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IgG removal significantly enhances detection of microarray allergen-specific IgE reactivity in patients' serum

To the Editor,

Allergen multiplex (AM) investigation is an excellent research tool and is now getting more importance in clinical practice¹. AM miniaturization using limiting amounts of each allergen suggested competition between IgE and IgG, but data in humans are scarce^{2,3}.

We investigated the effect of IgG depletion on specific (s)IgE levels measured with two AM. First, protein G was used to remove IgG from serum samples of 23 allergic patients (**Supp. Data**). While IgG depletion did not affect marker protein (albumin and ferritin) concentrations, it was associated with a decrease in total IgE concentrations (median: -35%; range -11 to -72%; $p < 0.0001$) (**Supp. Table 1**). This could be due to the presence of IgE/IgG aggregates or anti-IgE IgG⁴. Serum samples, before and after IgG depletion, were tested for sIgE with the ImmunoCAP™ ISAC (Thermo Fisher, Uppsala, Sweden). The ratio of post-depletion to intact (p/i) sIgE levels was calculated for each allergen. sIgE variations greater than 25% were considered for further analysis (i.e. ratio values between 0.76 and 1.24 were excluded), according to reported intra-assay variations^{5,6}.

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The 23 samples yielded a total of 719 p/i sIgE ratio values, which could be categorized as increased sIgE reactivity, including negative-to-positive sIgE transition, and decreased sIgE reactivity (**Supp. Table 2**).

Even without correction for the loss in total IgE during protein G treatment, sIgE reactivity was revealed for 90 molecular allergens in 19 patients, of whom 10 had never received allergenic immunotherapy (AIT) (**Supp. Table 1**). Fifty molecular allergens from 32 sources were concerned: 14 food, 7 pollen, 3 animal dander, 3 dust mite, 3 insect, 2 mold. sIgE revealed by IgG depletion was >0.5 ISU-E, was confirmed by singleplex ImmunoCAP when available, and was clinically relevant (**Figure 1, Supp. Table 1**). As induction of blocking antibodies is an important mode of action of AIT, we focused on five patients who had completed successful AIT for cow's milk (CM). In these patients, singleplex ImmunoCAP measurements demonstrated an increase in sIgE, sIgG₄, and sIgG to CM extract and molecules following AIT (**Figure 2A-B**). ISAC investigation showed increased post-depletion sIgE reactivity to CM allergens but no CM false negative with intact serum (**Figure 2C-D**). In contrast, sIgE to clinically relevant allergens unrelated to AIT was revealed by IgG depletion, and concerned mainly airborne molecules (**Supp. Table 1**).

In most cases, IgG removal in samples from the 23 patients led to an increase in sIgE levels. This was observed with both food and airborne allergens, and significantly higher for the former (**Supp. Figure 1**).

In 12 patients, post-depletion sIgE reactivity decreased for 43 different molecular allergens and a total of 51 occurrences. We did not identify features of these allergens which might have been associated with the presence, absence, category (food, airborne), glycosylation status, or the magnitude of sIgE decrease. The allergens most frequently concerned were: nMus m 1, nAct d 1, nAct d 2, nJug r 2, rMer a 1.

Next, we wondered whether IgG depletion also affected other AM. Six serum samples out of 23 were tested with ALEX Allergy Explorer (MacroArray Diagnostics GmbH, Wien, Austria). Compared to ISAC, where molecular allergens are spotted onto glass slides, ALEX contains extracts and molecular allergens coupled to nanobeads, themselves absorbed on a nitrocellulose support⁷. Results obtained with ALEX confirmed the effect of IgG depletion, with a frequent increase of sIgE to molecular allergens but also to extracts (**Supp. Table 1**). Similar to ISAC, the impact of IgG competition on sIgE levels to molecular allergens was stronger for food than for airborne molecules (**Supp. Figure 1**). All but two (Bos d 6; Cla h 8) false negative ISAC sIgE were detected with ALEX in intact serum (**Supp. Table 1**).

Regarding extracts, IgG depletion induced a mean four- to five-fold increase in sIgE concentrations, with no significant difference between food and airborne (data not shown). We speculate that this could be related to the presence of shared cross-reactive and panallergen components in plant food and pollen extracts, although this hypothesis does not account for CM, egg, or fish allergens. However, sensitization

to clinically relevant sources was less likely to be missed with ALEX than with ISAC thanks to the presence of extracts and molecular allergens from the same source, and possibly to higher amounts of allergen.

Taken together, IgG depletion increased apparent sIgE concentrations from both AM three to five-fold on average and up to 82-fold (**Supp. Table 1**).

The higher increase in sIgE reactivity to food molecular allergens with both AM suggests that sIgG to food are more abundant and/or of higher avidity than sIgG to airborne allergens. IgG competition was not restricted to AIT-related allergens (e.g. CM or egg white in patients #1-3 and 19-23), but was present for a wide variety of allergens. Environmental exposure induces humoral immune responses against allergens and is associated with the presence of high concentrations of IgG⁸. Thus, sIgE values obtained with AM result from the combination of concentrations and avidities of sIgG and sIgE. Weak correlations between the levels of IgG and IgE to mammalian epithelial allergens were reported⁹, but data are scarce for other molecular families. Moreover, the degree of competition between IgE and IgG can vary over time in a given individual, e.g. during AIT or spontaneous allergy resolution². Further research in this direction will also contribute to an important unresolved question: is *in vitro* competition between patients' sIgE and IgG correlated to the level of protection from allergic symptoms?

