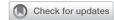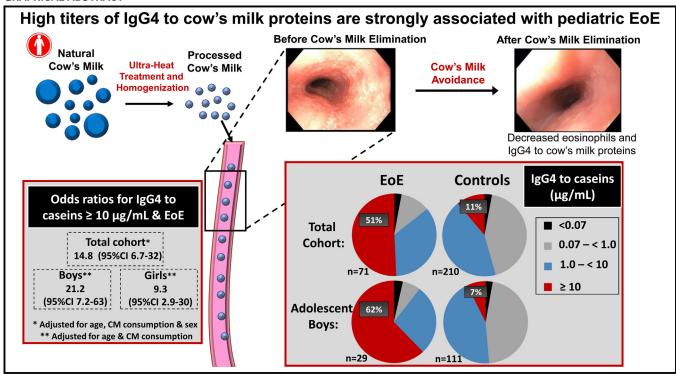# Specific IgG$_4$ antibodies to cow's milk proteins in pediatric patients with eosinophilic esophagitis

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GRAPHICAL ABSTRACT

High titers of IgG4 to cow's milk proteins are strongly associated with pediatric EoE

Background: Allergen-specific IgG_4 ($sIgG_4$) antibodies are often associated with tolerance, but $sIgG_4$ antibodies to causally relevant foods have been reported recently in adults with eosinophilic esophagitis (EoE). Prevalence and levels of food $sIgG_4$ are not well established in the general pediatric population.

Objective: We sought to investigate serum food $sIgG_4$ with component diagnostics in children with EoE and children from an unselected birth cohort and to explore the effects of sex, age, and milk consumption on $sIgG_4$ levels.

Methods: Sera from 71 pediatric patients with EoE and 210 early adolescent children from an unselected birth cohort (Project Viva) were assayed for $sIgG_4$ and specific IgE (sIgE) to major cow's milk (CM) proteins (α-lactalbumin, β-lactoglobulin, and caseins) and to wheat, soy, egg, and peanut proteins.

Results: In the EoE cohort high-titer $sIgG_4$ (≥10 μg/mL) to CM proteins was more common than in control sera and achieved odds ratios for EoE ranging from 5.5 to 8.4. sIgE levels to CM proteins were mostly 4 IU/mL or less in patients with EoE, such that $sIgG_4$/sIgE ratios were often 10,000 or greater. When adjusted for age and milk consumption, high-titer $sIgG_4$ to CM proteins was strongly associated with EoE, with an odds ratio of greater than 20 to all 3 CM proteins in boys.

Conclusions: $sIgG_4$ to CM proteins are common and high titer in children with EoE. Although it is not clear that this response is pathogenic, $sIgG_4$ levels imply that these antibodies are an important feature of the local immune response that gives rise to EoE. (J Allergy Clin Immunol 2018;142:139-48.)

Key words: Eosinophilic esophagitis, children, IgG_4 assays, cow's milk proteins, molecular allergens

Eosinophilic esophagitis (EoE) is a chronic disease characterized by eosinophil-rich esophageal inflammation.[1] The prevalence, estimated at 0.05% to 0.1% in the United States, appears to be increasing, and male subjects of all ages are disproportionately affected.[2] Patients with EoE, particularly children, can present with a spectrum of symptoms.[3-6] The histologic and clinical responses to elemental and elimination diets provide strong evidence that food antigens are important contributors to EoE.[7-9] Cow's milk (CM) has consistently been shown to be a dominant factor, with wheat, egg, soy, and peanut also often causally related.[10,11] However, the nature of the immune response to food and the role of this immune response in disease pathogenesis remains incompletely understood.

