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TITLE:

Allergen sensitization in a birth cohort at mid-childhood: focus on food component IgE and IgG4 responses

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Capsule Summary

Among 616 eight year old children in an unselected birth cohort the authors found strikingly different IgG4/IgE ratios to milk and peanut components. This finding was additionally related to discordant results between an extract and microchip component IgE assay.

Keywords

IgE, IgG4, sensitization, extract assay, ImmunoCAP, component assay, ISAC, eosinophilic esophagitis

Abbreviations

EoE – Eosinophilic esophagitis

ISAC - Immuno Solid-phase Allergen Chip

IgE – Immunoglobulin E

IgG4 – Immunoglobulin G4

IL-10 – Interleukin-10

To the Editor:

Standard IgE assays which measure a single allergen extract or component attached to a solid phase (such as ImmunoCAP) and microchip component assays (such as ISAC) represent two strikingly different approaches for measuring IgE antibodies to allergens (1). While the latter approach is commonly used to assess sensitization profiles in cohort studies, the microchip assay may be less sensitive than traditional allergen testing (2). This could have important consequences when trying to characterize the true prevalence of IgE responses in a population, or in situations where low-level IgE to allergen may be important, such as eosinophilic esophagitis (3, 4). We embarked on the present study to characterize the levels and prevalence of sensitization to common allergens in an unselected birth cohort at mid-childhood, to compare findings using a total extract assay (ImmunoCAP) and a microchip component assay (ISAC), and to relate differences in results from the two assays to specific IgE and IgG4 levels.

Project Viva is a longitudinal cohort of mother-child pairs enrolled at initial prenatal visits in urban and suburban areas of Eastern Massachusetts from 1999-2002. Study details and demographics have been previously described, but importantly there were no selection criteria relating to asthma or atopic disease at the time of enrollment of the mothers (5). The study design is available in the supplemental data (Fig E1). Sera from 616 children at the mid-childhood visit (median age 7.7 years) were available for assay. Allergen extract-specific IgE antibodies were measured by ImmunoCAP (Thermo Fisher Scientific/Phadia, Kalamazoo, MI) to 8 inhalants and 5 foods where values > 0.35 kU/L were considered positive. Our findings on sensitization prevalence were largely consistent with results from 6-9 year olds reported in NHANES 2005-06. In particular, sensitization to food, while largely low titer, was common (Fig 1A and Table E1) (6).

Samples that were positive for one or more of the 13 allergens were assayed by microchip component assay (ISAC, Thermo Fisher Scientific/Phadia) (3). Results of this analysis are semi-quantitative and expressed in standardized units, with 0.3 units representing the lower limit of detection. For dust mite, cat and peanut there was a convincing correlation between the results of the two testing techniques in terms of the prevalence of positive titers (Fig 1B). This was true even at low levels of sensitization (i.e – class 1 and 2). As a clinical correlation, we evaluated the relationship of the presence of IgE to peanut extract and IgE to component allergens with the answer to the question “Has your child had an allergic reaction to peanuts?” Out of the cohort there were 27 individuals who reported a history of such a reaction and univariate analysis revealed a significant odds ratio with the different testing modalities (Table E2). However, there were some allergens where the agreement between extract and microchip assays was not as strong (Table E3). Focusing on food our analysis

showed that the microchip assay detected positive results in less than 20% of the sera that were positive to milk, egg or wheat by extract testing (Fig 1B).

To address the possibility that the relevant components were not included on the microchip we assayed IgE to specific components derived from milk (Bos d 4, Bos d 5 and Bos d 8), peanut (Ara h 1, Ara h 2 and Ara h 8) and hen's egg (Gal d 1 and Gal d 2) with the 'singleplex' ImmunoCAP. These results showed that the specific component assays correlated more closely with extract testing for milk and egg as compared to microchip component testing, and this was true even at low-level sensitization (Fig 1C). As an example, testing for specific IgE to Bos d 4 (α -lactalbumin), Bos d 5 (β -lactoglobulin) and Bos d 8 (caseins) was positive for at least one of these components in 68 of the 81 cases that were positive by milk extract testing, whereas the microchip approach detected only a single positive out of these 81 samples despite incorporating the same components. This indicates that the relevant components are present on the microchip, but that the sensitivity of the microchip for IgE to cow's milk or hen's egg is much less than the specific component assays. Of note, our analysis of wheat was limited by the fact that not all of the components used on the microchip were available for the singleplex ImmunoCAP for wheat.

