Baloh et al. World Allergy Organization Journal (2024) 17:100940
http://doi.org/10.1016/j.waojou.2024.100940

WORLD ALLERGY ORGANIZATION JOURNAL

BRIEF RESEARCH REPORT

Open Access

Peanut-specific IgG subclasses as biomarkers of peanut allergy in LEAP study participants

Carolyn H. Baloh, MD[a,b]*, Noha Lim[c], Michelle Huffaker[d], Pooja Patel[e], Jody Tversky[e], George Du Toit, MB, BCh[f,g,h], Gideon Lack, MB, BCh[f,g,h], Tanya M. Laidlaw[a,b] and Donald W. MacGlashan[e]

ABSTRACT

Antigen-specific IgG2 and IgG3 are rarely measured in food allergy clinical trials despite known function in preventing mast cell and basophil activation. Our objective was to determine whether measuring peanut-specific IgG2 and IgG3 levels would correlate with peanut allergy status. Peanut-specific IgG subclasses were measured via ELISA assays in Learning Early About Peanut allergy (LEAP) trial participants at 5 years of age and were correlated with peanut allergy vs peanut sensitization vs non-peanut allergic and peanut consumption vs peanut avoidance. Peanut-specific IgG1, IgG2, IgG3, and IgG4 levels were significantly different between participants with peanut allergy vs peanut sensitization vs non-peanut allergic, and a multivariate logistic regression model and stepwise selection found that IgG1 most closely associated with peanut allergy status. Similarly, all subclasses differentiated those consuming vs those avoiding peanut, but subsequent modeling found that IgG4 most closely associated with consumption status. Amongst the peanut-specific IgG subclasses, IgG1 was the best biomarker for peanut allergy, while IgG4 was the best biomarker for peanut antigen exposure in this highly atopic cohort. Our study did not find added value from evaluating peanut-specific IgG 2 and 3 as biomarkers of peanut allergy, although they did correlate with peanut allergy. Subsequent studies should assess the value of adding IgG subclasses to multivariate models predicting peanut allergy status.

Keywords: Peanut hypersensitivity, Immunoglobulin G (IgG), Biomarkers, Basophils, Mast cells, Enzyme-linked immunosorbent assay

INTRODUCTION

Many allergen immunotherapy (AIT) clinical trials have included measurements of antigen-specific immunoglobulin G1 (IgG1) and immunoglobulin G4 (IgG4), but few have assessed immunoglobulin G2 (IgG2) or immunoglobulin G3 (IgG3).[1] This omission has largely been due to a lack of reliable assays and a lack of understanding for how each subclass could inhibit mast cell or basophil activation.[1] We now know that memory B cells producing IgG antibodies can be precursors to IgE antibodies, IgG can block binding of antigen to IgE, and IgG can interact with inhibitory receptors on mast cells and basophils (CD32b, FcγRIIb), preventing activation and release of mediators including histamine.

Allergen-specific IgG4, which is often measured in clinical trials of AIT, has been shown to increase with AIT[2] and is thought to have an important role

[a]Immune Tolerance Network, Benaroya Research Institute at Virginia Mason, Seattle, WA, USA
*Corresponding author. 60 Fenwood Rd, BTM/Hale Building, 5th floor, Boston, MA 02115, USA. E-mail: cbaloh@immunetolerance.org
Full list of author information is available at the end of the article

http://doi.org/10.1016/j.waojou.2024.100940
Received 9 January 2024; Received in revised from 6 June 2024; Accepted 11 July 2024

in the early response to AIT by blocking IgE antibodies. However, IgG4 only comprises ~10% of all antigen-specific IgG in patients with allergic rhinitis and doesn't significantly prevent activation of basophils, directly or indirectly.[1,3] Plasma both from peanut-allergic individuals who have been treated with oral immunotherapy and from individuals who are only sensitized but not clinically allergic to peanut can inhibit mast cell activation. However, depletion of IgG4 from those plasma samples does not fully restore mast cell activation,[4] suggesting that other IgG subclasses could also be important in the process of tolerance.