In conclusion, we report direct evidence that IgG competition hampers sIgE binding to a limiting amount of allergen. Virtually any allergenic molecule or extract can be affected, as demonstrated with two widely used AM. We also demonstrate that IgG competition can completely block sIgE binding, resulting in false-negative reports. In terms of clinical practice, the proof of false negative results for major food and airborne molecular allergens is probably the most salient finding. We believe that this information is worth being considered for AM-based precision medicine approaches and large-scale studies of IgE sensitization profiles.

CONFLICT OF INTEREST

JB reports personal fees from Chiesi, Cipla, Hikma, Menarini, Mundipharma, Mylan, Novartis, Purina, Sanofi-Aventis, Takeda, Teva, Uriach, other from KYomed-Innov, outside the submitted work. The other authors have nothing to disclose. JV reports personal fees from Thermo Fisher Scientific, personal fees from Meda Pharma (Mylan), personal fees from Beckman Coulter, personal fees from Sanofi, outside the submitted work.

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AUTHOR CONTRIBUTIONS

YS, MM, FM, SP, CC, SM, CB, GC and PA performed the experiments. CN and MBA contributed clinical and laboratory data about patients. YS, NC, MM, AMB, JV and PA analyzed and interpreted the results. PA, JV and JB wrote the manuscript. All authors reviewed the draft of the manuscript and provided intellectual input.

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REFERENCES

1. Ansotegui IJ, Melioli G, Canonica GW, Caraballo L, Villa E, Ebisawa M, *et al.* IgE allergy diagnostics and other relevant tests in allergy, a World Allergy Organization position paper. *World Allergy Organ J* 2020;13:100080.
2. Wollmann E, Lupinek C, Kundi M, Selb R, Niederberger V, Valenta R. Reduction in allergen-specific IgE binding as measured by microarray: A possible surrogate marker for effects of specific immunotherapy. *J Allergy Clin Immunol* 2015;136:806.
3. Schmid JM, Würtzen PA, Dahl R, Hoffmann HJ. Pretreatment IgE sensitization patterns determine the molecular profile of the IgG4 response during up dosing of subcutaneous immunotherapy with timothy grass pollen extract. *J Allergy Clin Immunol* 2016;137:562.
4. Chan Y-C, Ramadini F, Santos AF, Pillai P, Ohm-Laursen L, Harper CE, *et al.* "Auto-anti-IgE": Naturally occurring IgG anti-IgE antibodies may inhibit allergen-induced basophil activation. *J Allergy Clin Immunol* 2014;134:1394.

5. Martinez-Aranguren R, Lizaso MT, Goikoetxea MJ, Garcia BE, Cabreira-Freitag P, Trellez O, *et al.* Is the determination of Specific IgE against components using ISAC 112 a reproducible technique? *PLoS One* 2014;9:e88394.
6. van Hage M, Schmid-Grendelmeier P, Skevaki C, Plebani M, Canonica W, Kleine-Tebbe J, *et al.* Performance evaluation of ImmunoCAP® ISAC 112: a multi-site study. *Clin Chem Lab Med* 2017;55:571.
7. Heffler E, Puggioni F, Peveri S, Montagni M, Canonica GW, Melioli G. Extended IgE profile based on an allergen microarray: a novel tool for precision medicine in allergy diagnosis. *World Allergy Organ J* 2018;11:7. .
8. Schwartz A, Panetta V, Cappella A, Hofmaier S, Hatzler L, Rohrbach A, *et al.* IgG and IgG4 allergenic molecules in early childhood by route of exposure and current and future IgE sensitization: Results from the Multicentre Allergy Study birth cohort. *Allergy Clin Immunol* 2016;138:1426.
9. Curin M, Swoboda I, Wollmann E, Lupinek C, Spitzauer S, van Hage M, *et al.* Microarrayed dog, cat, and horse allergens show weak correlation between allergen-specific IgE and IgG responses. *J Allergy Clin Immunol* 2014;133:918.

