Predicting probability of tolerating discrete amounts of peanut protein in allergic children using epitope-specific IgE antibody profiling

Maria Saprun | Paul Kearney | Clive Hayward | Heather Butter | Robert Gets | Scott H. Sicherer | Paul J. Turner
Dianne E. Campbell | Hugh A. Sampson

ARTICLE SUMMARY

• Existing diagnostic testing is not predictive of severity or the threshold dose of clinical reactivity, and many patients still require an Oral Food Challenge (OFC). While OFCs are very useful for making an allergy diagnosis and determining clinical reactivity, they often cause anaphylaxis, which can increase patient anxiety, and are time and resource intensive.¹

• An extensive validation was performed across 5 cohorts (all with confirmed oral food challenge results) across six different countries. Cohorts used: BOP1, OPIA, CAFETERIA, CoFAR6, and PEPITES with specimens from Australia, UK, US, Ireland, and Germany.

• This paper reports the first validated algorithm using two key peanut specific IgE epitopes to predict probabilities of reaction to different amounts of peanut in allergic subjects and may provide a useful clinical substitute for peanut oral food challenges.

• Using the algorithm, subjects were assigned into "high", "moderate", or "low" dose reactivity groups. On average, subjects in the "high" group were 4 times more likely to tolerate a specific dose, compared to the "low" group. For example, 88% of patients in the high dose reactivity group were able to tolerate ≥144 mg of peanut protein whereas only 29% were able to tolerate the same amount in the low dose reactivity group.²

CLINICAL CONSIDERATIONS

• The new epitope test offers more granular information to help clinicians stratify treatment and peanut avoidance plans for their patients.

• See below for summary of clinical considerations based on threshold reactivity level¹

<table>
<thead>
<tr>
<th>allergenis peanut diagnostic result</th>
<th>clinical considerations¹</th>
</tr>
</thead>
</table>
| likely allergic - low dose reactor | • inform or avoid oral food challenge to reduce risk of anaphylaxis  
|                                    | • confirm strict avoidance of peanut  
|                                     | • consider immunotherapy to reduce risk of reaction |
| likely allergic - moderate dose reactor | • consider a single oral food challenge (30 to 100 mg) to reduce anxiety and improve quality of life  
|                                    | • less stringent avoidance of peanut regime  
|                                     | • consider inclusions of precautionary labeled foods such as "May contain peanut"  
|                                     | • consider immunotherapy to reduce risk of reaction |
| likely allergic - high dose reactor | • consider a single oral food challenge (100 to 300 mg) to reduce anxiety and improve quality of life  
|                                     | • less stringent avoidance of peanut regime  
|                                     | • consider inclusions of precautionary labeled foods such as "May contain peanut"  
|                                     | • consider starting immunotherapy at higher doses to shorten time to maintenance dose |
| unlikely allergic                   | • oral food challenge to rule out the diagnosis of peanut allergy |

HOW TO ORDER TESTING

• Visit allergenis.com and complete the account set up form
• Choose your phlebotomy preference (in-office or mobile phlebotomy)
• Place your order through our online platform
• Receive the results

ATTEND A WEBINAR

Upcoming webinars to learn more about the clinical utility of the thresholds.

Clinical Utility of Thresholds in Patient Management

Dr. Hugh Sampson from the Icahn School of Medicine at Mount Sinai

November 21, 2022 @ 2 pm EST

Scan to register.

REFERENCES


Visit allergenis.com for more information or to start ordering.
SEB-induced IL-13 production in CLA⁺ memory T cells defines Th2 high and Th2 low responders in atopic dermatitis

To the Editor,

Staphylococcus aureus, memory skin-homing cutaneous lymphocyte-associated antigen (CLA)⁺ T cells and IL-13 constitute relevant players in atopic dermatitis (AD) pathogenesis.¹ Since circulating CLA⁺ T cells reflect cutaneous abnormalities present in human inflammatory skin diseases,² an ex vivo coculture model made of purified circulating CLA⁺ effector and central memory T cells and autologous lesional epidermal cells was established. We show a CLA-dependent production of IL-13 upon activation with staphylococcal enterotoxin B (SEB) that allows the differentiation of the Th2 high and Th2 low groups, with distinct clinical correlations between both groups, within a clinically homogeneous population of adult non-treated moderate-to-severe AD patients.

Our results showed that IL-13, together with IL-4, IL-17A, IL-22, CCL17, and CCL22, was preferentially produced by circulating memory CLA⁺ T cells upon activation with SEB in the presence of autologous lesional epidermal cells (Figure 1A). Interestingly, SEB activation of the CLA⁺/Epi cocultures resulted in a predominant IL-13 production among the Th2 cytokines (IL-13, IL-4, IL-5) (Figure 1B). The amount of IL-5 and IFN-γ produced by SEB-activated CLA⁻ T cells was higher or similar than that by CLA⁺ T cells, respectively, suggesting their relationship to extracutaneous sites. This model is stimulus-specific since polyclonal activators such as PMA/Ionomycin and CD3/CD28 are not CLA-specific (Figure S1A), and epidermal cells contributed to the T-cell activation (Figure S1B).