IgG1 is elevated in patients with allergic diseases and IgG1-producing memory B cells are precursors to high-affinity IgE production, a key factor in allergic responses.[5] IgG1 is the most prevalent IgG subclass and has the capability to block allergen binding to IgE.[3] IgG2 and IgG3 are also both present in appreciable amounts in patients with allergic rhinitis and inhibit CD32b on mast cells and basophils in vitro.[1,3] Given the functional activity of IgG2 and IgG3 in allergic rhinitis, we asked whether antigen-specific IgG2 and IgG3 levels were biomarkers of peanut allergy.

RESULTS AND DISCUSSION

In this study we sought to measure all 4 peanut-specific IgG subclasses in Learning Early About Peanut allergy (LEAP) study participants. The LEAP study was a randomized trial of early peanut introduction at 4–11 months of age compared to peanut avoidance.[6] Plasma samples and clinical data were obtained at the primary outcome timepoint (5 years of age). An ELISA assay to measure peanut-specific IgG 1, 2, 3, and 4 was developed as described previously,[1] except that whole peanut extract (ALK) was used, additional data on methods available in supplement. The study was intended to determine which peanut-specific IgG subclass(es) were the best biomarkers of peanut allergy. Peanut allergy was defined as a positive oral food challenge (OFC) at 5 years of age. Peanut-sensitized only was defined as having a peanut skin prick test (SPT) wheal $\geq$3mm or a peanut-specific IgE (psIgE) $\geq$0.35 and a negative OFC. Peanut non-allergic was defined as a peanut SPT wheal <3mm or psIgE <0.35 and a negative OFC. Statistical analyses were done in R with ANOVA or Student's T test, and multivariate modeling was performed with logistic regression and stepAIC.

Plasma samples were available from 516 of the original 628 LEAP participants at 5 years of age and assayed. Of these, 53 (10%) were peanut allergic, 105 (20%) were peanut-sensitized only, and 358 (70%) were non-allergic at 5 years of age (Table 1). Age and gender were similar across the three clinical groups. However, eczema severity (Scoring Atopic Dermatitis (SCORAD)), egg SPT responses, egg-specific IgE, and psIgE were significantly different across the groups at 4–11 months. These clinical differences were not seen between peanut allergic and non-allergic in the original 628 LEAP participants; however, they did not have a third sensitized group which likely contributed to the difference in our sub-cohort. Additional analysis comparing those who passed versus those who failed their OFC showed no differences in demographics between the subset of 516 and the original 628 participants (data not shown).

Across 516 participants, we found that peanut-specific IgG1 was the most abundant IgG subclass and IgG4 was the least abundant (Fig. 1) (mean and standard deviation of IgG1: 51% $\pm$ 20; IgG2: 24% $\pm$ 17; G3: 22% $\pm$ 16; G4: 3% $\pm$ 5, actual values in Supplemental Table 1). Although the biological or clinical significance of this finding is unknown, we found that 16% of the plasma samples had peanut-specific IgG3 levels that were greater than their peanut-specific IgG1 levels, of these N = 10 were allergic, N = 7 were sensitized only, and N = 66 were non-allergic. This differs from findings of ragweed and dust mite allergy results where antigen-specific IgG3 levels are always considerably less than antigen-specific IgG1 levels.[1] We then assessed levels of peanut-specific IgG1, IgG2, IgG3, and IgG4 according to clinical peanut allergy status. A 3-way ANOVA indicated that levels of all four peanut-specific IgG subclasses were significantly different according to clinical status ($p < 0.001$ for all ANOVAs). Pairwise comparisons showed that IgG1 levels were significantly lower between non-allergic and sensitized ($p < 0.001$) and between non-allergic and allergic ($p < 0.001$), but not between allergic and sensitized participants. IgG2 and IgG4 levels were significantly lower only in non-allergic compared