There are multiple lines of evidence arguing against a pathogenic role for food-specific IgE (sIgE) antibodies in patients with EoE, including (1) the lack of immediate symptoms on food ingestion, (2) the poor response to anti-IgE therapy observed in clinical trials, and (3) the low predictive value of skin prick tests or the Immuno Solid-Phase Allergen Chip (ISAC) microarray for identifying causative foods.[3-6,11-15] However, a role for IgE cannot be entirely excluded. Our group has demonstrated that food sIgE is common in patients with EoE[12] and that sIgE assays that use high-capacity ImmunoCAPs demonstrate positive results to CM proteins (α-lactalbumin [Bos d 4], β-lactoglobulin [Bos d 5], and caseins [Bos d 8]) in the majority of patients with EoE.[16] Moreover, we have reported that low-titer sIgE to CM (0.10-1.0 IU/mL) is associated with histologic remission in pediatric patients with EoE on a CM elimination diet.[15]

Another antibody isotype that has drawn attention in patients with EoE is IgG_4. Although IgG_4 has structural features that promote anti-inflammatory activity and is often considered a mediator of allergen tolerance,[17,18] 2 groups have recently reported the presence of specific IgG_4 ($sIgG_4$) antibodies to food extracts, including CM and wheat, in sera and esophageal tissue biopsy specimens of adults with EoE.[13,19] The clinical significance of those results is not clear, in part because the prevalence of $sIgG_4$ to food proteins in the general population is not well established and because there has not been a parallel analysis with sIgE. Moreover, the $sIgG_4$ response to foods has not been reported in children with EoE, nor have results been stratified to assess for a difference between male and female subjects.

To address these shortcomings, we investigated serum IgE and IgG_4 responses to CM, wheat, egg, soy, and peanut by using molecular allergen-based assays in pediatric patients with EoE. We compared the results with those of similarly aged children from a Boston-based birth cohort (Project Viva) unselected for any atopic risk or disease, resulting in a natural population including allergic and nonallergic children.[20] We also extended the analysis to explore the effects of sex, age, and milk consumption on $sIgG_4$ levels.

METHODS
Subjects

Sera were collected after achieving informed consent at Nationwide Children's Hospital (Columbus, Ohio [n = 35]) or the University of Virginia (Charlottesville, Va [n = 36]) from 71 children with newly established or active EoE in whom diagnosis had been confirmed by esophageal biopsies demonstrating 15 or more eosinophils per high-power field (eos/hpf). The control subjects included 210 children randomly selected from a Boston-based birth cohort (Project Viva) study, which included blood collection from 647 children in the early teenage years. Inclusion criteria and enrollment details for the control subjects and groups for secondary analysis are located in the Methods section in this article's Online Repository at www.jacionline.org.

Immunoassays

Using the ImmunoCAP 250 instrument (Thermo Fisher Scientific, Uppsala, Sweden), sera were assayed for sIgE to CM, wheat, egg white, soy, and peanut extracts; total IgE; $sIgG_4$ to gluten, gliadins, nBos d 4, nBos d 5, nBos d 6, nBos d 8, bovine lactoferrin (Bos d LF), galactose-α-1,3-galactose (α-gal), rTri a 14, rTri a 19, nGal d 1, nGal d 2, nGal d 4, nGly m 4, nGly m 5, nGly m 6, rAra h 1, rAra h 2, rAra h 3, rAra h 8, and rAra h 9; and total IgG_4 (see Table E1 in this article's Online Repository). Only sera that were positive for sIgE to whole CM extract were assayed for sIgE to individual CM proteins. Assays for lactoferrin and α-gal were accomplished by coupling biotinylated lactoferrin purified from CM (Sigma-Aldrich, St Louis, Mo) or α-gal linked to human serum albumin (V-Labs, Covington, La) to a streptavidin ImmunoCAP.[21]

TABLE I. Summary of demographics and characteristics in patients with EoE and control subjects

