Published in final edited form as:

J Allergy Clin Immunol. 2014 December ; 134(6): 1310–1317.e6. doi:10.1016/j.jaci.2014.05.042.

Oral immunotherapy induces IgG antibodies that act via FcγRIIb to suppress IgE-mediated hypersensitivity

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Abstract

Background—Food anaphylaxis is triggered by specific IgE antibodies. Paradoxically, some individuals with significant IgE levels can ingest allergenic foods without incident. Similarly, subjects completing oral immunotherapy (OIT) tolerate food challenges despite persistent high-titer food-specific IgE.

Objective—To test whether IgG antibodies induced by food immunotherapy prevent food-induced anaphylaxis, and whether this occurs via the inhibitory receptor FcγRIIb.

Methods—Food allergy-susceptible *Il4raF709* mice were enterally sensitized to ovalbumin (OVA). Similarly sensitized IgE-deficient *Il4raF709/IgE^{-/-}* mice, which can ingest OVA without anaphylaxis, were subjected to a high-dose enteral OVA desensitization protocol (OIT). Sera from both groups were tested for the ability to activate or inhibit bone marrow mast cells (BMMC) exposed to allergen, or to passively transfer allergy to naïve hosts. In parallel experiments, sera obtained from peanut allergic patients before and after undergoing OIT were interrogated for their ability to enhance or suppress peanut-induced activation in an indirect assay using basophils from non-allergic donors.

Results—*Il4raF709* mice exhibited strong OVA-specific IgE responses. Their sera efficiently sensitized BMMC for activation by antigen challenge. Sera from *Il4raF709/IgE^{-/-}* mice subjected to OVA OIT suppressed BMMC responses. This inhibition was IgG-mediated and FcγRIIb-dependent. Similarly, pre-OIT, but not post-OIT sera from patients efficiently sensitized basophils

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Conflict of Interest Statement

None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript. DTU is currently an employee at Genentech.

for peanut-induced activation. IgG antibodies in post-OIT sera suppressed basophil activation by pre-OIT sera. This inhibition was blocked by antibodies against FcγRII.

Conclusion—Food-specific IgG antibodies, such as those induced during OIT, inhibit IgE-mediated reactions. Strategies that favor IgG responses might prove useful in the management of food allergy.

Keywords

Food allergy; tolerance; anaphylaxis; IgE; desensitization; oral immunotherapy

Introduction

Food allergy is a growing health problem affecting children and adults worldwide¹. Clinical evaluation for this disorder centers on assessment of food allergen-specific IgE antibodies, and anaphylaxis arises when these antibodies activate mast cells and basophils via FcεRI. However the correlation between allergen-specific IgE levels and susceptibility to food-induced reactions is imperfect^{2, 3}. This inconsistency may be due to other immune parameters that are not usually evaluated, such as food-specific IgG antibodies or food-specific cellular immunity that might protect against or suppress food-induced reactions, including anaphylaxis, the most significant manifestation of food allergy. A more complete understanding of these additional parameters affecting food sensitivity and tolerance is needed in order to enhance the evaluation and care of these patients.

A large body of evidence suggests that food-specific IgG antibodies might play a major role in protection against food-induced reactions. Injection immunotherapy to aeroallergens is known to induce allergen-specific IgG4 antibodies, which have been postulated to function by blocking allergen interaction with FcεRI-bound IgE⁴. A similar mechanism might be operative in food allergy. Recovery from milk protein allergy has been associated with elevated IgG levels^{5, 6} and Caubet and colleagues reported that egg protein-specific IgG4 antibodies are predictive of successful baked egg challenge⁷. In patients successfully completing OIT or sublingual immunotherapy (SLIT), desensitization is also associated with a strong food-specific IgG antibody response. Skripak and colleagues demonstrated that patients undergoing milk OIT exhibited no change in milk-specific IgE but had a marked induction of milk-specific IgG4⁸. Similarly, we found that OIT to milk, facilitated by a short course of omalizumab treatment, resulted in a 15-fold induction of milk-specific IgG4⁹. Burks and colleagues reported similar increases in food-specific IgG4 in egg and peanut OIT and SLIT^{10–13}. Although the association between IgG induction and food desensitization suggests a mechanistic effect, a suppressive function of the IgG antibodies induced in these clinical allergic responses has not been directly demonstrated.

In this study, we evaluated the suppressive functions of IgG antibodies induced during food allergen desensitization, using both experimental mouse models of food-induced anaphylaxis, and analysis of sera from patients successfully completing peanut OIT.

Materials and methods

Sensitization of mice

Mice used in this study are described in the Online Repository. For sensitization, *Il4raF709* and *Il4raF709/IgE^{-/-}* were treated intragastrically (*i.g.*) with sterile PBS or 250µg OVA (Sigma-Aldrich, St. Louis, MO) plus 20µg cholera toxin (List Biological Laboratories Inc., Campbell, CA), once a week for three weeks. Mice were challenged with 150mg OVA *i.g.* to induce anaphylaxis. Some *Il4raF709/IgE^{-/-}* mice were subsequently subjected to three weeks of daily desensitization therapy with 100mg OVA *i-g*

