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In vitro supporting diagnostic tools in plant-food allergy

To the Editor,

Nonspecific lipid transfer proteins (nsLTP) are commonly responsible for plant-food allergy, in some cases life-threatening. Pru p 3 (a nsLTP) is the major allergen of peach and one of the main triggers of severe allergic reactions in Mediterranean population. Currently, there are several diagnostic methods available like serum specific immunoglobulin E (sIgE) and skin prick test (SPT), but the only confirmatory diagnostic tool still requires oral food challenge (OFC), with the risks associated with it.[1] To solve these drawbacks, in vitro diagnostic tools like basophil activation test (BAT) or mast cell activation test (MAT) have recently emerged as functional tests to support the diagnosis.[2,3] In this study, we analyze the possible added value of BAT and MAT in diagnostic of nsLTP-allergy.

To address it, we evaluated a cohort of 21 patients with nsLTP-allergy sensitized to Pru p 3 and 16 healthy controls (Table 1 and Table S1). Serum total IgE (tIgE) and sIgE levels were evaluated by ImmunoCAP. Moreover, BAT and MAT were carried out using Pru p 3 at eight ten-fold concentrations to establish the optimal allergen concentrations (for maximal and specific cellular activation) using flow cytometry. Figure S1 shows flow gating strategy used for BAT (Figure S1A) and MAT (Figure S1B). After evaluating the percentage of activated cells (%CD63$^+$), 0.1 and 0.01 μg/mL concentrations of Pru p 3 were selected as optimal for both methods (Figure 1A,B). Besides, all individuals included in this study were responder to BAT.

BAT displayed great values of sensitivity (90.5%) and specificity (100%), with high positive/negative predictive values (PPV, NPV) (≥88%) (Figure S2), according to our previous results.[2] However, our study differs with the results found in other studies performed in a population of patients sensitized to LTP in the Mediterranean area.[4] These differences may be due to the subject inclusion criteria, since in Decuyper and collaborators study, criteria were sensitization to Pru p 3 and/or Mal d 3, but was not confirmed by OFC. Moreover, receiver operating characteristic (ROC) curve analysis from BAT to Pru p 3 included both asymptomatic and symptomatic sensitized patients whereas in our study, this ROC curve analysis included data from healthy individuals and allergic patients confirmed by OFC.

Regarding sIgE for Pru p 3, it had a perfect sensitivity (100%), but no so good specificity (81.25%) (Figure S2).

On the contrary, for MAT, Laboratory of Allergic Diseases 2 (LAD2) human mast cells were challenged with Pru p 3 (Figure 1B).

TABLE 1 Clinical and demographic characteristic of LTP-allergic patients.

	Healthy controls ($n=16$)	LTP-allergic patients				
		All ($n=21$)	OAS ($n=11$)	URT/ANG ($n=7$)	ANAPH ($n=3$)	p-value
Age (years)[†]	42.81 ± 13.26	36.09 ± 8.34	34.00 ± 6.37	42.14 ± 9.02	29.67 ± 5.86	N.S.
Female (%)	68.75	76.19	81.81	71.43	66.67	N.S.
SPT (mm^2)[‡]	<7	75 (43.5–105.5)	80 (48–112)	75 (45–100)	40 (25–77)	N.S.
Total IgE (kU/L)[‡]	98.9 (59.38–189.3)	200 (91.9–391.5)	200 (89.3–272)	197 (94.5–598)	218 (88.4–242)	N.S.
Specific IgE Pru p 3 (kU/L)[‡]	0.025 (0.0025–0.1725)	10.3 (3.53–18.8)	7.74 (2.51–17.9)	13.8 (3.54–18.4)	19.2 (4.21–30.6)	***, *, **, *
Ratio sIgE/tIgE	0 (0–0)	0.06 (0.02–0.12)	0.04 (0.01–0.09)	0.06 (0.02–0.14)	0.13 (0.02–0.22)	***, **, *, *
BAT Positive (%)	12.5	90.45	90.91	85.71	100	****, ****, **, *
MAT Positive (%)	12.5	85.71	81.82	85.71	100	****, ***, **, *

Note: All significant p-values were obtained by comparisons between Control vs. All/ Control vs OAS/ Control vs URT/ANG/ Control vs. ANAPH, respectively.