Demographics/characteristics	Patients with EoE (n = 71)*	Control subjects (n = 210)	P value
Age (y), median (range)	11.0 (2.0-18.0)	12.9 (12.0-16.1)	.002‡
Female sex, no. (%)	23 (32.4)	99 (47.1)	.03†
BMI (kg/m^2), mean (SD)	20.3 (5.6)	21.0 (4.6)	.30‡
Race/ethnicity			
Asian	0	7	.20†
Black	3	33	.01†
Hispanic	1	9	.46†
White	63	130	<.001†
Other	4	31	.06†
Peak eosinophil count/hpf, median (IQR)	40 (25-60)	NA	NA
Food impaction Sx, no. (%)	12 (16.9)	NA	NA
Dysphagia Sx, no. (%)	37 (52.1)	NA	NA
Vomiting Sx, no. (%)	22 (31.0)	NA	NA
Abdominal pain Sx, no. (%)	26 (36.6)	NA	NA
Reflux Sx, no. (%)	20 (28.2)	NA	NA
Asthma Hx, no. (%)	22 (31.0)	55 (26.2)	.43†
Rhinitis Hx, no. (%)	41 (57.7)	41 (20.5)	<.001†
Eczema Hx, no. (%)	25 (35.2)	41 (20.2)	.02†
Food allergy Hx, no. (%)	20 (28.2)	25 (11.9)	.001†
Family Hx of EoE/reflux, no. (%)	14 (19.7)	NA	NA
Consumes CM or dairy, no. (%)	53 (74.6)	205 (98.1)‖	<.001†
Hx of acute reactions to CM, no. (%)	6 (8.5)	3 (0.4)	.01†
Total IgE (IU/mL), GM (95% CI)	122 (80.4-157)	68.0 (56.3-82.2)	.01§
CM sIgE, detectable, no. (%)¶	54 (76)	76 (36)	<.001†
CM sIgE (IU/mL), GM (95% CI)	1.17 (0.74-1.85)	0.28 (0.24-0.33)	<.001§

BMI, Body mass index; *GM*, geometric mean; *Hx*, history; *IQR*, interquartile range; *Sx*, symptoms.

*Sixty-five of 71 patients with EoE had been treated with a proton pump inhibitor.

†, ‡, and §P values were calculated by using the χ^2 or Fisher exact test, the Student *t* test, or the Mann-Whitney test, respectively.

‖Of the 210 subjects, 209 answered the questionnaire regarding dairy consumption; 1 or more servings per week of CM or dairy was considered positive.

¶Defined as 0.1 IU/mL or greater.

IgG$_4$ results were expressed in micrograms per milliliter (positive = ≥0.07 μg/mL). IgE results were expressed in international units per milliliter (positive = ≥0.10 IU/mL). For quantitative comparison of sIgE and sIgG$_4$ results, 1 IU/mL IgE was converted to 2.42 ng/mL.[22] Details on total IgG$_4$ measurements and other specific IgG antibody assays are provided in the Methods section in this article's Online Repository.

Statistics

Demographic/clinical data and antibody titers were compared between patients with EoE and control subject by using the Mann-Whitney, Student *t*, or χ^2 tests (or the Fisher exact test, where appropriate). Differences in antibody titers were assessed across multiple groups by using the Kruskal-Wallis test. Changes in antibody titers before and after elimination diets were evaluated with the Wilcoxon signed-rank test. Associations between CM sIgE and sIgG$_4$ levels and EoE status were assessed by using logistic regression analyses. Models were adjusted for age, sex, and milk consumption. Milk consumption was assessed by means of questionnaire or clinical history and dichotomized as yes or no. Statistics were performed and visualized with STATA SE/11 software (StataCorp, College Station, Tex) and GraphPad Prism software (version 7; GraphPad Software, La Jolla, Calif).

RESULTS

Subjects' demographics and clinical history

Children with EoE and control children were similar with respect to age, body mass index, and asthma history, although the EoE group included children with a wider range of ages (Table I). In keeping with the male dominance of this disease, boys were a larger percentage of the EoE cohort. Both groups were predominately white; however, the proportion of nonwhite subjects was

significantly greater among the control subjects compared with patients with EoE. Total IgE levels and reported histories of rhinitis, eczema, and food allergy were greater among children with EoE than control children.