To address the hypothesis that the discrepancy in results between specific and microchip component assays could be explained by the presence of 'blocking' antibodies, we assayed specific IgG4 to milk, egg and peanut components in a subset of the sensitized samples. For milk and egg there was abundant IgG4 to the same components that were recognized by IgE (Fig 2A and B). The analysis revealed that milk (& egg) components have a much higher IgG4/IgE ratio (Fig E2), as well as a distinct IgG4/IgE 'signature' as compared to peanut components (Fig 2C).

Taken together these results suggest that microchip assays are most likely to be discordant with extract or specific component assays for allergens with high concomitant IgG4 levels, such as milk and egg. Allergen-specific differences in the ratio of specific IgG4/IgE are also consistent with the hypothesis that sensitization to milk and egg occur via a different route than peanut. This latter observation is limited, however, by the fact we do not have detailed dietary history on these subjects to account for consumption differences.

The results described here highlight potential problems with component assays that should be considered when using component-based assays. These include:

1. Component assays are only possible if the relevant allergen epitopes have been identified, purified and included on the microchip or the specific component assay (see www.allergen.org).

2. Quantities of allergen used in assays vary markedly, with estimates ranging from less than 1 picogram on a microchip to 1 microgram or more on a solid-phase singleplex assay.
3. With lower quantities of allergen, i.e.-microchip techniques, the potential effects of IgG4 or IgG1 'blocking' antibodies become much greater (for example, in the case of abundant IgG4 to milk as seen in Fig 2a).
4. With higher quantities of purified allergen, i.e.-singleplex component assays, minor impurities can give erroneous positive signals.
5. When IgE is directed against a minor component of the extract, the component assay can give a result that is much higher than the result with the extract (for example, Ara h 8 as seen in Fig 2a).

This work builds on a number of earlier reports describing allergen-specific IgG4 and performance characteristics of allergen multiplex assays (7-9). We also emphasize that this work does not address the suitability of multiplex component testing for evaluating immediate hypersensitivity reactions (which typically involve high titer specific IgE). The results demonstrate a striking difference between the response to peanut and milk proteins within the cohort. It also demonstrates limitations of microchip assays for determining IgE sensitization to some allergens, a finding that could be particularly important in disease states such as eosinophilic esophagitis (EoE) where skin testing has had little utility but it has been shown that low titer IgE to food allergens may be relevant to diagnosis and management (4).

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Figure Legends

Fig 1. **A**, IgE sensitization to inhalant and food allergen extracts in 6-10 year olds (n = 616) as measured by ImmunoCAP. **B**, Comparison of IgE results from microchip assay with results from the extract assay. **C**, Comparison of food-specific components with ImmunoCAP singleplex and ISAC. Class sensitization (on x-axis) was determined by extract testing and statistics reflect the χ^2 test with Yates correction.

Fig 2. **A**, Representative results of samples that were tested for IgE and IgG4 to cow's milk and peanut components by ImmunoCAP, and for IgE to components by ISAC. **B**, Specific IgG4 levels from samples that were positive to the component-relevant food specific IgE by extract testing where threshold of detection of specific IgG4 was 0.07 $\mu\text{g/ml}$. Data represents geometric means where error bars are the 95% CI. Values less than the threshold of detection are shown below the dotted line and are not included in the geometric mean. **C**, Milk (n=20) and peanut (n=23)-specific components were plotted for IgE and IgG4. Where IgE and IgG4 were less than the detection limit they were depicted with the values 0.25 kU/L and 0.04 $\mu\text{g/mL}$, respectively.