Abbreviations: ANAPH, anaphylaxis; BAT, basophil activation test; MAT, mast cell activation test; N.S., not significant; OAS, oral allergy syndrome; sIgE, specific IgE; SPT, skin prick test; tIgE, total IgE; URT/ANG, urticaria/angioedema.

[†]Mean ± SD,

[‡]Median (Q1-Q3).

*$p<0.05$; **$p<0.01$; ***$p<0.001$; ****$p<0.0001$.

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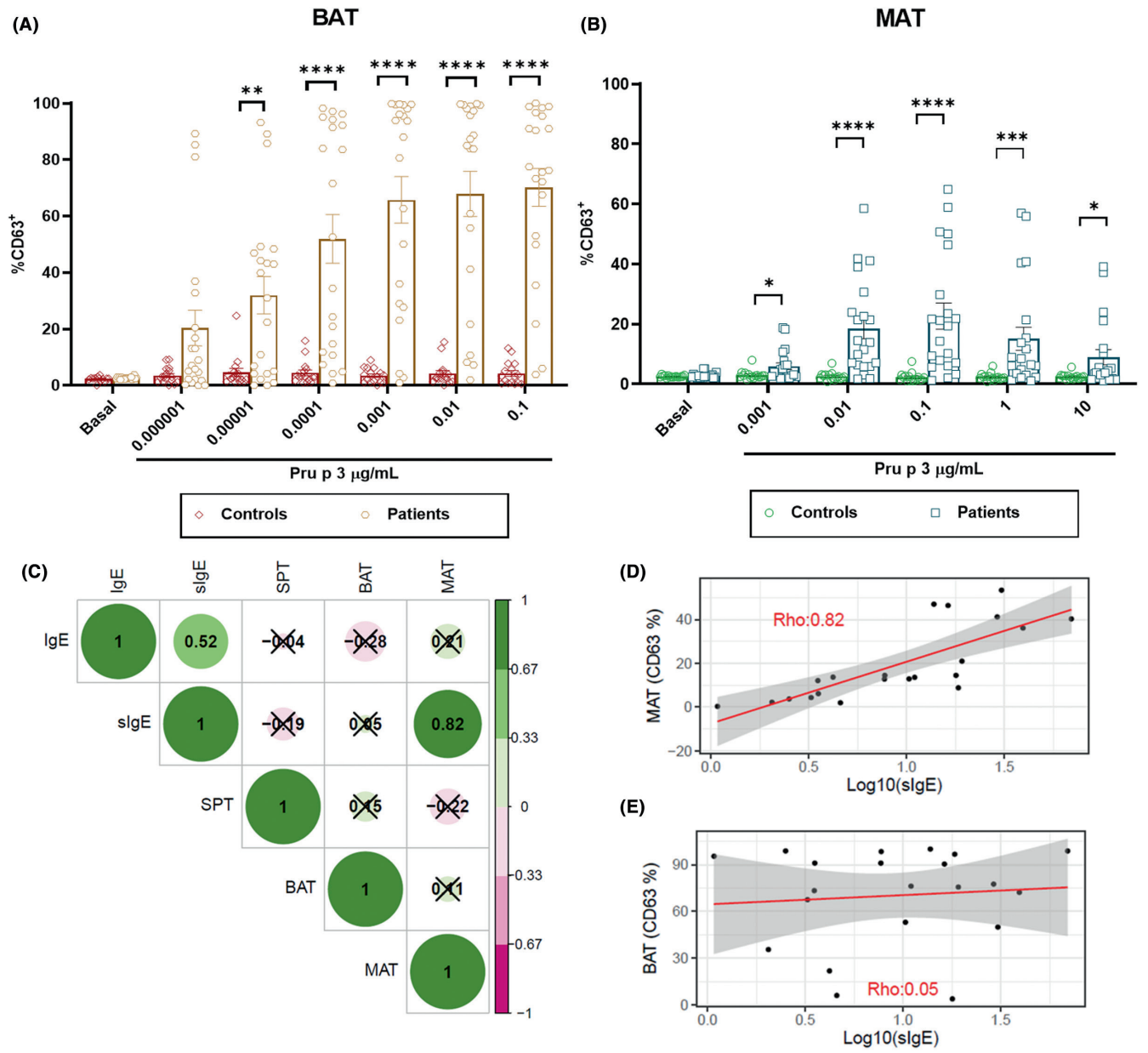