Study population

In order to probe the induction of suppressive IgG antibodies in human subjects undergoing OIT we used sera from our recently published trial of peanut OIT in high-risk peanut-allergic subjects¹⁴. Thirteen patients with IgE-mediated peanut allergy with histories of significant symptoms including urticaria, vomiting and/or anaphylaxis were enrolled in the study. The subjects included 8 boys and 5 girls, ranging in age from 8–16 years. The median peanut-specific IgE level was 229 kU/L. All children had a skin prick wheal of at least 8.5 mm to peanut extract, and failed an initial double-blind placebo-controlled food challenge on week 0 at peanut protein doses of 50 mg or less (given as peanut flour). After 12 weeks of pre-treatment with omalizumab, patients were started on oral immunotherapy and 12 of 13 reached 2000 mg peanut on week 32. Patients continued their daily ingestion of 2000–4000 mg peanut protein daily. Sera used on this study were taken from patients on weeks 0 and 52. The Institutional Review Board at Boston Children's Hospital approved the clinical protocol, and written informed consent from all participants and/or their parents was obtained before participation in the study. The trial was registered on ClinicalTrials.gov (NCT01290913).

Human basophil activation analysis

Basophil activation tests were performed utilizing the Flow CAST Basophil Activation Test kit (Bühlmann Laboratories, Schönenbuch, Switzerland) per manufacturer's instructions. Briefly, 50µl aliquots of non-peanut allergic whole blood were sensitized with 20% pre- or post-OIT patient sera, IgG-depleted sera, eluted IgG fraction equal to original serum levels and/or anti-CD32 antibody (2µg/ml, FUN-2, Biolegend, San Diego, CA) in 100uL basophil stimulation buffer at 37°C for 2 hours. Anti-FcεRI mAb was used as a positive control for basophil activation. Samples were incubated for 10min at 37°C with 2(µg/ml complete peanut extract and Flow CAST staining reagent (including CCR3 and CD63). After erythrocyte lysis, cells were washed and subjected to flow cytometry. Basophils were identified as SSC^{low}CCR3⁺ cells and activation was measured by CD63 expression. In independent control experiments, cells in this gate were confirmed to be uniformly CD123⁺CD3⁻CD19⁻FcεRI⁺IgE⁺c-Kit⁻. A minimum of 200 basophils was obtained for each sample. Peripheral blood from non-peanut allergic adult donors was obtained with approval from the Institutional Review Board of Boston Children's Hospital.

Statistics

Statistical analyses were performed using Prism GraphPad Version 5.0f (GraphPad Software, San Diego, CA). Unpaired *t* tests or ANOVA with Bonferroni post-tests was used for comparisons between unlinked groups, including mouse BMMC experiments and data from pooled patient sera. Repeated measures ANOVA with Bonferroni post-tests was used to analyze data from individual patient basophil activation tests. Repeated measures two-way ANOVA was applied to anaphylaxis data. Since peanut-specific IgG values were spread across several orders of magnitude, the data were transformed by taking the logarithm prior to statistical analysis with paired *t* tests. *P* values are indicated in the figures using the shorthand **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001.

Results

IgG antibodies generated during food allergen ingestion inhibit IgE-mediated mast cell activation and systemic anaphylaxis

We have previously used a genetic approach, targeted insertion of an activated form of the IL-4 receptor α -chain (IL-4R α), to develop *Il4raF709* mice that are both susceptible to enteral allergen sensitization and capable of mounting robust systemic anaphylaxis upon ingestion challenge¹⁵. Food-induced anaphylaxis in these animals is completely IgE-dependent: neither IgE^{-/-} nor Fc ϵ RI α ^{-/-} animals carrying the *Il4raF709* allele exhibit allergic responses following ingestion challenge. We reasoned that IgE-deficient IgE^{-/-}/*Il4raF709* mice, which retain intact IgG antibody responses, would be useful for analysis of the biological effects of IgG antibodies generated in response to high-dose food allergen ingestion in an oral desensitization (OD) protocol.

IgE^{-/-}/*Il4raF709* and *Il4raF709* mice were enterally sensitized to ovalbumin (OVA) by low-dose gavage weekly over three weeks. Challenge was performed by OVA gavage one week after the last sensitizing dose and anaphylaxis assessed by measuring core body temperature using implanted thermal transponders. As expected, *Il4raF709* but not IgE^{-/-}/*Il4raF709* mice enterally sensitized to ovalbumin (OVA) displayed robust anaphylactic responses with intense and sustained temperature drops and two deaths after oral OVA challenge (Fig 1A). This anaphylactic response in *Il4raF709* animals was accompanied by a large increase in plasma levels of the mast cell-specific protease, mMCP-1, indicative of intense mast cell activation (Fig 1B)¹⁶. No evidence of mast cell activation was detected in IgE^{-/-}/*Il4raF709* mice. Although OVA-sensitized IgE^{-/-}/*Il4raF709* mice exhibited evidence of immune sensitization, including OVA-specific Th2 responses and mast cell expansion, they had no anti-OVA IgE (Fig E1 and data not shown).