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Table E1.

	allergen (phadia #)	class 0 (≤ 0.35 kU/L)	class 1 (0.36-0.7 kU/L)	class 2 (>0.7 -3.5 kU/L)	class 3 (>3.5 -17.5 kU/L)	class 4 (>17.5 -50 kU/L)	class 5 (>50 -100 kU/L)	class 6 (>100 kU/L)	not measured	total # measured
Indoor	D. farinae (d2)	430	18	46	43	29	30	20	0	616
	cat dander (e1)	490	7	35	42	26	10	6	0	616
	dog dander (e5)	492	23	54	28	7	2	0	10	606
	cockroach (i6)	553	26	18	6	3	0	0	10	606
outdoor	rye grass (g5)	525	24	35	17	4	1	0	10	606
	ragweed (w1)	538	13	37	11	7	0	0	10	606
	Alternaria (m6)	565	5	15	7	4	1	0	19	597
	Aspergillus (m3)	576	9	10	2	0	0	0	19	597
food	milk (f2)	506	53	40	3	0	0	0	14	602
	egg white (f1)	556	26	18	6	0	0	0	10	606
	wheat (f4)	518	39	27	10	1	0	0	21	595
	peanut (f13)	531	15	37	18	6	3	6	0	616
	soybean (f14)	548	13	20	9	4	1	0	21	595

Table E2.

Peanut Allergic Reactions in the Viva Cohort*

Extract, ImmunoCAP	<u># positive of peanut allergic</u>	<u># positive of total</u>	<u>OR (95% CI)</u>
Peanut (f13)	21/25	82/284	17 (5.6 – 52)
Dust Mite (d2)	16/25	176/284	1.1 (0.5 – 2.6)
Cat (e1)	15/25	121/284	2.2 (0.9 – 5.0)
Components, by ISAC	<u># positive of peanut allergic</u>	<u># positive of total</u>	<u>OR (95% CI)</u>
Ara h 1	15/25	21/284	63 (20 – 197)
Ara h 2	15/25	23/284	47 (16 – 137)
Ara h 8	5/25	40/284	1.6 (0.6 – 4.5)

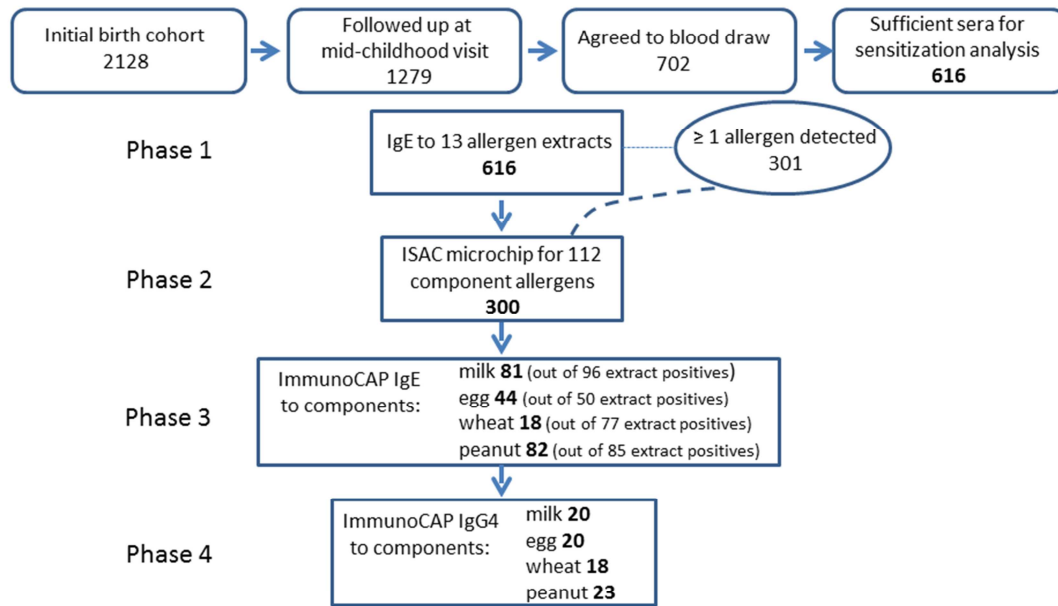
* “has your child ever had an allergic reaction to peanut?”