Figure legends

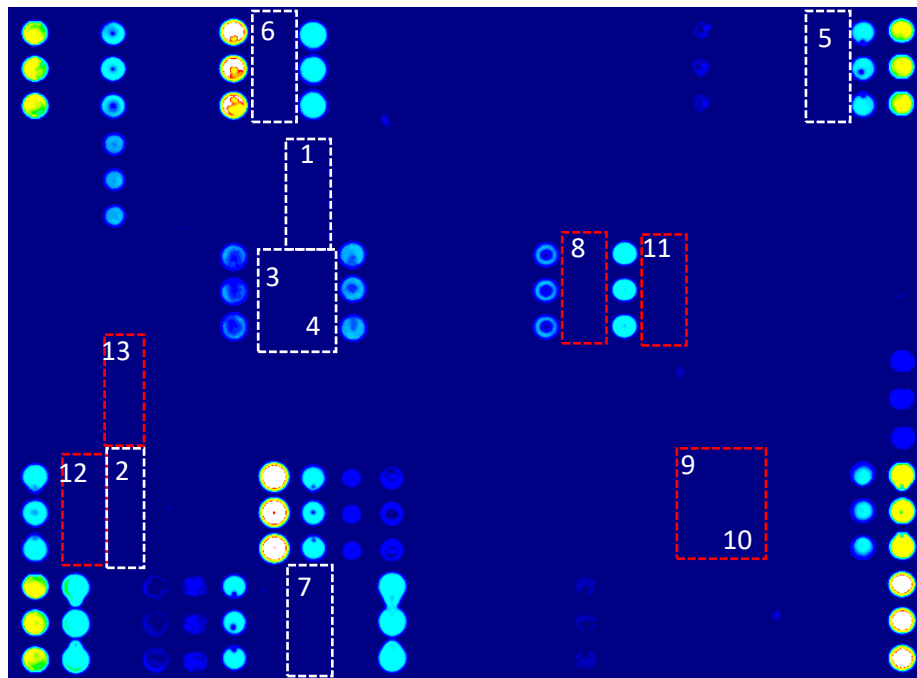
Figure 1: ImmunoCAP ISAC allergen microarray testing of intact or IgG-depleted serum

Data from patient #14 sample tested as intact serum (upper panel) and IgG-depleted serum (lower panel). Clinically relevant IgE reactivities revealed only in the IgG-depleted serum are shown in frames (white: PR-10 family, red: other allergen families). Corresponding ISU-E levels from the laboratory report are displayed.

Figure 2: ImmunoCAP ISAC allergen microarray testing of intact or IgG-depleted serum

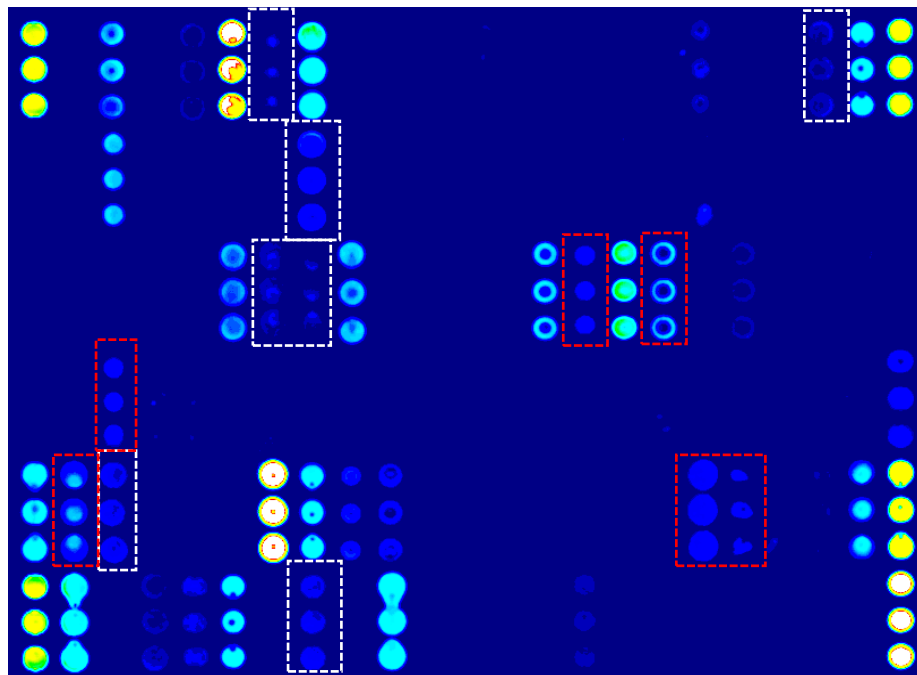
Samples were drawn before (t1) and after AIT (t2) in five children successfully treated with CM AIT. Singleplex sIgE, sIgG₄ and sIgG were measured at t1 and t2. ImmunoCAP ISAC was performed at t2 with intact and IgG-depleted serum. **A.** Variation of sIgE to CM extract (left) and casein (right). **B.** Variation of sIgG₄ to CM extract (left) and casein (right) and of sIgG to CM extract. **C.** Images of ISAC spots of CM molecular allergens showing sIgE signal in intact and IgG-depleted serum. **D.** Comparison of ISAC results of sIgE to CM molecular allergens for intact and IgG-depleted serum.

