < .001$), and between specific IgE to OVA and to OVM ($r_s = 0.77$; $P < .001$). Most subjects had detectable IgE to both OVM and OVA; 5 subjects (20%) reacting to baked egg had high levels of IgE to OVA and undetectable IgE to OVM.

As previously reported,[14] OVA- and OVM-specific $IgG_4$ levels did not differ significantly between subjects reactive and tolerant to baked egg ($P = .78$ and $P = .77$, respectively). The correlation between specific IgE and specific $IgG_4$ was modest, although significant for OVA ($r_s = 0.33$; $P < .001$) and OVM ($r_s = 0.53$; $P < .001$).

A subgroup of subjects was found to have undetectable $IgG_4$ to OVM and/or to OVA. Nine of the 23 subjects (39.1%) with undetectable $IgG_4$ to both OVM and OVA never knowingly ate egg before the OFC, whereas only 8 subjects out of the 84 (9.5%) with detectable specific $IgG_4$ to OVA and/or to OVM had never knowingly eaten egg before ($P = .002$). The majority of patients (55 of 62; 88.7%) who reported a reaction to egg in the past had positive specific $IgG_4$ to OVM and/or OVA.

## OVA- and OVM-specific IgE/IgG4 ratios in different groups of subjects

OVA- and OVM-specific IgE/$IgG_4$ ratios were significantly higher in baked egg–reactive subjects (group A) than in baked egg–tolerant subjects (groups B and C) ($P = .001$ and $P = .003$, respectively) (Fig 1). Of note, differences in OVA- and OVM-specific ratio between groups remain significant after adjusting for specific IgE to OVA and OVM by analysis of covariance using ranks ($P < .03$). The correlation coefficient between the IgE/$IgG_4$ ratio to OVA and to OVM was high ($r_s = 0.73$; $P < .001$), as well as