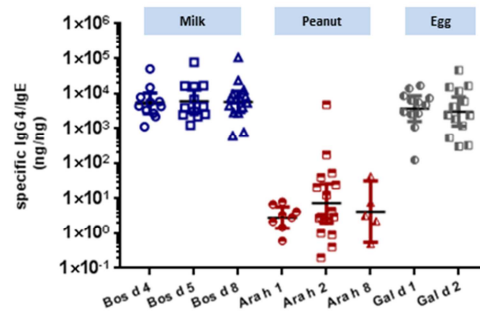
n = 27 reported reactions of 616 in the total cohort;

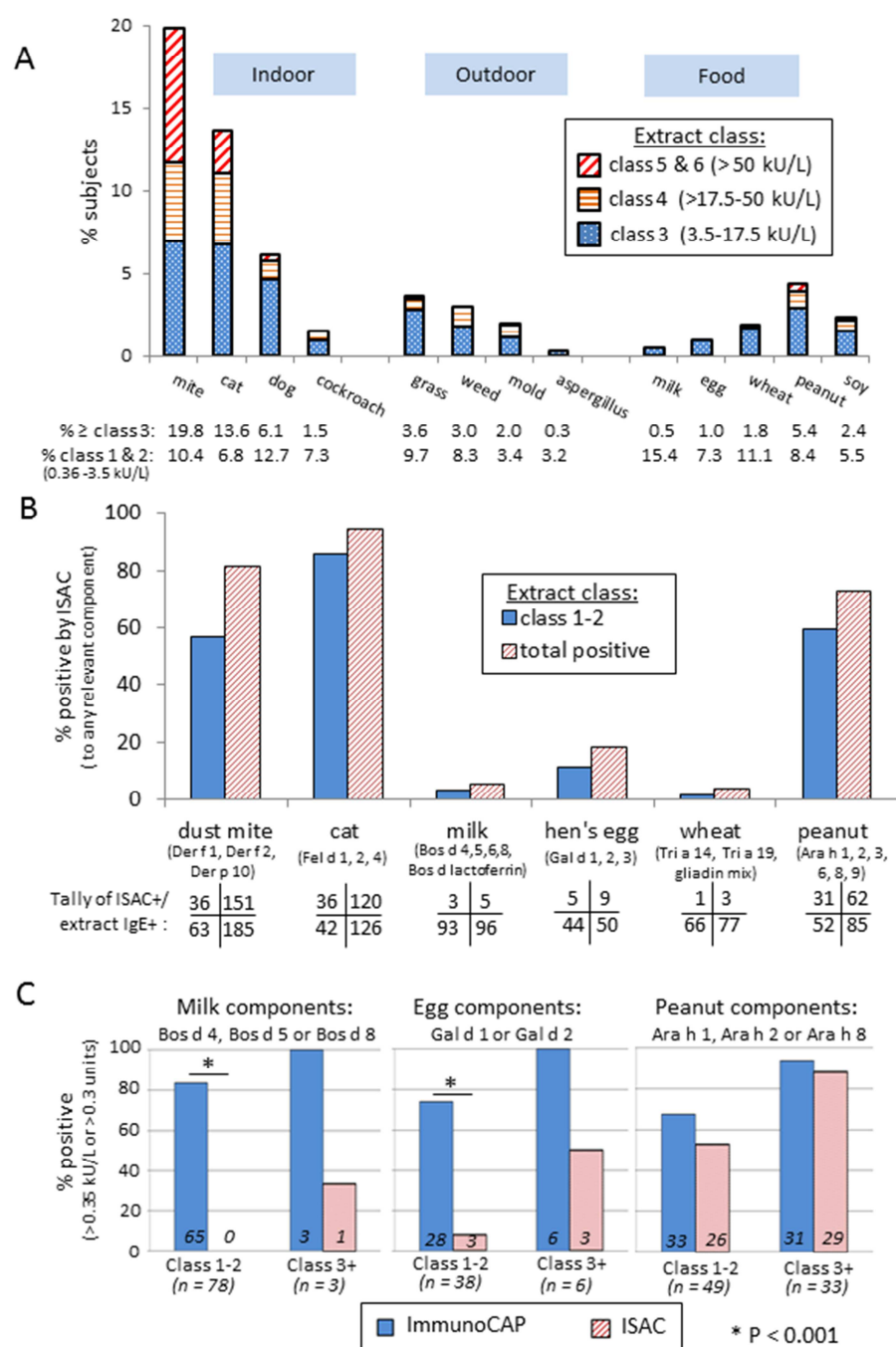
n = 284 individuals out of the 300 whose sera was tested by ISAC and responded to the question;