As IgE^{-/-}/*Il4raF709* mice tolerated the oral OVA challenge, they were then treated with high enteral doses of OVA daily for an additional three weeks. We reasoned that oral desensitization in an IgE-free system would be similar to human OIT performed under cover of omalizumab^{14, 17}. The ability of pooled sera from IgE^{+/+}/*Il4raF709* or high-dose OVA-treated IgE^{-/-}/*Il4raF709* mice to sensitize mast cells was assessed using bone marrow mast cells (BMMC) from wild-type mice. Sensitized BMMC were challenged *in vitro* with OVA and activation was detected by measuring surface expression of LAMP-1, a sensitive

indicator of granule extrusion^{18, 19}. Data are presented both as flow cytometry plots for a single representative experiment (Fig 2) and as mean values for replicate activation assays (Fig E2). Exposure of BMMC to OVA alone had no effect (Fig 2A), while treatment with an anti-FcεRI antibody induced expression of LAMP-1 (Fig 2B). Consistent with the anaphylaxis results *in vivo*, IgE^{+/+} sera sensitized BMMC for robust activation to OVA ($51.2 \pm 1.99\%$ LAMP-1 positive) while IgE^{-/-} sera conferred no OVA sensitivity either before or after desensitization (Fig 2C,D, E2 and data not shown). OVA challenge of BMMC sensitized with a mixture of sera from IgE^{-/-} and IgE^{+/+} animals resulted in a strikingly weak response ($4.12 \pm 0.958\%$) (Fig 2E, E2), a finding that indicated that sera from high-dose OVA-fed IgE^{-/-} mice not only failed to sensitize mast cells for activation but exerted a suppressive effect.

The hypothesis that IgG antibodies mediated the observed inhibition was tested by sensitization of BMMC with the same sera following IgG depletion. Removal of IgG from IgE^{-/-} sera abrogated the suppressive activity (Fig 2G, E2). Replacement of the IgG fraction with equivalent amounts of purified IgG prepared from the same sera fully restored suppression (Fig 2H, E2). Taken together, these results establish that IgE antibodies drive mast cell activation and anaphylaxis in *Il4raF709* mice, and that IgG antibodies produced following allergen ingestion exert a suppressive function.

IgG-mediated inhibition of food allergen mast cell activation and anaphylaxis occurs via an FcγRIIb-dependent mechanism

Two non-mutually exclusive models have been proposed whereby IgG antibodies might inhibit IgE-mediated mast cell activation and hypersensitivity responses^{20–22}. In the first putative mechanism, IgG antibodies exert a “blocking” function, binding allergens in the extracellular milieu, masking their epitopes and preventing their recognition by IgE antibodies. In an alternative model, IgG antibodies bound to Fcγ receptors bind to the same allergenic proteins as FcεRI-bound IgE resulting in delivery of an inhibitory signal. To discriminate between these mechanisms we took advantage of FcγRIIb^{-/-} mice as well as BMMC cultured from the same animals. Consistent with a role for FcγRIIb, sensitization of FcγRIIb^{-/-} BMMC with a mixture of sera from IgE^{+/+} and IgE^{-/-} mice resulted in strong activation (Fig 2G, Fig 3A). The suppressive effect of the IgE^{-/-} serum was completely eliminated. We observed that the response of BMMC sensitized with only IgE^{+/+} serum was in fact enhanced in FcγRIIb^{-/-} BMMC, suggesting that elimination of inhibitory signals provided by IgG antibodies contained in this same serum triggered amplified degranulation.

A dose response analysis of the ability of IgG to inhibit wild-type vs FcγRIIb^{-/-} BMMC activation using monoclonal TNP-specific antibodies revealed that, at high doses, inhibition could be achieved even in BMMC lacking inhibitory IgG receptors, suggesting some contribution of a steric blocking effect (Fig 3B). However the IgG concentration required to mediate 50% inhibition of IgE-induced activation was >10-fold higher for FcγRIIb^{-/-} BMMC, indicating that most of the inhibitory effect is mediated at the level of the Fc receptor.

To test the relevance of FcγRIIb-mediated inhibition of IgE-induced mast cell activation to anaphylactic responses *in vivo*, we passively sensitized wild-type or FcγRIIb^{-/-} mice with

sera from IgE^{+/+} and/or IgE^{-/-} mice enterally sensitized to OVA. OVA challenge of both wild-type and FcγRIIb^{-/-} recipients of IgE^{+/+} serum resulted in robust anaphylactic responses (Fig 3C). In contrast, wild-type, but not FcγRIIb^{-/-} recipients of IgE^{+/+} plus IgE^{-/-} sera exhibited significant protection from hypothermia, a finding which is supportive of FcγRIIb-dependent IgG-mediated suppression of responses to OVA challenge in systemic anaphylaxis.

Detection of inhibitory antibodies in the sera of peanut OIT subjects

We have recently reported the results of peanut OIT performed under cover of omalizumab in a group of 13 high-risk subjects¹⁴. Patients had a median peanut-specific IgE level of 229 kUA/L and reacted to peanut flour challenge doses of 100 mg or less (Table 1). Twelve patients eventually reached a dose of 4000 mg peanut flour over a median time of 8 weeks. We reasoned that these highly peanut sensitive subjects, all of whom successfully ingested significantly more peanut after OIT, would provide an ideal group in which to assess the hypothesis that inhibitory IgG antibodies are induced in the course of OIT.

Pre-OIT sera (W0) and post-OIT sera (W52) were used for this analysis. At the 52-week time point, subjects had been off omalizumab for 8 months (more than 10 elimination half-lives for omalizumab). Two of 13 patients withdrew from the study and were not included in our evaluation. As the ability to detect suppressive activity in post-OIT sera was dependent on a minimum 30% basophil activation by pre-treatment sera, we additionally excluded three patients (see Table 1) for whom basophil activation fell below this threshold.