FIGURE 1 Activation and correlation analysis of in vitro tools in plant-food allergy diagnosis. Flow cytometry levels of %CD63$^+$ cells in a dose–response curve for BAT at six ten-fold concentrations (A) and MAT at five ten-fold concentrations (B) in allergic patients and healthy controls. Concentrations represented in figures were those producing relevant and significant differences between controls and patients. (C) Spearman correlation matrix between tIgE, sIgE to Pru p 3, SPT area, %CD63$^+$ cells in BAT and MAT. An X in Spearman correlation matrix indicates non-significant correlation ($P > .05$). Scatterplots showing all individual sample correlations between %CD63 positive cells in MAT (D) BAT (E) and serum sIgE levels. Allergic patients ($n = 21$), healthy controls ($n = 16$), (mean ± SEM); $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

All procedures were performed with the same batch of cultured LAD2 cells. MAT had a great sensitivity (85.71%) and specificity (94.74%) with high PPV and NPV (Figure S2). Interestingly, from the two patients that tested negative in BAT, one of them was rescued as positive in MAT. Thus, the combination of BAT and MAT increased sensitivity up to 95% compared to the techniques themselves (around 85%) while maintaining a good specificity (93.75%).

MAT showed a very strong correlation between Pru p 3 sIgE levels and %CD63$^+$ challenged at 0.01 and 0.1 μg/mL (Spearman $r = 0.82$ and 0.79, respectively) but not with tIgE, SPT area or BAT (Figures 1C,D and S3). On the contrary, BAT showed no correlation with either sIgE, tIgE, or SPT area (Figures 1C,E and S3). Our results suggest that, as expected, MAT is more related to sIgE levels than BAT, because MAT uses LAD2 cells which are passively sensitized

with patient's sera whereas BAT is based on sIgE already bound to FCεRI basophils. However, neither of them correlated according to the severity of the patient's allergic reactions.[5]

MAT has been previously described as a useful technique in the diagnosis of peanut allergy by using human blood-derived mast cells[6] or LAD2 cells.[7] However, we demonstrate for the first time the utility of MAT in the diagnosis of nsLTP-allergy with higher specificity and PPV compared to sIgE determination. MAT and BAT can be used as complementary tools in the diagnosis of LTP-allergy, as the combination of both increased the sensitivity up to 95%. Additionally, BAT needs to be done routinely with fresh blood, but MAT can be used with stored serum and when BAT fails (after anaphylactic reaction or in non-responders),[5] which has an added value despite the difficulty of these cellular techniques. One strength of this study is the performance of the OFC to confirm peach allergy. However, it should be noted that the severity grade classification of patients may be underestimated as OFC must be stopped at the first objective reaction according to clinical guidelines and patient safety.

In conclusion, both BAT and MAT can be used as reliable alternatives to sIgE measurement when the allergen is not commercially available to be evaluated. Further experiments to test the ability of MAT in higher cohorts and for other allergens are necessary.

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CONFLICT OF INTEREST STATEMENT

None of the authors have a conflict of interest to disclose.

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REFERENCES

1. Goikoetxea MJ, Berroa F, Cabrera-Freitag P, et al. Do skin prick test and in vitro techniques diagnose sensitization to peach lipid transfer protein and profilin equally well in allergy to plant food and pollen? *J Investig Allergol Clin Immunol*. 2015;25(4):283-287.
2. Cañas JA, Perez-Sanchez N, Lopera-Doblas L, et al. Basophil activation test utility as a diagnostic tool in LTP allergy. *Int J Mol Sci*. 2022;23(9):4979.
3. Chirumbolo S, Bjorklund G, Vella A. Mast cell activation test versus basophil activation test and related competing issues. *J Allergy Clin Immunol*. 2018;142(3):1018-1019.
4. Decuyper II, Pascal M, Van Gasse AL, et al. Performance of basophil activation test and specific IgG4 as diagnostic tools in nonspecific

lipid transfer protein allergy: Antwerp-Barcelona comparison. *Allergy*. 2020;75(3):616-624.
5. Santos AF, Alpan O, Hoffmann HJ. Basophil activation test: mechanisms and considerations for use in clinical trials and clinical practice. *Allergy*. 2021;76(8):2420-2432.
6. Bahri R, Custovic A, Korosec P, et al. Mast cell activation test in the diagnosis of allergic disease and anaphylaxis. *J Allergy Clin Immunol*. 2018;142(2):485-496.
7. Ji C, Huang Y, Yeung LH, et al. Ara h 2-specific IgE presence rather than its function is the best predictor of mast cell activation in children. *J Allergy Clin Immunol Pract*. 2023;11(4):1154-1161.