Patients were stratified based on the median of the IL-13 response in the SEB-induced CLA⁺ T-cell AD cocultures (Figure 1C), and we found differentiated T-cell responses to SEB between the Th2 high and the Th2 low groups (Figure 1D). Although both groups were clinically homogeneous (Figure S1C), this stratification suggested differential immunological mechanisms between both groups, since they not only differed in terms of in vitro stimulation, but also in terms of severity, plasma markers, IgE levels against S. aureus and mRNA expression from cutaneous lesions.

In the Th2 high group, in contrast to the Th2 low, the IL-13 response by SEB CLA⁺ T cells directly correlated with EASI score and plasma levels of CCL17 and sII-2R (Figure 2A-C). This group also showed a direct correlation between anti-S. aureus IgE levels and SEB-induced CLA⁺ T-cell-mediated IL-13 response in vitro (Figure 2D). The mRNA expression from lesional skin biopsies was similar between both groups (Figure S2A), but the IL-13 produced by SEB-stimulated CLA⁺/Epi cocultures directly correlated with CCL26 (Figure 2E) and inversely correlated with LCN2 mRNA expression in the Th2 high group (Figure 2F). Additionally, the IL-13/IL-17A and IL-13/IFN-γ ratios in the SEB-stimulated CLA⁺ T-cell cocultures were higher in the Th2 high than the Th2 low group (Figure S2B), supporting the type 2 signature and the lowered type 1 immunity in the Th2 high group, which may facilitate S. aureus infection.

The study has a few limitations. We did not study the presence of S. aureus in the skin of the AD patients and the number of patients was not very high but we found consistent significant results on the relationship between the SEB-induced CLA⁺ T-cell IL-13 response and clinical features of the patients.

The novelty of our results relies on the separation of the Th2 high and low populations, corresponding with disease activity, based on the CLA⁺ T-cell IL-13 response to SEB, which are key mediators in AD pathogenesis.⁴ Interestingly, the existence of Th2 high and low groups in non-treated moderate-to-severe AD patients has been shown by serum proteomic profiling.⁵ In conclusion, we consider that this new and translational approach allows obtaining readouts on cytokine production that complement current studies based on transcriptomics and flow cytometry and may help to explore the complex heterogeneity of AD pathophysiology from a more functionally point of view.
FIGURE 1 Production of AD-associated mediators by SEB-activated cocultures of CLA+ T cells and lesional epidermal cells and stratification into the Th2 high and Th2 low groups. (A) Quantification (pg/ml) of IL-13, IL-4, IL-5, IL-17A, IL-22, IFN-γ, CCL17, and CCL22 in 24-hour cocultures in basal conditions or stimulated with SEB (n = 35 for IL-13/4/17A and IFN-γ, n = 30 for IL-5, n = 29 for IL-22, and n = 20 for CCL17/22). (B) Th2 cytokines produced by SEB-induced CLA+ T-cell cocultures presented by column bars and the median ± 95% CI. (C) IL-13 levels in AD (n = 35) and control subjects (n = 8). Dotted line indicates the median of SEB-induced CLA+ T-cell IL-13 response in AD. (D) Cytokine response (pg/ml) by SEB-activated CLA+ T-cell cocultures was compared between the Th2 high and the Th2 low groups. Abbreviations: AD, atopic dermatitis; CLA, cutaneous lymphocyte-associated antigen T cells; Epi, epidermal cells suspension; HC, healthy controls; M, untreated; SEB, staphylococcal enterotoxin B. **: p < .01; ***: p < .001; ****: p < .0001
The study was funded by FIS/ISICIII (Ministerio de Economía y Competitividad e Instituto de Salud Carlos III) 2021 (PI21/01179 and PI21/00335) and Fondo Europeo del Desarrollo Regional (FEDER). Additionally, Sans-De San Nicolás L was granted by a PhD fellowship from the Agency for Management of University and Research Grants of the Generalitat de Catalunya (FI-SDUR 2020); De Jesús-Gil C was granted by a PhD fellowship from the Agency for Management of University and Research Grants of the Generalitat de Catalunya (FI-SDUR 2020).

**FUNDING INFORMATION**

The study was funded by FIS/ISICIII (Ministerio de Economía y Competitividad e Instituto de Salud Carlos III) 2021 (PI21/01179 and PI21/00335) and Fondo Europeo del Desarrollo Regional (FEDER). Additionally, Sans-De San Nicolás L was granted by a PhD fellowship from the Agency for Management of University and Research Grants of the Generalitat de Catalunya (FI-SDUR 2020); De Jesús-Gil C was granted by a PhD fellowship from the Agency for Management of University and Research Grants of the Generalitat de Catalunya (FI-SDUR 2020).