	Allergic (n = 53)	Sensitized (n = 105)	Non-allergic (n = 358)	p-value	Total (n = 516)
Age at enrollment (months)	7.7 (1.94)	7.6 (1.73)	7.9 (1.73)	0.501	7.8 (1.76)
Male, n (%)	36 (67.9)	66 (62.9)	201 (56.1)	0.168	303 (58.7)
SCORAD	40 (20.2)	38.5 (18.4)	31.1 (18.2)	<0.001	33.5 (18.7)
Cumulative Peanut Amount (g)	0.1 (0, 0.85)	9.35 (9.35, 9.35)	5 (5, 5.5)	<0.001	5 (5, 9.35)
Reaction Dose (g)	0.175 (0.1, 1)				0.175 (0.1, 1)
Peanut SPT (mm)	0 (0, 2)	0 (0, 0)	0 (0, 0)	<0.001	0 (0, 0)
Raw hens egg white SPT (mm)	10 (6, 16)	11 (5, 14)	8 (0, 12)	<0.001	9 (0, 13)
Pasteurized hens egg white SPT (mm)	5 (3, 7)	4 (2, 6)	3 (0, 5)	<0.001	3 (0, 6)
Hens egg white-specific IgE (kAU/L)	2.48 (0.33, 10.4)	2.6 (0.41, 12.25)	0.52 (0.05, 2.38)	<0.001	0.88 (0.08, 3.94)
psIgE (kAU/L)	0.51 (0.12, 2.47)	0.09 (0.02, 1.39)	0.02 (0.01, 0.1)	<0.001	0.03 (0.01, 0.25)

Table 1. Baseline demographics of LEAP cohort subset by peanut allergy status at 5 years of age. *Age & SCORAD are shown as mean (SD) while other data are shown as mean (IQR). Abbreviations: Scoring Atopic Dermatitis (SCORAD), skin prick test (SPT), peanut-specific IgE (psIgE), Learning Early About Peanut allergy (LEAP).*

to sensitized participants ($p < 0.001$ for both). IgG3 levels were significantly lower only in non-allergic compared to allergic participants ($p = 0.002$) (Fig. 2A).

Levels of the IgG subclasses were then examined according to peanut consumption or avoidance to determine if consumption was a confounding factor. Participants in the peanut-

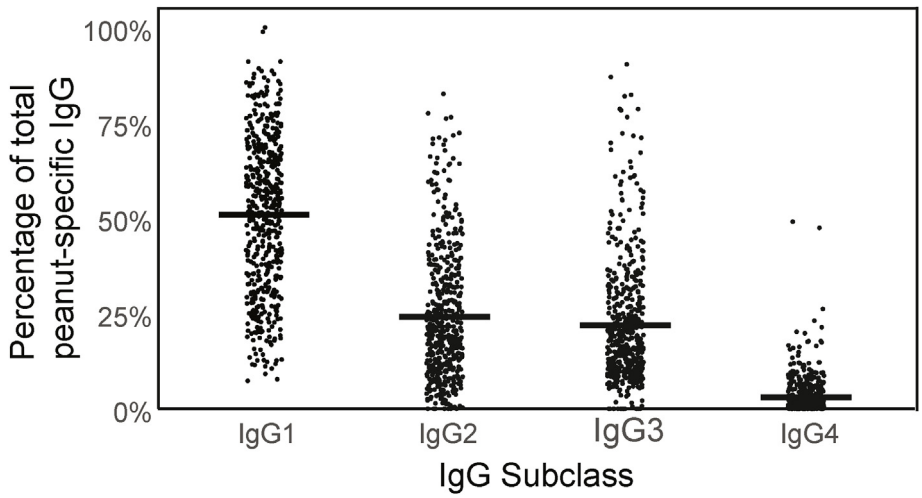

Fig. 1 Peanut-specific IgG subclass abundance in 516 LEAP participants. Peanut-specific IgG1, IgG2, IgG3, and IgG4 percentages as a fraction of total peanut-specific IgG are shown for each participant. Bars indicate mean for each IgG subclass. Abbreviation: Learning Early About Peanut allergy (LEAP).