Peanut OIT induces allergen-specific suppressive IgG antibodies that act via FcγRIIb

We evaluated peanut-specific IgG by subclass and observed significant increases in all of them (Fig 4). The more than 2-log increases in peanut-specific IgG2 and IgG4 were most striking, and, in the face of falling IgE levels, the peanut G4:E ratio increased by more than 3 logs. Significant rises were evident for IgG1 and IgG3 as well. Peanut-specific IgA also increased (Fig E3). Basophil assays were employed to analyze the effects of these peanut-specific antibodies on IgE-mediated activation. Previous investigations have demonstrated decreased basophil sensitivity to antigen challenge following peanut OIT²³. In order to determine whether such hyporesponsiveness might be the result of alterations in the balance of activating versus inhibitory antibodies interacting with Fc receptors, we developed an *indirect* assay analogous to the BMMC system used in the murine model. Basophils from normal, non-peanut-allergic, donors (hence uniform in their intrinsic susceptibility to activation) were incubated with study sera and then exposed to peanut antigen. Expression of CD63, a granule membrane protein, which is rapidly induced by FcεRI signaling and closely linked to anaphylactic degranulation²⁴, was used as an indicator of activation. In preliminary and control experiments this assay consistently gave a unimodal increase in CD63 expression in all but three of the study patients, with negligible expression on resting cells or on cells exposed to peanut allergen but not sensitized by allergic serum. The ability of the sera to activate basophils was concentration-dependent (Fig E4).

Basophils were incubated with the following combinations of sera prior to peanut stimulation and activation analysis: 1) pre-OIT (W0), 2) post-OIT (W52), 3) a mixture of

pre- and post-OIT (W0 + W52) and 4) W0 + W52 + anti-Fc γ RII antibody (W0 + W52 + α CD32). Data are presented for a representative patient (3) (Fig 5) as well as for all subjects (Fig 6A). Additional informative manipulations and controls were performed using pooled sera from all subjects tested (Fig 6B), and individual patient sera (Fig E5). Exposure of donor basophils to peanut alone resulted in no appreciable activation (Fig 5A). Fc ϵ RI cross-linking with a polyclonal serum induced 62–92.9% CD63 expression (Fig 5A and data not shown). Pre-OIT sera sensitized basophils for 63.33 $\pm$ 5.64% activation (Fig 6A). W52 sera conferred markedly less activation (32.53 $\pm$ 6.06%), a finding that is striking in light of the fact that the W52 study subjects still had markedly elevated peanut-specific IgE levels, with a median peanut-specific IgE of 62.1 kU_A/L.

When basophils were exposed to both the same amount of W0 serum used under the initial activating conditions along with an additional equal amount of W52 serum, a marked reduction in responsiveness was observed (45.81 $\pm$ 5.10% vs 63.33 $\pm$ 5.64%) (Figs 5, 6), consistent with a suppressive activity within the W52 sera. This suppressive activity was lessened when W52 sera were passed over protein G sepharose to remove IgG antibodies (Figs 6B and E5) and was restored (P <0.001) by replacement of the IgG fractions. The suppression exerted by post-OIT sera was similarly ablated by addition of anti-CD32 antibodies. These findings provide strong evidence that peanut OIT induces suppressive IgG antibody responses that directly inhibit Fc ϵ RI-mediated basophil activation via an Fc γ RIIb-dependent mechanism.

Discussion

Previous investigations have established that both natural loss of food allergen sensitivity and successful completion of OIT protocols are associated with the induction of food allergen-specific IgG responses^{5–9, 11–13}. However the functional effects of food-specific IgG antibodies induced during OIT on allergen:IgE-mediated hypersensitivity reactions have not been evaluated. This study provides evidence that food allergen administration, as occurs in OIT, drives IgG production and that the IgG antibodies formed during such responses can suppress IgE-mediated responses, including anaphylaxis.

We used both a mouse model system and sera from a human OIT trial to test the hypothesis that food-specific IgG antibodies act via Fc γ RIIb to inhibit Fc ϵ RI-mediated activation. Application of the murine model system allowed for highly consistent conditions of food allergen exposure in a genetically homogeneous population and for analysis of the effects of sera on primary mast cells. The utilization of Fc γ RIIb^{−/−} mice and their mast cells permitted a direct genetic test of the contribution of this receptor to the alterations in mast cell responsiveness to pre- versus post-OD sera. A key novel observation in this study was that IgE^{−/−}/*Il4ra*F709 mice not only failed to express anaphylaxis but that their sera, taken following repeated high-dose OVA gavage, also suppressed IgE-mediated anaphylaxis. This provides strong evidence that IgG antibodies generated in the course of allergen ingestion and acting via Fc γ RIIb can suppress anaphylactic reactions. It is important to note, however, that IgE-deficient mice are not equivalent to omalizumab-treated patients. Unlike patients, the mice have lacked IgE throughout their lives with possible effects on the development, homeostasis and function of Fc ϵ RI⁺ cells, including mast cells and dendritic cells.

Furthermore, the IgE^{-/-} animals have absolutely no FcεRI-bound IgE whereas omalizumab does not completely remove IgE from cellular receptors in patients.