ISAC™ with intact serum



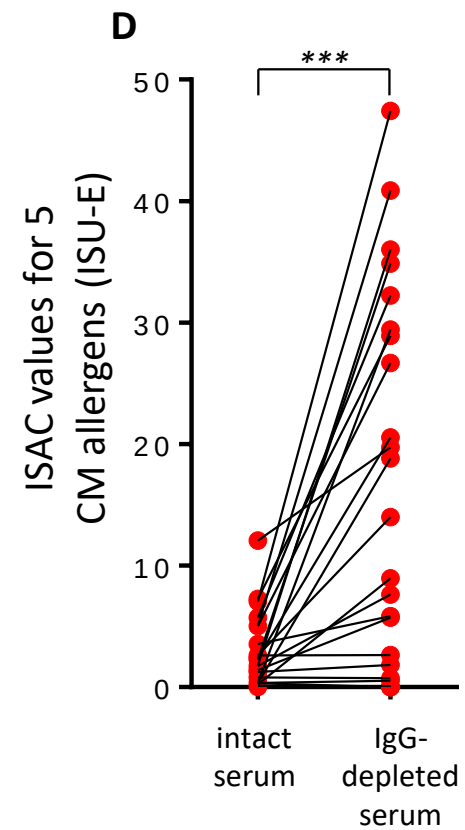
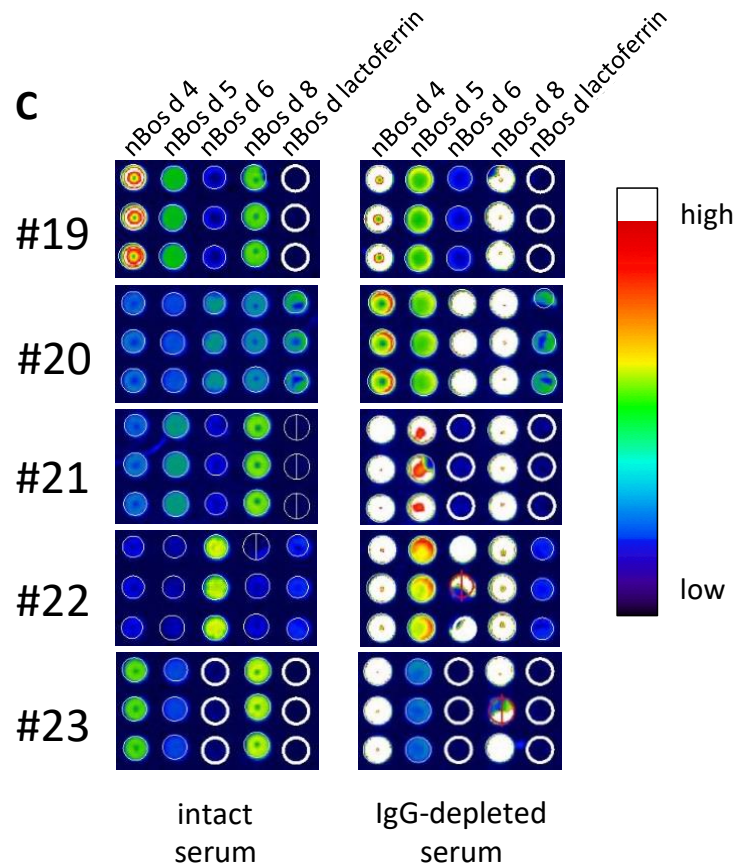
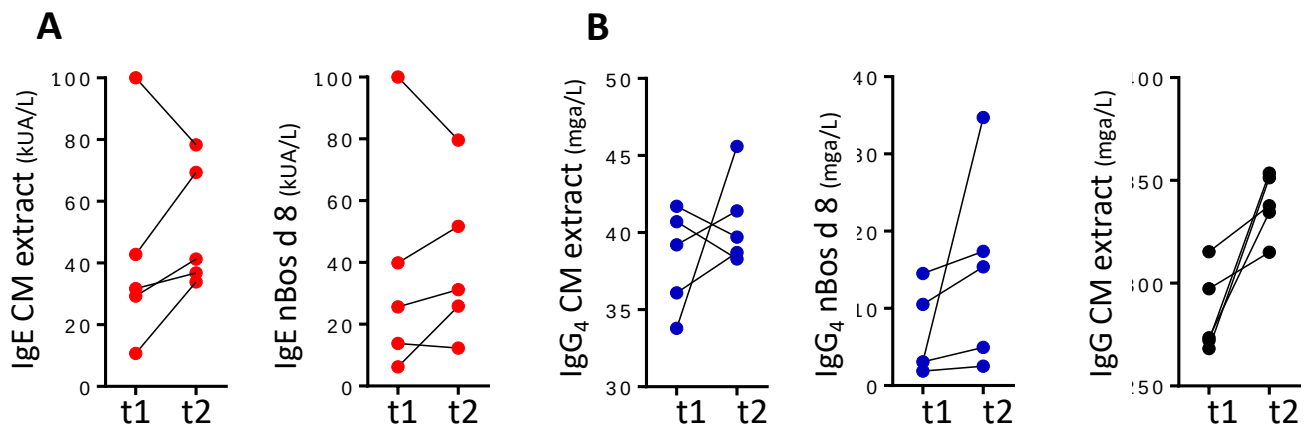
1	Bet v 1	} <0.1 ISU-E
2	Mal d 1	
3	Cor a 1.0101	
4	Cor a 1.0401	
5	Ara h 8	
6	Aln g 1	
7	Pru p 1	
8	Cyn d 1	
9	Phl p 1	
10	Phl p 11	
11	Der f 1	
12	Lep d 2	
13	Fel d 1	