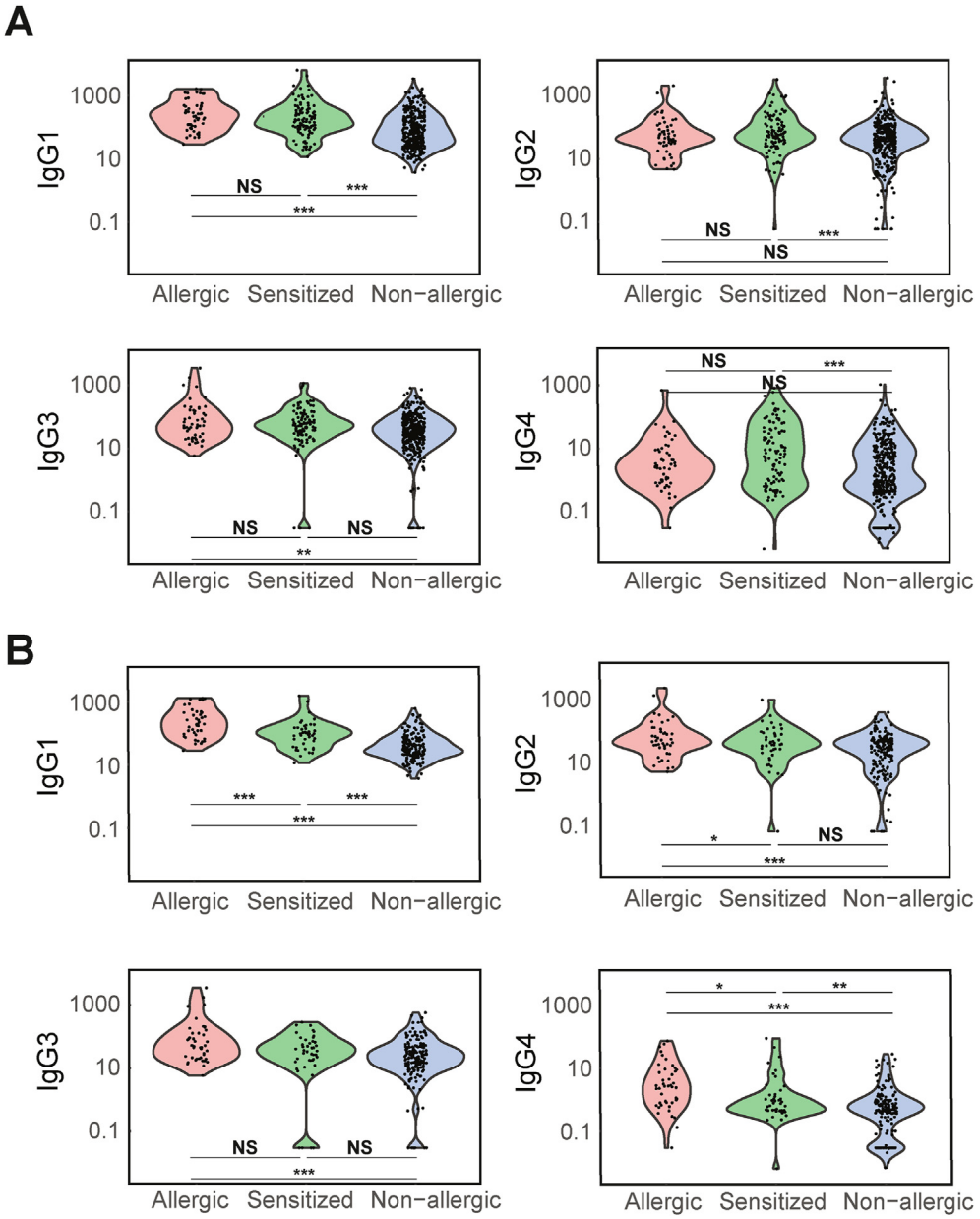

Fig. 2 Peanut-specific IgG subclass levels by clinical status. A. Entire cohort (N = 516). B. Peanut avoidance arm only (N = 263). Peanut-specific IgG subclass levels (ng/mL) are expressed for each participant subdivided by clinical status: peanut allergic (pink), only peanut sensitized (green), and not peanut allergic or sensitized (blue). 3-way ANOVA p-value <0.001 for all panels of figure. Pair-wise comparisons are indicated as *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, NS $p > 0.05$.