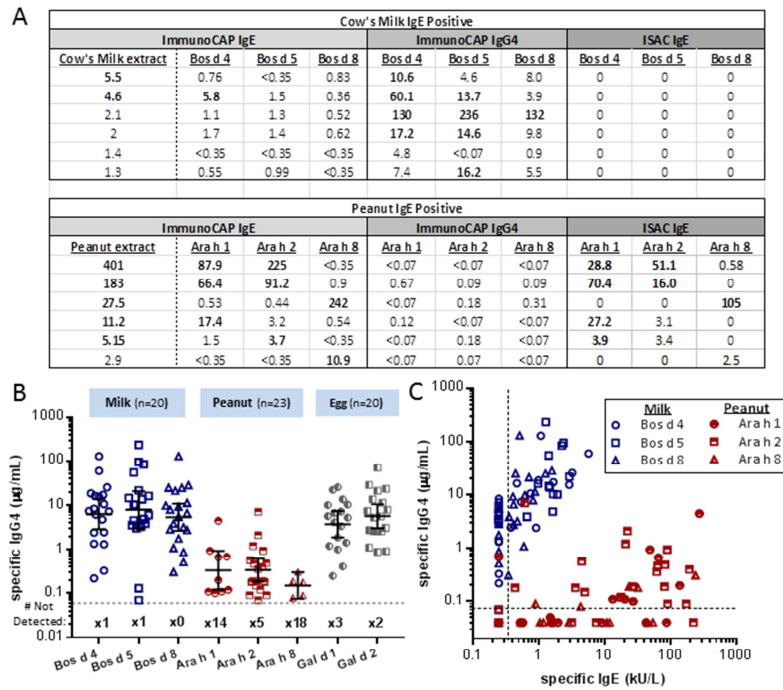
25 of the 27 positive responses (peanut allergic) were in the n= 284 (total)