ISAC™ with IgG-depleted serum



1	Bet v 1	2.9	ISU-E
2	Mal d 1	1.4	ISU-E
3	Cor a 1.0101	0.6	ISU-E
4	Cor a 1.0401	0.4	ISU-E
5	Ara h 8	0.6	ISU-E
6	Aln g 1	0.3	ISU-E
7	Pru p 1	1.2	ISU-E
8	Cyn d 1	1.8	ISU-E
9	Phl p 1	1.7	ISU-E
10	Phl p 11	2.1	ISU-E
11	Der f 1	6.3	ISU-E
12	Lep d 2	5.2	ISU-E
13	Fel d 1	3.1	ISU-E





ratio of sIgE reactivity in
IgG-depleted versus intact serum

100

10

1

$p = 0.003$

$p = 0.005$

food

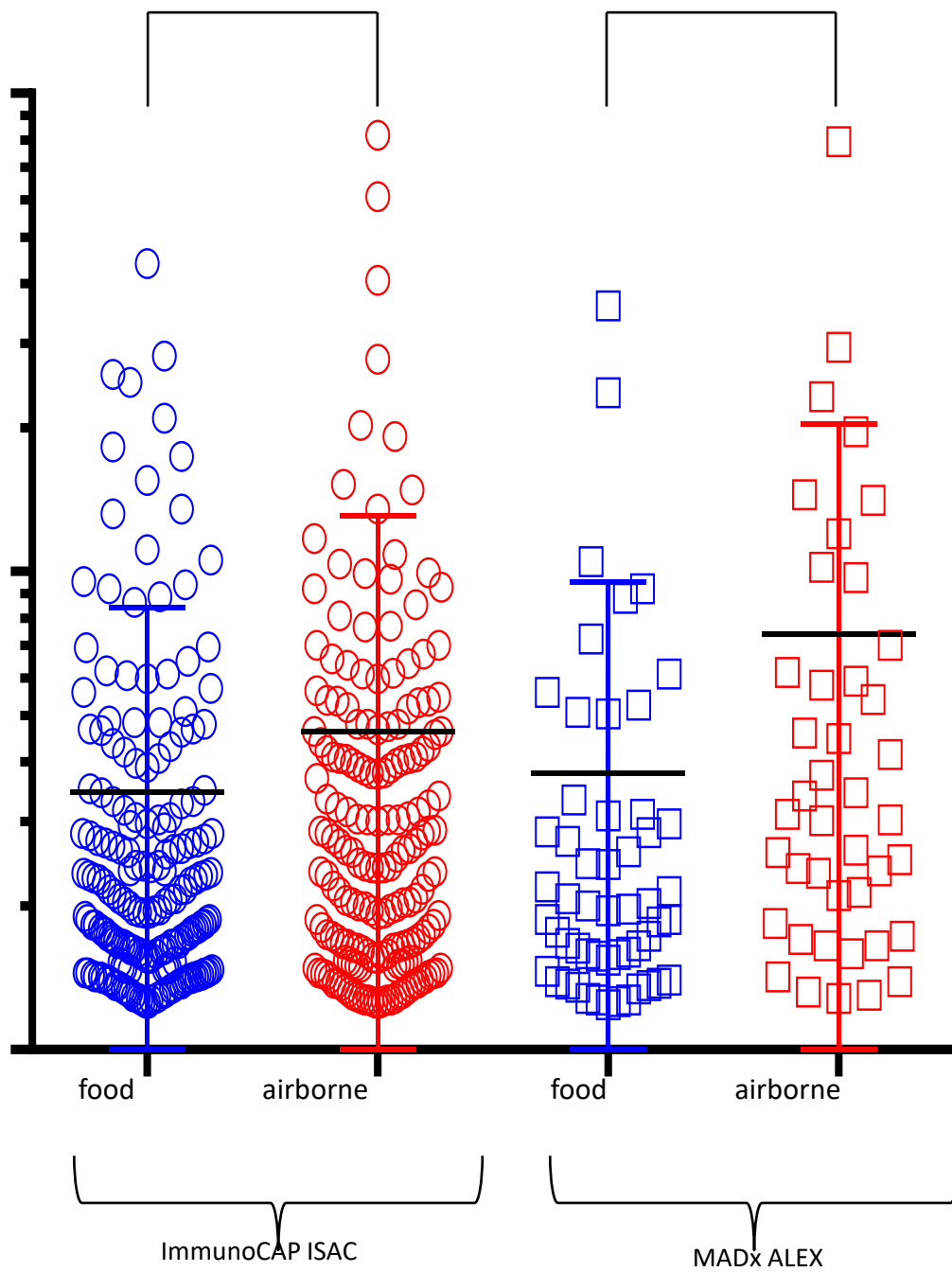
airborne

food

airborne

ImmunoCAP ISAC

MADx ALEX



#	Age	Sex	Allergy history				Analysis of sera with sIgE reactivity revealed after IgG removal				Total IgE (kIU/L)		
			Current food allergy	Other IgE-related diseases	Resolved allergies	AIT	sIgE reactivity revealed by IgG removal (ISAC ISU-E)	Intact serum sIgE reactivity (ALEX kU _A /L)	ImmunoCAP™ singleplex (kU _A /L)	Highest increases in ISAC sIgE reactivity post IgG-depletion	intact serum	post-IgG removal	ratio
1	35	M	avocado, celery, SES, OAS raw plant food	AD, AR, Asthma	none	none	Ara h 2 (<0.1), Ara h 9 (0.43), Bla g 1 (1.19), Bla g 2 (0.74)	Ara h 2 (1.41), Ara h 9 (4.43), Bla g 1 (1.41), Bla g 2 (0.79)	Ara h 2 (5.7), Ara h 9 (5.4)	Bla g 2, Cor a 8, Bla g 1, Ves v 5, Fel d 2	5256	4520	0.86
2	2	F	pistachio	none	EW, nuts	CM (ongoing)	Gly m 6 (0.65), Ana o 2 (0.36)	Gly m 6 (0.69), Gly m (0.27), Ana o (9.2)	Gly m 6 (5.6), Ana o 3 (4.6)	Ses i 1, Bos d 4, Cor a 9, Gal d 2, Gly m 6	195	60	0.31
3	9	M	nuts, PN, SES, shrimp	AD, Asthma	CM, EW	PN (ongoing)	Bos d 4 (3.11), Tri a 14 (0.58), Jug r 3 (0.47)	Bos d 4 (0.49), Tri a (0.31), Bos d milk (3.7), Jug r (0.55)	Bos d milk (16.6), Jug r (1.0)	Bos d 8, Bos d 5, Gal d 1, Bos d 4, Gal d 2	6906	3808	0.55
4	12	F	CM, nuts, PN, SES	Asthma	EW	CM (failed)	Bos d 6 (0.49), Bos d lactoferrin (0.33)	Bos d 6 (<0.1), Bos d milk (33)	Bos d milk (>100)	Act d 2, Gal d 2, Equ c 1, Mus m 1, Fel d 1	17927	12509	0.70
5	15	M	none	EoE	none	none	Bos d 4 (0.38), Bos d 5 (0.9), Bos d 8 (2.97), Tri a 14 (0.42)	Bos d 4 (0.19), Bos d 5 (0.36), Bos d 8 (0.54), Bos d milk (0.36)	Bos d 4 (0.33), Bos d 5 (0.65), Bos d 8 (2.2)	Bos d 8, Ara h 9, Bos d 6, Pru p 3, Gly m 4	895	793	0.89
6	45	M	CM, EW, nuts, PN, OAS raw plant foods	AD, Asthma	none	none	Ber e 1 (0.72), Pen m 2 (0.64), Fel d 4 (0.53), Art v 1 (0.57), Api m 4 (1.21)	N.A.	N.A.	Gly m 4, Act d 8, Ole e 9, Cor a 1.01, Api m 4	11719	7815	0.67
7	33	F	OAS raw plant foods	AD, Asthma	none	none	Cla h 8 (0.69), Api m 4 (0.56)	N.A.	N.A.	Gly m 4, Ara h 8, Api g 1, Gal d 1, Gal d 3	8928	7022	0.79