consumption arm had significantly higher levels of all 4 IgG subclasses compared to those in the peanut-avoidance arm ($p < 0.001$ for all) (Fig. 3). To better assess differences in IgG subclasses by allergy status, a 3-way ANOVA was performed within the peanut-avoidance subgroup. Levels of all 4 IgG subclasses were significantly different between the peanut allergic, peanut sensitized, and non-allergic participants ($p < 0.001$ for all comparisons). Subsequent pairwise comparisons confirmed that peanut-specific IgG1, IgG2, IgG3, and IgG4 levels were significantly lower in peanut non-allergic versus allergic ($p < 0.001$ for each comparison). Peanut-specific IgG1 and IgG4 were also significantly lower in non-allergic versus peanut-sensitized ($p < 0.001$ & $p = 0.003$). Peanut-specific IgG1, IgG2, and IgG4 were significantly lower in peanut sensitized versus peanut allergic ($p < 0.001$, $p = 0.04$, $p = 0.03$, respectively). (Fig. 2B). We hypothesize that the IgG subclasses

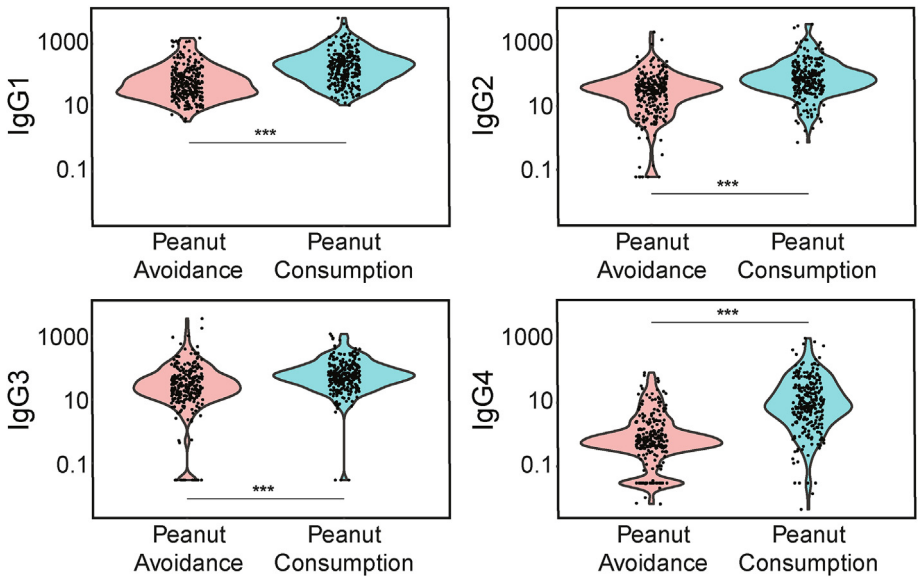

Fig. 3 Peanut-specific IgG subclass levels by clinical trial randomization group. Peanut-specific IgG subclass levels are expressed for each participant subdivided by randomization group (N = 516): peanut consumption (blue), peanut avoidance (pink). Pair-wise comparisons are listed on the graphs. Significance is indicated with * $p < 0.05$, **$p < 0.01$, ***$p < 0.001$, NS $p > 0.05$.

are higher in peanut allergic individuals because IgG expressing plasma cells are a reservoir for IgE expressing plasma cells. It is also possible that an increase in IgG expressing plasma cells is the body's defense mechanism against allergy which is overwhelmed by the IgE in allergic participants. Many of these relationships did not hold true when looking at the peanut-consumption arm as all IgG subclasses only differentiated sensitized only from non-allergic (Supplemental Fig. 1).