		class 0	class 1 - 2	class 3 - 4	class 5 - 6
Dust mite (d2)	sample assayed by CAP, n = 616	430	64	72	50
<i>Der f 1, Der f 2, Der p 10</i>	samples also run for ISAC, n = 300	115	63	72	50
	# of samples positive for at least one ISAC component	6	36	66	49
	% positive in ISAC compared to CAP	5.2	57.1	91.7	98.0
Cat dander (e1)	samples assayed by CAP, n = 616	490	42	68	16
<i>Fel d 1, Fel d 2, Fel d 4</i>	samples also run for ISAC, n = 300	174	42	68	16
	# of samples positive for at least one ISAC component	15	36	68	16
	% positive in ISAC compared to CAP	8.6	85.7	100.0	100.0
Dog dander (e5)	samples assayed by CAP, n = 606	492	77	35	2
<i>Can f 1, Can f 2, Can f 5</i>	samples also run for ISAC, n = 296	182	77	35	2
	# of samples positive for at least one ISAC component	1	24	25	2
	% positive in ISAC compared to CAP	0.5	31.2	71.4	100.0
Cockroach (i6)	samples assayed by CAP, n = 606	553	44	9	0
<i>Bla g 1, Bla g 2, Bla g 5, Bla g 7</i>	samples also run for ISAC, n = 296	243	44	9	0
	# of samples positive for at least one ISAC component	3	6	8	0
	% positive in ISAC compared to CAP	1.2	13.6	88.9	
Ragweed (w1)	samples assayed by CAP, n = 606	538	50	18	0
<i>Amb a 1</i>	samples also run for ISAC, n = 296	228	50	18	0
	# of samples positive for at least one ISAC component	2	10	11	0
	% positive in ISAC compared to CAP	0.9	20.0	61.1	
Rye grass (g5)	samples assayed by CAP, n = 606	525	59	21	1
<i>Cyn d 1, Phl p 1, Phl p 2, Phl p 4, Phl p 5, Phl p 6, Phl p 11</i>	samples also run for ISAC, n = 296	215	59	21	1
	# of samples positive for at least one ISAC component	9	41	20	1
	% positive in ISAC compared to CAP	4.2	69.5	95.2	100.0
Alternaria (m6)	samples assayed by CAP, n = 597	565	20	11	1
<i>Alt a 1, Alt a 6</i>	samples also run for ISAC, n = 292	260	20	11	1
	# of samples positive for at least one ISAC component	1	4	11	1
	% positive in ISAC compared to CAP	0.4	20.0	100.0	100.0
Aspergillus (m3)	samples assayed by CAP, n = 597	576	19	2	0
<i>Asp f 1, Asp f 3, Asp f 6</i>	samples also run for ISAC, n = 292	271	19	2	0
	# of samples positive for at least one ISAC component	0	1	0	0
	% positive in ISAC compared to CAP	0.0	5.3	0.0	
Milk (f2)	samples assayed by CAP, n = 602	506	93	3	0
<i>Bos d 4, Bos d 5, Bos d 6</i>	samples also run for ISAC, n = 291	195	93	3	0
<i>Bos d 8, Bos d lactoferrin</i>	# of samples positive for at least one ISAC component	0	3	2	0
	% positive in ISAC compared to CAP	0.0	3.2	66.7	
Wheat (f4)	samples assayed by CAP, n = 595	518	66	11	0
<i>Tri a 14, Tri a 19, Tri a aA/TI</i>	samples also run for ISAC, n = 290	213	66	11	0
	# of samples positive for at least one ISAC component	0	1	2	0
	% positive in ISAC compared to CAP	0.0	1.5	18.2	
Hen egg white (f1)	samples assayed by CAP, n = 606	556	44	6	0
<i>Gal d 1, Gal d 2, Gal d 3</i>	samples also run for ISAC, n = 292	242	44	6	0
	# of samples positive for at least one ISAC component	7	5	4	0
	% positive in ISAC compared to CAP	2.9	11.4	66.7	
Soybean (f14)	samples assayed by CAP, n = 595	548	33	13	1
<i>Gly m 4, Gly m 5, Gly m 6</i>	samples also run for ISAC, n = 291	244	33	13	1
	# of samples positive for at least one ISAC component	20	18	12	1
	% positive in ISAC compared to CAP	8.2	54.5	92.3	100.0
Peanut (f13)	samples assayed by CAP, n = 616	531	52	24	9
<i>Ara h 1, Ara h 2, Ara h 3</i>	samples also run for ISAC, n = 300	215	52	24	9
<i>Ara h 6, Ara h 8, Ara h 9</i>	# of samples positive for at least one ISAC component	6	31	22	9
	% positive in ISAC compared to CAP	2.8	59.6	91.7	100.0









Supplemental Material

Table E1. IgE sensitization to 8 aeroallergens and 5 foods by ImmunoCAP extract, stratified by sensitization class. In cases with insufficient sera not all allergens were tested.

Table E2: Analysis of relationship between history of peanut reactions and serum testing for peanut extract and specific components to Ara h 1, Ara h 2 and Ara h 8 with microchip (from n = 300). * Reactions were assessed based on the answer to the question 'has your child ever had an allergic reaction to peanut?'

Table E3. Comparison of IgE to common allergens using extract and ISAC. Classes shown are for extract where class 0 = < 0.36 kU/L, class 1-2 = 0.36-3.5 kU/L, class 3-4 = 3.51-50 kU/L and class 5-6 = > 50 kU/L.

Fig E1. Overview of study design. The number of individuals or sera that were tested are depicted. Sera that were tested are represented in bold.

Fig E2. Component-specific IgG4 to IgE ratio in sera that were positive to the component-relevant food by extract testing. In cases where IgG4 or IgE was not detected a ratio could not be calculated. IgE was normalized with the formula $1 \text{ kU/L} = 2.4 \text{ ng/mL}$.