8	40	M	CM, PN	AD, Asthma	none	none	Act d 1 (0.44), Alt a 6 (1.16), Bos d 5 (1.53), Gal d 2 (1.11), Pen m 1 (1.93), Ber e 1 (0.92), Bet v 4 (0.80), Phi p 7 (0.91), Cla h 8 (0.34)	Bos d milk (8.55), Act d 1 (0.17), Alt a 6 (<0.1), Bos d 5 (0.23), Gal d 2 (0.12), Pen m 1 (18.3), Phi p 7 (0.1), Cla h (0.1), Cla h 8 (0.02)	N.A.	Tri a ATI, Par j 2, Ara h 8, Ole e 9, Bos d 5	29837	16550	0.89
9	25	M	none	AD, Asthma	none	none	Gal d 5 (0.61), Cor a 8 (0.86), Art v 1 (0.85), Bla g 1 (0.37), Fel d 2 (0.51), Par j 2 (13.2), Pol d 5 (0.60)	N.A.	N.A.	Ole e 9, Par j 2, Mal d 1, Alt a 6, Pru p 3	21503	11614	0.54

10	50	F	none	AC, AR, Asthma	none	none	none	N.A.	N.A.	Cup a 1, Par j 2, Fel d 1, Cry j 1, Ole e 1	187	136	0.73
11	17	F	legumes	AD, Asthma	none	none	none	N.A.	N.A.	Act d 8, Api g 1, Cor a 1.01, Ara h 8, Mus m 1	580	401	0.69
12	18	F	EW, kiwi, nuts, peach, SES, shrimp	AD, Asthma	CM	none	Equ c 3 (0.60)	N.A.	N.A.	Cry j 1, <u>Bos d 8</u> , Pen m 2, Equ c 3, Der f 1	665	347	0.52
13	4	F	none	AD	none	none	Ara h 2 (0.86), Pla a 3 (0.73)	N.A.	Ara h 2 (0.88)	Ara h 2, Jug r 3, Pla a 3, Jug r 2, Pru p 1	2673	1775	0.66
14	9	M	Legumes	AD	PN	cypress (ongoing)	Bet v 1 (2.86), Pru p 1 (1.22), Ara h 8 (0.59), Mal d 1 (1.40), Cor a 1 (0.56), Blo t 5 (0.99), Cyn d 1 (1.83), Lep d 2 (5.22), Phl p 1 (1.68), Phl p 11 (2.09)	N.A.	Ara h 8 (0.20)	Lep d 2, Bet v 1, Phl p 11, <u>Cyn d 1</u> , Phl p 1	2396	1204	0.50
15	13	M	fish, SES, wheat	EoE	none	none	none	N.A.	N.A.	Jug r 2, Phl p 12, Ara h 9, Pla a 3, Jug r 3	2476	1354	0.55
16	4	F	PN	AD, Asthma	none	none	Pen m 1 (0.34)	N.A.	N.A.	Fel d 1, Ara h 1, Ara h 2, Ara h 3, Ara h 6	248	182	0.73
17	2	F	legumes, nuts, PN, SES	none	CM	none	Ara h 1 (0.37), Cor a 9 (0.37), Gal d 1 (0.32), Gal d 2 (0.34), Ses i 1 (0.39)	N.A.	Ara h 1 (0.42), Cor a 9 (0.59), Gal d 1 (0.19), Ses i (0.60)	Ara h 2, Jug r 1, Ara h 6, Der p 2, Der f 2	309	202	0.65
18	10	M	kiwi, PN, legumes, SES	EoE	none	none	none	N.A.	N.A.	Alt a 6, Ara h 2, Der p 1, Fel d 1, Phl p 4	208	110	0.53
19	1	M	goat & sheep milk, hazelnut	AD, Asthma	CM	CM (comple ted)	Can f 1 (0.85), Der f 1 (0.67), Der f 2 (1.53), Der p 1 (0.71), Der p 2 (1.86)	N.A.	<i>Can f (1.6)</i>	Der p 2, <u>Bos d 8</u> , Der f 2, Can f 1, Jug r 3	351	223	0.64
20	6	F	goat & sheep milk	Asthma	CM	CM (comple ted)	Der f 1 (0.55), Der p 1 (0.68), Der p 2 (0.58), Equ c 3 (1.26), Lep d 2 (0.95)	N.A.	N.A.	<u>Bos d 8</u> , Fel d 2, <u>Bos d 6</u> , Can f 3, <u>Bos d 4</u>	440	272	0.62