Multivariate logistic regression model and stepwise selection found that peanut-specific IgG1 was the best biomarker of peanut allergy and that the addition of any other combination of subclasses did not improve the model (Table 2). Peanut-specific IgG4 was the subclass most associated with peanut consumption and adding other subclasses to the model did not significantly improve the relationship. Allergen-specific IgG4 has similarly been shown to increase after chronic exposure to antigens in populations of beekeepers, cat owners, animal laboratory workers, and in those undergoing aeroallergen or food AIT.[7]

One study limitation is the high level of atopy as most LEAP participants had moderate eczema, and it is unknown how eczema impacts IgG2 and IgG3 levels. Additionally, CD32b expression and ELISAs to explore relative inhibition from IgG subclasses will be an important area of future exploration.

Overall, amongst the peanut-specific IgG subclasses, IgG1 was the best biomarker for peanut allergy, while IgG4 was the best biomarker for peanut antigen exposure. We did not find additional value in measuring IgG2 or IgG3. A previous study utilized LEAP participant data to develop an algorithm to predict peanut allergy, which did not include IgG subclasses but was highly predictive of peanut allergy (error rate 2.8%, area under the curve 0.99).[8] The variables included in this model in order from most to least important were

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	1.49013	0.07386	20.176	<0.001
log(G1)	−0.16161	0.01737	−9.304	<0.001

Table 2. Model coefficients of final model

peanut SPT wheal, Ara h 2 specific IgE, peanut-specific IgE, Ara h 1 specific IgE, and Ara h 3 specific IgE. A future project could examine the IgG subclasses in the context of a multi-parameter model.

Abbreviations

(AIT), Allergen immunotherapy; (IgG1), Immunoglobulin G1; (IgG2), Immunoglobulin G2; (IgG3), Immunoglobulin G3; (IgG4), Immunoglobulin G4; (LEAP), Learning Early About Peanut allergy; (SPT), Skin prick test; (OFC), Oral food challenge; (psIgE), Peanut-specific immunoglobulin E; (SCORAD), Scoring Atopic Dermatitis.

Funding

Research reported in this publication was supported by the United States National Institute of Allergy and Infectious Diseases (NIAID) of the National Institutes of Health (NIH) under Award Number UM1AI109565. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

Availability of data and materials

Data will be made publicly available at the website itntrialshare.org.

Author contributions

CHB, NL, TML, DM contributed to study design.
DM performed study assays.

CHB, NL performed data analysis.
CHB, NL, MH, PP, JT, GDT, GL, TML, DM contributed to data interpretation and manuscript preparation.
CHB wrote manuscript.

Ethics approval

Samples were used from the Learning Early About Peanut allergy (LEAP) Study (NCT00329784). Samples were selected in accordance with consent for future studies in LEAP Study consent. IRB approval of LEAP study can be found separately.

Authors' consent for publication

All authors consent for publication of this manuscript.

Declaration of competing interest

Carolyn H. Baloh, MD: no conflicts of interest to report.
Noha Lim, PhD: no conflicts of interest to report.
Michelle F. Huffaker, MD: no conflicts of interest to report.
Pooja Patel, MD: no conflicts of interest to report.
Jody Tversky, MD: reports research funding from Regeneron and AstraZenica.
George du Toit, MB BCh: reports grants from National Institute of Allergy and Infectious Diseases (NIAID, NIH), Food Allergy & Research Education (FARE), MRC & Asthma UK Centre, UK Dept of Health through NIHR, Action Medical Research and National Peanut Board. Scientific Advisory Board member Aimmune. Investigator on pharma-sponsored allergy studies (Aimmune, and DBV Technologies). Scientific advisor to Aimmune, DBV and Novartis.
Gideon Lack, MB BCh: reports grants from National Institute of Allergy and Infectious Diseases (NIAID, NIH), other from Food Allergy & Research Education (FARE), other from MRC & Asthma UK Centre, other from UK Dept of Health through NIHR, other from National Peanut Board (NPB), other from The Davis Foundation, during the conduct of the study; shareholder in DBV Technologies, and Mighty Mission Me, personal fees from Novartis, personal fees from Sanofi-Genyzme, personal fees from Regeneron, personal fees from ALK-Abello, personal fees from Lurie Children's Hospital, outside the submitted work.
Tanya M. Laidlaw, MD: reports grants from National Institute of Allergy and Infectious Diseases (NIAID, NIH), and has served on scientific advisory boards for GlaxoSmithKline, Sanofi-Genzyme, Novartis, Eli Lilly, and Regeneron.
Donald W. MacGlashan Jr, MD, PhD: No conflicts of interest to report.