All of the patients with EoE studied here had active EoE; that is, an esophageal biopsy specimen had shown 15 or more eosinophils/hpf from 1 or more esophageal biopsy specimens at the time of enrollment, with a median peak eosinophil count of 40 eosinophils/hpf (interquartile range [IQR], 25-60 eosinophils/hpf). All but 6 patients with EoE had been treated with a proton pump inhibitor.[23]

The most commonly reported gastrointestinal symptom among the patients with EoE was dysphagia, followed by abdominal pain, vomiting, reflux, and food impaction. A family history of EoE or reflux was common (20%) among patients with EoE. Approximately three quarters of the patients with EoE (n = 53/71) reported CM consumption compared with 98% in the control population. Of the 18 patients with EoE avoiding CM, 2 were exclusively avoiding CM, whereas 16 were avoiding CM and at least 1 other food. The questionnaire used to assess clinical history for the control children did not include questions relating to gastrointestinal symptoms, and esophagoscopy was not performed on the control subjects.

Screening for sIgG$_4$ to molecular food allergens in patients with EoE

To begin to define the nature of the IgG$_4$ response to food allergens in patients with EoE, we investigated sera from a subset of
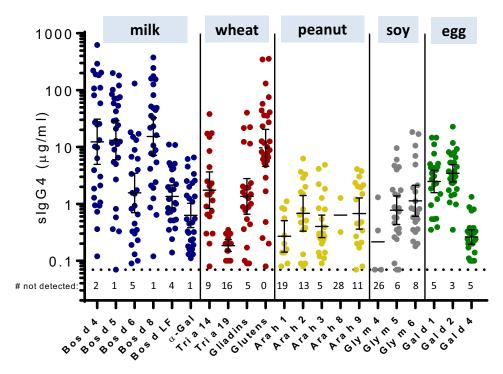
FIG 1. sIgG$_4$ levels (geometric mean [95% CI]) to 5 CM, 2 wheat, 5 peanut, 3 soy, and 3 egg proteins, as well as to α-gal (CM-related), gliadins (wheat), and gluten (wheat), in 30 pediatric patients with EoE. *Numbers below the dotted line* indicate negative values (<0.07 μg/mL).

patients with EoE (n = 30/71). The sera were assayed for sIgG$_4$ to 21 food allergens from CM, wheat, peanut, soy, and egg (see Table E1). The highest titers of sIgG$_4$ were seen to Bos d 4, Bos d 5, Bos d 8, and gluten, although sIgG$_4$ to other food proteins were also common (Fig 1). Bos d 4 and Bos d 5 are purified proteins, whereas both Bos d 8 and gluten represent mixtures of closely related proteins. Because of the pronounced magnitude of the sIgG$_4$ to CM proteins and the fact that CM is often reported to be the most common causal food in patients with EoE,[7,8] subsequent assays focused on the response to CM. The specificity of the IgG$_4$ assays for Bos d 4, Bos d 5, and Bos d 8 was supported by the results of absorption studies using purified proteins conjugated to Sepharose beads (see Fig E1, *A*, in this article's Online Repository). Quantitative accuracy was validated by means of comparison to RIAs using 3 different anti-IgG$_4$ mAbs (see Fig E1, *B*). Further studies using Sepharose conjugated to protein G or mAbs (to bind serum IgG or IgG$_4$, respectively) demonstrated that the IgG response to CM proteins in patients with EoE is predominantly IgG$_4$ (see Fig E2 and Table E2 in this article's Online Repository).

Characterizing sIgG$_4$ to CM proteins in children with EoE and control children

We next extended the investigation of CM proteins to include all 71 patients with EoE and the 210 control subjects. Although antibodies to at least 1 CM protein were detectable in almost all of the subjects in both groups (≥97%), levels of sIgG$_4$ to Bos d 4, Bos d 5, and Bos d 8 were significantly greater in patients with EoE than in control subjects (each $P < .001$; Table II).