**FIGURE 2** In the Th2 high group, SEB-triggered CLA+/Epi IL-13 response directly correlates with EASI, CCL17, sIL-2R, and anti-S. aureus IgE plasma levels and CCL26 mRNA expression in cutaneous lesions and inversely correlates with LCN2 mRNA expression in cutaneous lesions. IL-13 (pg/mL) from 24-hour cocultures was correlated with (A) EASI (n = 17 for Th2 high and n = 15 for Th2 low), (B) plasma CCL17 (n = 18 for Th2 high and n = 17 for Th2 low), (C) plasma sIL-2R (n = 18 for Th2 high and n = 17 for Th2 low), (D) anti-S. aureus IgE plasma levels (n = 17 for Th2 high and n = 17 for Th2 low), (E) CCL26 (n = 11 for Th2 high and n = 10 for Th2 low), and (F) LCN2 mRNA expression in lesional skin biopsies (n = 11 for Th2 high and n = 10 for Th2 low). Abbreviations: CLA, cutaneous lymphocyte-associated antigen T cells; Epi, epidermal cells suspension; M, untreated; SEB, staphylococcal enterotoxin B. ns: p > .05; *: p < .05; ***: p < .001.

**FIGURE 2** In the Th2 high group, SEB-triggered CLA+/Epi IL-13 response directly correlates with EASI, CCL17, sIL-2R, and anti-S. aureus IgE plasma levels and CCL26 mRNA expression in cutaneous lesions and inversely correlates with LCN2 mRNA expression in cutaneous lesions. IL-13 (pg/mL) from 24-hour cocultures was correlated with (A) EASI (n = 17 for Th2 high and n = 15 for Th2 low), (B) plasma CCL17 (n = 18 for Th2 high and n = 17 for Th2 low), (C) plasma sIL-2R (n = 18 for Th2 high and n = 17 for Th2 low), (D) anti-S. aureus IgE plasma levels (n = 17 for Th2 high and n = 17 for Th2 low), (E) CCL26 (n = 11 for Th2 high and n = 10 for Th2 low), and (F) LCN2 mRNA expression in lesional skin biopsies (n = 11 for Th2 high and n = 10 for Th2 low). Abbreviations: CLA, cutaneous lymphocyte-associated antigen T cells; Epi, epidermal cells suspension; M, untreated; SEB, staphylococcal enterotoxin B. ns: p > .05; *: p < .05; ***: p < .001.
University and Research Grants of the Generalitat de Catalunya (FI-2018), co-financed with FEDER; García-Jiménez I was granted by a PhD fellowship from the Universitat de Barcelona (PREDOCS-UB 2020).

ACKNOWLEDGEMENTS
We are grateful to all individuals who participated in our study.

CONFLICT OF INTEREST
Antonio Guilabert is a consultant for Sanofi, Almirall, and AbbVie. Laia Curto-Barredo is a consultant for Sanofi, AbbVie, Leo Pharma, and Lilly. Esther Serra-Baldrich is a consultant for Sanofi, Almirall, Leo Pharma, Pfizer, Galderma, and Lilly. Michael D. Howell is an employee and shareholder of DermTech. The rest of authors declare no conflict of interests.

Lídia Sans-De San Nicolàs1
Ignasi Figueras-Nart2
Montserrat Bonfill-Orti2
Carmen De Jesús-Gil1
Irene García-Jiménez1
Antonio Guilabert3
Laia Curto-Barredo4
Marta Bertolin-Colilla4
Marta Ferran4
Esther Serra-Baldrich5
Anna Zalewska-Janowska6
Yui-Hsi Wang7,8
Michael D. Howell9
Ramon M. Pujol4
Luis F. Santamaria-Babi1

1Grup d’Immunologia Translacional, Departament de Biologia Cel·lular, Fisiologia i Immunologia, Facultat de Biologia, Universitat de Barcelona (UB), Parc Científic de Barcelona (PCB), Barcelona, Spain
2Departament de Dermatologia, Hospital de Bellvitge, Universitat de Barcelona (UB), L’Hospitalet de Llobregat, Spain
3Departament de Dermatologia, Hospital General de Granollers, Granollers, Spain
4Departament de Dermatologia, Hospital del Mar, Institut Hospital del Mar d’Investigacions Médiques (IMIM), Universitat Autònoma de Barcelona (UAB), Barcelona, Spain
5Departament de Dermatologia, Hospital de la Santa Creu i Sant Pau, Universitat Autònoma de Barcelona (UAB), Barcelona, Spain
6Psychodermatology Department, Rheumatology and Clinical Immunology, Medical University of Lodz, Lodz, Poland
7Division of Allergy and Immunology, Cincinnati Children’s Hospital Medical Center, Cincinnati, Ohio, USA
8Type 2 Inflammation and Fibrosis Cluster, Immunology and Inflammation Research, Sanofi, Cambridge, Massachusetts, USA
9DermTech, Inc, La Jolla, California, USA

Correspondence
Luis F. Santamaria-Babi, Translational Immunology, Parc Científic de Barcelona, Baldiri i Reixac, 10, 08028 Barcelona, Spain.
Email: luis.santamaria@ub.edu

ORCID
Lídia Sans-De San Nicolàs https://orcid.org/0000-0002-2828-2842
Luis F. Santamaria-Babi https://orcid.org/0000-0002-1674-6654

REFERENCES

SUPPORTING INFORMATION
Additional supporting information can be found online in the Supporting Information section at the end of this article.