21	2	M	EW, kiwi, nuts, PN, SES	AD, Asthma	CM	CM (completed)	Alt a 1 (3.33), Ana o 2 (1.56)	N.A.	<i>Ana o (31.9), Ana o 3 (27.6), Alt a 1 (0.16)</i>	<u>Bos d 4</u> , Alt a 1, Gal d 2, Cor a 9, Tri a 14	2680	1977	0.74
22	0.5	M	none	Asthma	CM	CM (completed)	Der f 1 (0.52), Lep d 2 (0.81)	N.A.	N.A.	<u>Bos d 4</u> , <u>Bos d 8</u> , <u>Bos d 5</u> , Equ c 3, <u>Bos d 6</u>	404	114	0.28
23	6	F	goat & sheep milk	AR, Asthma	CM	CM (completed)	19 allergens; e.g. Phl p 1 (6.29), Der f 2 (8.48), Der p 1 (7.47)	N.A.	<i>Der f (0.16)</i>	Der f 2, Der p 2, Der p 1, Phl p 1, Fel d 1	336	182	0.54

Table S1. Clinical and laboratory data of the sample population.

Patients were recruited either from Marseille (#10 – #18) or Toulouse (all others) teaching hospitals. Patients #19 to #23 were studied after completion of successful oral allergen immunotherapy for cow's milk allergy. For each patient, the rightmost column shows the five molecular allergens with maximal increases of sIgE reactivity (ISAC) after IgG-depletion (underlined: molecular allergens related to AIT or resolved allergies). *Italics*: allergenic extracts (ALEX and singleplex ImmunoCAP), e.g. *Gly m* = soy extract.

AC: allergic conjunctivitis; AD: atopic dermatitis; AIT: allergen immunotherapy; AR: allergic rhinitis; CM: cow's milk; EW: egg white; N.A. not assessed; OAS: oral allergy syndrome; PN: peanut; SES: sesame.

Effect of IgG depletion	Number of values (n) mean / median (<i>range</i>)		
	ISAC molecular allergens	ALEX molecular allergens	ALEX allergen extracts
Specific IgE increase (p/i sIgE ratio >1.24)	n = 417 3.92 / 2.03 (1.26 - 82.0)	n = 145 5.45 / 2.39 (1.26 - 79.3)	n = 148 4.82 / 2.29 (1.26 - 40.8)
Specific IgE decrease (p/i sIgE ratio <0.76)	n = 51 0.56 / 0.63 (0.06 - 0.75)	n = 35 0.37 / 0.41 (0.02 - 0.75)	n = 61 0.46 / 0.51 (0.01 - 0.75)

Supp. Table 2. Summary of IgG depletion results.

Ratio of post- IgG depletion vs intact (p/i) sIgE were calculated for all possible comparisons in 23 (ImmunoCAP ISAC) or 6 (MADx ALEX) samples. The number of ratio values is shown after exclusion of those between 0.76 and 1.24 (*i.e.* when the change in sIgE apparent concentration induced by IgG-depletion is less than 25%; total number of ratio values before exclusion was 719).