Our studies on the high-risk peanut-allergic OIT subjects indicate that the same mechanisms are operative in food-allergic patients and are therefore clinically relevant. These subjects exhibited marked increases in peanut-specific IgG antibodies and were uniformly able to tolerate 160–400 times the dose tolerated before desensitization. This was despite the presence of peanut-specific IgE levels normally associated with a high risk of peanut reaction after desensitization at week 52 of the trial. Based on our CD32 blocking assays, this was likely due to the FcγRIIb-mediated suppression of IgE signals.

The function of IgG antibodies in modulating immune responses to allergens has been most extensively studied in the setting of subcutaneous immunotherapy SCIT^{4, 22}. Almost 80 years ago, using Prausnitz-Küstner passive cutaneous skin testing analyses, Cooke and colleagues demonstrated that serum from subjects completing immunotherapy contained a suppressive activity that inhibited passive sensitization by the “reagin” contained in pre-treatment sera²⁵. This inhibitory factor was eventually identified as IgG and the ability of immunotherapy preparations to induce IgG vs. IgE responses has been used to guide the development of optimal extracts for SCIT²⁶.

Lichtenstein demonstrated a correlation between induction of blocking IgG and the reduction of allergen-specific IgE in patients undergoing SCIT²⁷. SCIT induces predominantly IgG1 and IgG4 responses, with IgG4 responses becoming a focus of particular interest because of their very consistent induction, correlation with clinical improvement and unique biochemical characteristics²⁸. IgG4 antibodies undergo immunoglobulin chain reassortment generating chimeric structures with dual specificity, a property that prevents immune complex formation²⁹. It is notable, however, that IgG4 antibodies have a relatively low binding affinity for FcγRIIb³⁰ suggesting that the OIT-induced suppressive IgG activity observed in our study might be mediated by another isotype(s). We detected significant increases in all four IgG subclasses and additional studies will be required to assess whether they exert differential contributions to inhibit IgE responses. We also observed significant increases in peanut-specific IgA following OIT, and it is possible that IgA contributes to the inhibitory effect of post-OIT serum via allergen neutralization.

The ability of IgG antibodies to suppress IgE mediated anaphylaxis *in vivo* has previously been demonstrated by Strait and colleagues^{31, 32}. Our findings in the mouse model and in the human OIT serum analyses provide important evidence for the relevance of these inhibitory antibodies under physiologic conditions. In contrast to the findings of Strait and colleagues who found IgG suppression of allergic diarrhea to be FcγRIIb-independent, we observed that IgG-mediated inhibition of food allergy in both in the mouse model and in the basophil reporter system to be mediated by CD32 (FcγRII) with the murine experiments implicating FcγRIIb. The reason for this discrepancy may reside in differing mass amounts of IgG present in active vs. reconstituted systems. High titer mono-specific IgG might provide a stoichiometric excess of IgG sufficient to make direct steric blocking of the

antigen:IgE:FcεRI interaction the prevailing mechanism, removing a requirement for functional FcγRIIb.

Negative regulation of FcεRI-mediated mast cell and basophil activation has previously been studied in a number of experimental systems³³. Inhibition of IgE signaling by IgG was first demonstrated by Daëron and colleagues in RBL-2H3 cells stably transfected with FcγRIIb cDNA²⁰. The same group subsequently reported that IgE-mediated activation of BMMC is similarly subject to suppression by IgG antibodies of the same specificity³⁴. Tam and colleagues demonstrated that crosslinking of FcεRI and FcγRIIb on human basophils using a bispecific antibody against human IgE and FcγRIIb inhibits basophil histamine release and, like in BMMC, the inhibitory effect of IgG antibodies seems to dominate over the activating response to IgG in human basophils³⁵.

The relevance of inhibitory IgG receptor signaling to allergic pathogenesis has been suggested by animal models showing augmented anaphylaxis and allergic rhinitis^{36–38}. One previous study evaluating the effects “blocking” antibodies induced during *Bet v 1* immunotherapy in birch allergic patients showed inhibition of basophil activation by allergen:IgG immune complexes but no effect of anti-CD32 implying a “blocking” IgG effect rather than receptor-mediated inhibition³⁹. In contrast to our study, this group used basophils from the allergic subjects themselves. We believe this suggests that the allergic state may alter responsiveness to negative signaling pathways, a fact that might account for the lack of complete correlation between the presence of allergen specific IgG and protection from allergic reactions.

Up until recently, the treatment of patients with established IgE-mediated food allergy was limited to counseling strict allergen avoidance. Recent trials of OIT have provided evidence that safe, graded food allergen administration is possible and can have very significant effect on patients’ ability to safely ingest allergenic foods. We believe that delineation of the mechanisms operative in successful OIT will provide important insights into new opportunities for the treatment of food allergy. The FcγRIIb-mediated effects of OIT-induced IgG antibodies described in our study, for instance, lead us to speculate that passive immunization⁴⁰ with polyclonal food-specific IgG, the application of small molecule inhibitors of FcγRIIb signaling regulators or the generation of recombinant heterobivalent constructs that crosslink FcεRI and FcγRIIb in an allergen-specific manner⁴¹ might all be strategies to pursue in the future. In the meantime, further characterization of the full spectrum of immunoregulatory mechanisms operative in controlling food allergy should remain a high priority.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Ms. Alanna Darling for expert technical assistance.