SUPPORTING INFORMATION

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

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Affinity matters for IgE-blocking activity of allergen-specific antibodies

To the Editor,

It is well recognized that allergen immunotherapy (AIT) triggers the production of IgE-blocking IgG and IgA antibodies (Abs) that prevent allergen-induced IgE-mediated effector cell activation.[1] Recently, passive immunotherapy with IgE-blocking human monoclonal Abs (mAbs) specific for the major allergens Fel d 1 (cat) or Bet v 1 (birch pollen) significantly reduced the respiratory symptoms of cat- and birch pollen-allergic individuals, respectively.[2,3] These studies involved cocktails comprising two to three mAbs recognizing diverse epitopes on either allergen with subnanomolar affinity.[2,4] Notably, the cocktail of two Fel d 1-specific mAbs displayed stronger inhibitory activity than a similar concentration of polyclonal Fel d 1-specific IgG Abs purified from individuals after clinically effective cat-AIT.[2] The latter bound Fel d 1 with an apparent affinity ranging from 2.7 to 3.9 nM. These observations suggested a connection of IgE-blocking bioactivity and affinity. However, this correlation was not yet experimentally demonstrated.

We previously reported that the reduction of apple-induced symptoms after 16-week sublingual immunotherapy with recombinant (r) Mal d 1 correlated with the induction of IgE-blocking IgG1 Abs.[5] Here, peripheral blood mononuclear cells from a successfully treated individual as documented by reduced allergic reactions in open food challenges who displayed Mal d 1-specific IgE-blocking IgG1 Abs served as source for engineering of specific mAbs by the Fab yeast display library technology (see Online Supplement). Eight monoclonal IgG1 Abs could be expressed at a yield of >20 mg/L and displayed monomeric profiles in size exclusion chromatography (SEC) (Figure S2A). The mAbs K1.1, K1.2, K2.4, and K2.9 showed strong binding to rMal d 1 in ELISA (Figure S2B). Their IgE-blocking activity was tested in basophil inhibition assays (see Online Supplement). mAbs were added to rMal d 1 at molar ratios of 1:1, 10:1, and 100:1, prior to addition to heparinized blood from untreated apple-allergic individuals collected after informed consent and ethical clearance by the local ethics committee (EK1344/2018). The murine mAbs BIP1 (Mal d 1-binder) and BIP3 (non-Mal d 1-binder) served as controls. Mean values of inhibition for each mAb were calculated per donor and summarized in Figure 1A. None of the mAbs reduced allergen-induced basophil activation at a 1:1 ratio. At 10-fold molar excess BIP1 displayed a stronger inhibitory activity than BIP3, reaching significant difference at a 100-fold excess. K1.1, K1.2, and K2.4 showed weak inhibitory activity at 10:1 which increased at 100:1. K2.9 enhanced basophil activation (data not shown) and was therefore excluded.

K1.1 and K2.4 were selected for affinity improvement by light chain pool expansion (all methods are described in the Online Supplement). Altogether, this approach resulted in five descendant mAbs with excellent expression characteristics, monodisperse SEC profiles (Figure 2A), and stronger rMal d 1-binding than the parental mAb in ELISA (Figure 2B). The amino acid sequences of the complementarity-determining regions (CDR) 3 of the light chains of the different descendants of K1.1 and K2.4 showed a strong consensus (Figure 2C). Surface plasmon resonance confirmed a twofold to fivefold enhanced affinity of the descendant mAb (Figures 2B and S3). In basophil inhibition assays, all descendants showed stronger IgE-blocking activity than the respective parental mAb that increased with the molar excess to allergen (Figure 1B).