***IgG removal significantly enhances detection of
microarray allergen specific IgE reactivity in patients' serum***

SUPPLEMENTARY DATA

Materials and Methods

Patients

Patients #1 - #9 and #19 - #23 were recruited from paediatric ward or adult pulmonology and allergology department in Toulouse teaching hospital. All patients or their legal representatives gave informed consent before collection of samples. Patients #10 to #18 were from paediatric or adult allergology departments of Marseille teaching hospitals and underwent allergen microarray investigations as part of their routine allergy diagnosis or follow-up in agreement with current EAACI and WAO guidelines (Matricardi PM *et al*, *Pediatr Allergy Immunol* 2016; Ansotegui IJ *et al*, *WAO J* 2020). As the present study concerned only previously generated clinical and laboratory data and additional experiments with excess serum, it was categorized as a type 3b non-interventional research, Art. L1121-1 CSP under the French law. The study was approved by internal review board of the University Hospitals of Marseille (2019-271, September 3rd, 2019) and by the Research Ethics Committee (Comité de Protection des Personnes) Sud-Ouest et Outre-Mer II, for samples collected in Toulouse Teaching Hospital (sample collection declaration DC20162804).

IgG depletion of serum

IgG removal from serum samples was obtained by three rounds of absorption cycles using Thermo Fisher protein G-coated magnetic beads (Rockford, IL). One hundred μ L of beads were previously washed twice by slowly vortexing for 30 seconds followed by a 1-minute centrifugation at 1500 rpm, with 300 μ L of a wash buffer composed of Tris (25mM) plus 0.05% of Tween 20 at pH 7.5. Five hundred μ L of serum sample were then added to 100 μ L of beads and incubated for one hour under rotation, in order for serum IgG to be captured by protein G-coated beads. Next, the beads were separated from the IgG-depleted serum using a magnet. This cycle of depletion was carried out three times to achieve complete IgG depletion. A total IgG assay was performed in the IgG-depleted samples using the Human IgG ELISA Kit (Abcam, Amsterdam, the Netherlands) according to the manufacturer's instructions. In IgG-depleted samples, IgG concentration was below 230 μ g/L. The purpose of this assay was to check the quality of depletion after the three cycles. IgG-depleted serum samples were stored at -80° C before use.

Albumin and ferritin dosages

Albumin and ferritin levels were determined in native and IgG-depleted serum samples with a Cobas c 501 analyzer (Roche Diagnostics, Mannheim, Germany). A bromocresol green method, using the CFAS calibrator (Roche Diagnostics) which is traceable to the international protein calibrator IRMM BCR470/CRM470, was used for albumin. Ferritin levels were measured through a particle-enhanced immunoturbidimetric assay using ferritin agglutination with latex particles coated with anti-ferritin antibodies, followed by turbidimetric determination of the precipitate at 570/800 nm.

IgE measurements and data analysis

Singleplex total or specific IgE measurements were performed with ImmunoCAP™ Phadia 250 systems (Thermo Fisher Scientific, Phadia AB, Uppsala, Sweden), in compliance with ISO 15189 standards. Multiplex determination of sIgE levels was performed with intact or IgG-depleted serum, using ImmunoCAP™ ISAC 112 IgE (ThermoFisher Scientific). Raw fluorescence data were acquired by a laser scanner (LuxScan 10K, Capital-Bio, Beijing, China) and were converted into ISU-E (ISAC Standardised Units for IgE) using Microarray Image Analyzer software (Thermo Fisher). Except for patient #15, intact and IgG-depleted samples of each patient were tested on 2 positions of the same ISAC slide. For each molecular allergen displayed on ISAC 112, the ratio of post/pre-depletion ISU-E levels

was calculated. Seventy pre-depletion sIgE values were changed from “0” to 0.3 ISU-E to allow ratio calculation. Finally, all ratio values between 0.76 and 1.24 were excluded from analysis, as small ISU-E variation could be due to the intra and inter-assay variations of the ImmunoCAP™ ISAC.

For patients #1-5 and #8, ALEX V1 allergen chips (Allergy Explorer, Macro Array Diagnostics, Vienna, Austria) was performed according to the manufacturer’s instructions. Briefly, 100µL of intact or IgG-depleted serum was incubated for 15 min at 25°C in presence of 400µL of cross-reactive carbohydrate determinants blocking reagent (MADx), before deposition of the diluted sample (500µL) on an ALEX biochip. After 120 min of incubation at ambient temperature, followed by 3 washes, ALEX chips were incubated for 30 min with an anti-human Fcε secondary antibody (MADx) labelled with alkaline phosphatase, washed 5 times before addition of colorimetric substrate. Reaction was allowed to develop for 8 minutes. Chip images were acquired with a CCD camera, and sIgE levels were converted into kU_A/L using the Raptor software (MADx). Similar to ISAC, ALEX data were analyzed by calculating the ratio of post/pre-IgG depletion sIgE levels, excluding those ratio values comprised between 0.76 and 1.24.

Statistical analysis

Non-parametric Mann-Whitney paired-tests were used to compare post and pre-depletion data (PRISM, GraphPad Software, La Jolla CA, USA).

Supp. Figure Legend:

Figure S1: IgG interference with IgE binding to multiplexed food or airborne molecular allergens.

Samples from 23 allergic patients were assayed by ImmunoCAP™ ISAC (left) and six out of these twenty-three were also assayed by Macroarray Diagnostics ALEX biochip (right). Each sample was tested intact (before IgG-depletion) and after IgG-depletion. For each molecular allergen, the post-depletion versus intact sIgE ratio was calculated as a surrogate for IgG interference with IgE binding.

IgG removal was associated with an increase in IgE reactivity which was significantly higher toward food molecular allergens (represented in blue) than for airborne allergens (red). *p* values are from non-parametric Mann-Whitney tests.