This project was supported by National Institutes of Health grants: NIAID R56 AI100889 (HCO), 5T32AI007512-24 (OTB, SL and JSZ) and UL1 RR 025758 (DTU), and by grants from the Thrasher Foundation

(DTU), the Bunning Food Allergy Project (DTU), the Jasmine and Paul Mashikian Fund (DTU), Genentech (DTU), Consejo Nacional de Ciencia y Tecnología (CONACyT) grant 207999 (JMT), and the Department of Defense: DOD 11-1-0553 (TAC).

Abbreviations

BMMC	Bone marrow mast cells
OIT	Oral Immunotherapy
OVA	Ovalbumin
SCIT	subcutaneous immunotherapy
SLIT	sublingual immunotherapy
WT	Wild-type
IgE^{-/-}	IgE-deficient

References

1. Burks AW, Tang M, Sicherer S, Muraro A, Eigenmann PA, Ebisawa M, et al. ICON: food allergy. *The Journal of allergy and clinical immunology*. 2012; 129:906–920. [PubMed: 22365653]
2. Caubet JC, Sampson HA. Beyond skin testing: state of the art and new horizons in food allergy diagnostic testing. *Immunology and allergy clinics of North America*. 2012; 32:97–109. [PubMed: 22244235]
3. Begin P, Nadeau KC. Diagnosis of food allergy. *Pediatric annals*. 2013; 42:102–109. [PubMed: 23718238]
4. Wisniewski J, Agrawal R, Woodfolk JA. Mechanisms of tolerance induction in allergic disease: integrating current and emerging concepts. *Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology*. 2013; 43:164–176. [PubMed: 23331558]
5. Savilahti EM, Rantanen V, Lin JS, Karinen S, Saarinen KM, Goldis M, et al. Early recovery from cow's milk allergy is associated with decreasing IgE and increasing IgG4 binding to cow's milk epitopes. *The Journal of allergy and clinical immunology*. 2010; 125:1315–1321. e9. [PubMed: 20462631]
6. James JM, Sampson HA. Immunologic changes associated with the development of tolerance in children with cow milk allergy. *The Journal of pediatrics*. 1992; 121:371–377. [PubMed: 1517910]
7. Caubet JC, Bencharitiwong R, Moshier E, Godbold JH, Sampson HA, Nowak-Wegrzyn A. Significance of ovomucoid- and ovalbumin-specific IgE/IgG(4) ratios in egg allergy. *The Journal of allergy and clinical immunology*. 2012; 129:739–747. [PubMed: 22277199]
8. Skripak JM, Nash SD, Rowley H, Brereton NH, Oh S, Hamilton RG, et al. A randomized, double-blind, placebo-controlled study of milk oral immunotherapy for cow's milk allergy. *The Journal of allergy and clinical immunology*. 2008; 122:1154–1160. [PubMed: 18951617]
9. Bedoret D, Singh AK, Shaw V, Hoyte EG, Hamilton R, DeKruyff RH, et al. Changes in antigen-specific T-cell number and function during oral desensitization in cow's milk allergy enabled with omalizumab. *Mucosal immunology*. 2012; 5:267–276. [PubMed: 22318492]
10. Jones SM, Pons L, Roberts JL, Scurlock AM, Perry TT, Kulis M, et al. Clinical efficacy and immune regulation with peanut oral immunotherapy. *The Journal of allergy and clinical immunology*. 2009; 124:292–300. e1–97. [PubMed: 19577283]
11. Kim EH, Bird JA, Kulis M, Laubach S, Pons L, Shreffler W, et al. Sublingual immunotherapy for peanut allergy: clinical and immunologic evidence of desensitization. *The Journal of allergy and clinical immunology*. 2011; 127:640–646. e1. [PubMed: 21281959]
12. Vickery BP, Lin J, Kulis M, Fu Z, Steele PH, Jones SM, et al. Peanut oral immunotherapy modifies IgE and IgG4 responses to major peanut allergens. *The Journal of allergy and clinical immunology*. 2013; 131:128–134. e1–3. [PubMed: 23199605]