Acknowledgments

The authors have no acknowledgments.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.waojou.2024.100940.

Author details

[a]Immune Tolerance Network, Benaroya Research Institute at Virginia Mason, Seattle, WA, USA. [b]Division of Allergy and Clinical Immunology, Brigham and Women's Hospital, Boston, MA, USA. [c]Immune Tolerance Network, Bethesda, MD, USA. [d]Immune Tolerance Network, University of California San Francisco, San Francisco, CA, USA. [e]Johns Hopkins University Department of Medicine, Johns Hopkins Asthma and Allergy Center, Baltimore, MD, USA. [f]Peter Gorer Department of Immunobiology, School of Immunology & Microbial Sciences, London, United Kingdom. [g]Children's Allergy Service, Guy's and St Thomas' NHS Foundation Trust, London, United Kingdom. [h]Pediatric Allergy Group, Department of Women and Children's Health, School of Life Course Sciences, King's College London, London, United Kingdom.

REFERENCES

1. MacGlashan Jr D, Alvarez-Arango S, Tversky J. Subclasses of allergen-specific IgG: serum IgG2 and IgG3 levels are not predicted by IgG1/IgG4 levels. *Clin Exp Allergy*. Aug 2021;51(8):1093–1095. https://doi.org/10.1111/cea.13977.
2. Qin L, Tang LF, Cheng L, Wang HY. The clinical significance of allergen-specific IgG4 in allergic diseases. *Front Immunol*.

2022;13, 1032909. https://doi.org/10.3389/fimmu.2022.1032909.

3. MacGlashan Jr D, Hamilton RG. Parameters determining the efficacy of CD32 to inhibit activation of FcepsilonRI in human basophils. *J Allergy Clin Immunol*. Apr 2016;137(4):1256–1258 e11. https://doi.org/10.1016/j.jaci.2015.10.043.
4. Santos AF, James LK, Bahnson HT, et al. IgG4 inhibits peanut-induced basophil and mast cell activation in peanut-tolerant children sensitized to peanut major allergens. *J Allergy Clin Immunol*. May 2015;135(5):1249–1256. https://doi.org/10.1016/j.jaci.2015.01.012.
5. Saunders SP, Ma EGM, Aranda CJ, Curotto de Lafaille MA. Non-classical B cell memory of allergic IgE responses. *Front Immunol*. 2019;10:715. https://doi.org/10.3389/fimmu.2019.00715.
6. Lack G, Plaut M, Sayre PH. Peanut consumption in infants at risk for peanut allergy. *N Engl J Med*. May 28 2015;372(22):2165. https://doi.org/10.1056/NEJMc1504021.
7. Platts-Mills TAE, Keshavarz B, Wilson JM, et al. An overview of the relevance of IgG4 antibodies in allergic disease with a focus on food allergens. *Children*. May 20 2021;8(5). https://doi.org/10.3390/children8050418.
8. Sever ML, Calatroni A, Roberts G, et al. Developing a prediction model for determination of peanut allergy status in the learning early about peanut allergy (LEAP) studies. *J Allergy Clin Immunol Pract*. Jul 2023;11(7):2217–2227 e9. https://doi.org/10.1016/j.jaip.2023.04.032.