13. Vickery BP, Pons L, Kulis M, Steele P, Jones SM, Burks AW. Individualized IgE-based dosing of egg oral immunotherapy and the development of tolerance. *Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology*. 2010; 105:444–450.
14. Schneider LC, Rachid R, Lebovidge J, Blood E, Mittal M, Umetsu DT. A pilot study of omalizumab to facilitate rapid oral desensitization in high-risk peanut-allergic patients. *The Journal of allergy and clinical immunology*. 2013; 132:1368–1374. [PubMed: 24176117]
15. Mathias CB, Hobson SA, Garcia-Lloret M, Lawson G, Poddighe D, Freyschmidt EJ, et al. IgE-mediated systemic anaphylaxis and impaired tolerance to food antigens in mice with enhanced IL-4 receptor signaling. *The Journal of allergy and clinical immunology*. 2011; 127:795–805. e1–6. [PubMed: 21167580]
16. Pemberton AD, Wright SH, Knight PA, Miller HR. Anaphylactic release of mucosal mast cell granule proteases: role of serpins in the differential clearance of mouse mast cell proteases-1 and -2. *J Immunol*. 2006; 176:899–904. [PubMed: 16393974]
17. Nadeau KC, Schneider LC, Hoyte L, Borrás I, Umetsu DT. Rapid oral desensitization in combination with omalizumab therapy in patients with cow's milk allergy. *The Journal of allergy and clinical immunology*. 2011; 127:1622–1624. [PubMed: 21546071]
18. Foger N, Jenckel A, Orinska Z, Lee KH, Chan AC, Bulfone-Paus S. Differential regulation of mast cell degranulation versus cytokine secretion by the actin regulatory proteins Coronin1a and Coronin1b. *J Exp Med*. 2011; 208:1777–1787. [PubMed: 21844203]
19. Leonard SA, Martos G, Wang W, Nowak-Węgrzyn A, Berin MC. Oral immunotherapy induces local protective mechanisms in the gastrointestinal mucosa. *J Allergy Clin Immunol*. 2012; 129:1579–1587. e1. [PubMed: 22554705]
20. Daron M, Latour S, Malbec O, Espinosa E, Pina P, Pasmans S, et al. The same tyrosine-based inhibition motif, in the intracytoplasmic domain of Fc gamma RIIB, regulates negatively BCR-, TCR-, and FcR-dependent cell activation. *Immunity*. 1995; 3:635–646. [PubMed: 7584153]
21. Daron M, Malbec O, Latour S, Arock M, Fridman WH. Regulation of high-affinity IgE receptor-mediated mast cell activation by murine low-affinity IgG receptors. *The Journal of clinical investigation*. 1995; 95:577–585. [PubMed: 7860741]
22. Flicker S, Valenta R. Renaissance of the blocking antibody concept in type I allergy. *International archives of allergy and immunology*. 2003; 132:13–24. [PubMed: 14555854]
23. Thyagarajan A, Jones SM, Calatroni A, Pons L, Kulis M, Woo CS, et al. Evidence of pathway-specific basophil anergy induced by peanut oral immunotherapy in peanut-allergic children. *Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology*. 2012; 42:1197–205. [PubMed: 22805467]
24. MacGlashan DW Jr. Basophil activation testing. *The Journal of allergy and clinical immunology*. 2013; 132:777–787. [PubMed: 23958648]
25. Cooke RA, Bernhard JH, Hebal S, Stull A. Serological evidence of immunity with co-existing sensitization in hay fever type of human allergy. *J Exp Med*. 1935; 62:733–750. [PubMed: 19870445]
26. Bousquet J, Lockey R, Malling HJ. Allergen immunotherapy: therapeutic vaccines for allergic diseases. A WHO position paper. *The Journal of allergy and clinical immunology*. 1998; 102:558–562. [PubMed: 9802362]
27. Lichtenstein LM, Holtzman NA, Burnett LS. A quantitative in vitro study of the chromatographic distribution and immunoglobulin characteristics of human blocking antibody. *Journal of immunology*. 1968; 101:317–324.
28. Aalberse RC, van der Gaag R, van Leeuwen J. Serologic aspects of IgG4 antibodies. I. Prolonged immunization results in an IgG4-restricted response. *Journal of immunology*. 1983; 130:722–726.
29. van der Neut Kolfschoten M, Schuurman J, Losen M, Bleeker WK, Martinez-Martinez P, Vermeulen E, et al. Anti-inflammatory activity of human IgG4 antibodies by dynamic Fab arm exchange. *Science*. 2007; 317:1554–1557. [PubMed: 17872445]
30. Smith KG, Clatworthy MR. FcγRIIB in autoimmunity and infection: evolutionary and therapeutic implications. *Nature reviews. Immunology*. 2010; 10:328–343. [PubMed: 20414206]

31. Strait RT, Morris SC, Finkelman FD. IgG-blocking antibodies inhibit IgE-mediated anaphylaxis in vivo through both antigen interception and Fc gamma RIIB cross-linking. *J Clin Invest.* 2006; 116:833–841. [PubMed: 16498503]
32. Kucuk ZY, Strait R, Khodoun MV, Mahler A, Hogan S, Finkelman FD. Induction and suppression of allergic diarrhea and systemic anaphylaxis in a murine model of food allergy. *The Journal of allergy and clinical immunology.* 2012; 129:1343–1348. [PubMed: 22465213]
33. Malbec O, Daeron M. The mast cell IgG receptors and their roles in tissue inflammation. *Immunol Rev.* 2007; 217:206–221. [PubMed: 17498061]
34. Daeron M, Malbec O, Latour S, Arock M, Fridman WH. Regulation of high-affinity IgE receptor-mediated mast cell activation by murine low-affinity IgG receptors. *J Clin Invest.* 1995; 95:577–585. [PubMed: 7860741]
35. Tam SW, Demissie S, Thomas D, Daeron M. A bispecific antibody against human IgE and human FcgammaRII that inhibits antigen-induced histamine release by human mast cells and basophils. *Allergy.* 2004; 59:772–780. [PubMed: 15180766]
36. Takai T, Ono M, Hikida M, Ohmori H, Ravetch JV. Augmented humoral and anaphylactic responses in Fc gamma RII-deficient mice. *Nature.* 1996; 379:346–349. [PubMed: 8552190]
37. Ujike A, Ishikawa Y, Ono M, Yuasa T, Yoshino T, Fukumoto M, et al. Modulation of immunoglobulin (Ig)E-mediated systemic anaphylaxis by low-affinity Fc receptors for IgG. *J Exp Med.* 1999; 189:1573–1579. [PubMed: 10330436]
38. Watanabe T, Okano M, Hattori H, Yoshino T, Ohno N, Ohta N, et al. Roles of FcgammaRIIB in nasal eosinophilia and IgE production in murine allergic rhinitis. *Am J Respir Crit Care Med.* 2004; 169:105–112. [PubMed: 14525800]
39. Ejrnaes AM, Svenson M, Lund G, Larsen JN, Jacobi H. Inhibition of rBet v 1-induced basophil histamine release with specific immunotherapy -induced serum immunoglobulin G: no evidence that FcgammaRIIB signalling is important. *Clin Exp Allergy.* 2006; 36:273–282. [PubMed: 16499637]
40. Flicker S, Gadermaier E, Madritsch C, Valenta R. Passive immunization with allergen-specific antibodies. *Current topics in microbiology and immunology.* 2011; 352:141–159. [PubMed: 21681684]
41. Liu Y, Sun Y, Chang LJ, Li N, Li H, Yu Y, et al. Blockade of peanut allergy with a novel Ara h 2-Fcgamma fusion protein in mice. *The Journal of allergy and clinical immunology.* 2013; 131:213–221. e1–5. [PubMed: 23199607]

Clinical implications

OIT induces IgG antibodies that can act via the inhibitory IgG receptor, Fc γ RIIb, to block IgE-mediated immediate hypersensitivity reactions.

Capsule Summary

Allergists have sought an explanation for the paradox that some subjects who have successfully completed OIT tolerate allergenic foods despite the persistence of high-titer food-specific IgE. This study reveals that IgG antibodies induced following OIT can inhibit IgE-mediated hypersensitivity responses.

Figure 1. Food allergen-induced anaphylaxis and mast cell activation is IgE-dependent
(A) Systemic anaphylaxis (core body temperature) following enteral challenge of OVA-sensitized *Il4raF709* and *IgE^{-/-}/Il4raF709* mice. ‡Death. **(B)** Plasma levels of mouse mast cell protease-1 (mMCP-1) ($n=5-8$). Data are representative of 3 experiments.

Figure 2. Antigen-induced activation of mast cells sensitized with sera of OVA-fed mice
 OVA-induced LAMP-1 on serum-sensitized BMMC. (A) No serum. (B) Anti-FcεRI control. (C) IgE^{+/+} / *Il4raF709* serum. (D) high-dose OVA-fed IgE^{-/-} / *Il4raF709* serum. (E) IgE^{+/+} plus IgE^{-/-} sera. (F) FcγRIIb^{-/-} BMMC treated as in E. (G) WT BMMC, IgE^{+/+} plus IgG-depleted IgE^{-/-}. (H) As in G, with IgG added back. Representative plots from one of five experiments.

Figure 3. IgG inhibits mast cell activation via FcγRIIb

(A) OVA-induced activation of WT or FcγRIIb^{-/-} BMMC sensitized with IgE^{+/+} or high-dose OVA-fed IgE^{-/-} sera. (B) mAb-sensitized BMMC activated with TNP. Mean±SEM, one of 3 experiments. (C) Anaphylaxis (core temperature) in OVA-challenged WT or FcγRIIb^{-/-} recipients of IgE^{+/+} ± high-dose OVA-fed IgE^{-/-} sera. Mean±SEM ($n=3-5$), one of two experiments.

Figure 4. Peanut-specific IgG levels increase following OIT

ELIS A analysis of peanut-specific IgG levels in serum samples taken before and after OIT.

(A) IgG1, (B) IgG2, (C) IgG3, (D) IgG4, (E) IgG (all isotypes), (F) ratio of IgG4 to IgE.

Circles represent individual patient values, with boxplots overlaid.

Figure 5. Peanut OIT induces IgG that inhibits basophil activation

Basophil activation was measured by flow cytometry in peanut-challenged whole blood samples from a non-allergic donor. Basophils were sensitized with sera taken before or after OIT and stained for CD63. W0: pre-OIT, week 0. W52: post-OIT at 52 weeks. αCD32 : αCD32 antibody.

Figure 6. IgG inhibits basophil activation by allergic sera via CD32

(A) Peanut-induced activation of basophils sensitized with serum from individual patients ($n=11$), with boxplots overlaid. (B) CD63 upregulation in basophils sensitized with serum pooled from all patients. Data are mean $\pm$ SEM of independent replicates ($n=4$). W52D: W52 depleted of IgG.

Subject	Age (y)	Sex	W0 Peanut-specific IgE (kU _A /L)	W52 Peanut-specific IgE (kU _A /L)	W0 skin test wheal (mm)	W52 skin test wheal (mm)
1	8	M	436	85	12.5	11.5
2*	8	M	58	38.2	20.5	8
3	9	F	617	578	15	5
5	14	M	150	62.1	16.5	7
6	14	M	229	84.1	10.5	3.5
8	14	F	290	>100	24	8.5
9*	7	M	65	17	9.5	5.5
10	10	F	327	61.7	9.5	6
11*	11	F	21	16.5	8.5	4
12	12	F	307	42.9	24.5	6
13	8	M	172	92.5	18	Not Done

Of the 13 subjects initially enrolled in the study, subjects 4 and 7 withdrew prior to completion of the 52-week protocol. Their clinical information is not presented here.
F, Female; *M*, male.

* Subjects not included in basophil activation analysis, secondary to <30% basophil activation by W0 patient serum.