We also compared sIgG$_4$ levels to CM proteins in the 2 groups as a function of whether subjects produced sIgE to CM (Table I).

sIgG$_4$ levels to Bos d 4, Bos d 5, and Bos d 8 were significantly greater in CM-sensitized children with EoE than in control children, regardless of whether the control children were sensitized (Fig 2). For children with EoE who were not sensitized to CM, this response only achieved significance when compared with Bos d 5 and Bos d 8 in nonsensitized control subjects. As an additional control, sIgE and sIgG$_4$ levels to CM proteins were compared with those in a cohort of children (n = 10) with a history of IgE-mediated anaphylaxis to CM (and who were not consuming dairy). The magnitude of the sIgG$_4$ responses were much greater in children with EoE than children with CM-induced anaphylaxis (see Table E3 in this article's Online Repository).

Because sIgG$_4$ titers to CM proteins in patients with EoE are very high, a direct comparison of the quantities of sIgG$_4$ with total IgG$_4$ was possible. There was a nonsignificant trend toward higher total IgG$_4$ levels among patients with EoE compared with control subjects (see Fig E3, *A*, in this article's Online Repository). Direct measurement of sIgG$_4$ to CM is limited by technical issues relating to background binding; however, a surrogate measurement for sIgG$_4$ to CM was obtained by summing the titers of sIgG$_4$ to Bos d 4, Bos d 5, and Bos d 8.[24] Expressing sIgG$_4$ to these 3 CM proteins as a percentage of total IgG$_4$ demonstrated that these antibodies represent a higher proportion of serum IgG$_4$ in patients with EoE compared with control subjects ($P < .001$; see Fig E3, *B*).

Characterization of CM sIgE titers, IgG$_4$/IgE ratios, and associations with EoE

Recently, our group has reported that IgE antibodies to food, specifically low- to moderate-titer sIgE to CM, is associated with

TABLE II. Analysis of sIgG$_4$ titers to CM proteins in children with EoE and control children

	Prevalence of detectable sIgG$_4$, no. (%)*			sIgG$_4$ titer (µg/mL), GM (95% CI)‡		
CM protein	Patients with EoE (n = 71)	Control subjects (n = 210)	P value†	Patients with EoE	Control subjects	P value§
Bos d 4	66 (93)	146 (70)	<.001	10.8 (6.57-17.7)	1.46 (1.08-1.9)	<.001
Bos d 5	69 (97)	184 (88)	.05	11.4 (7.68-17.0)	2.59 (2.03-3.30)	<.001
Bos d 6	61 (86)	131 (62)	<.001	1.28 (0.82-1.99)	0.51 (0.41-0.65)	<.001
Bos d 8	69 (97)	204 (97)	.70	10.7 (7.07-16.3)	1.43 (1.15-1.77)	<.001
Any	69 (97)	208 (99)	.58	—	—	—

GM, Geometric mean.
*Detectable sIgG$_4$ was defined as values of 0.07 µg/mL or greater.
†*P* values were calculated by using the Fisher exact test.
‡GM (95% CI) was calculated by using positive values (values of sIgG$_4$ ≥0.07 µg/mL or sIgE ≥0.1 IU/mL).
§*P* values were calculated by using the Mann-Whitney test.
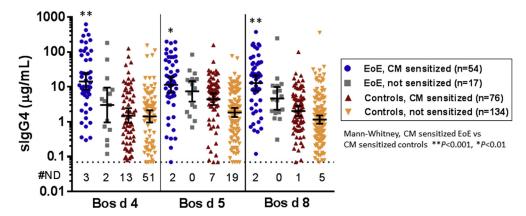

FIG 2. sIgG$_4$ levels (geometric mean [95% CI]) to Bos d 4, Bos d 5, and Bos d 8 in 71 patients with EoE and 210 control subjects with or without CM sensitization. *Values below the dotted line* indicate the number not detectable *(#ND)* and were excluded from calculation of the geometric mean. Statistical analysis was performed with the Mann-Whitney test.

EoE among children scheduled for esophagoscopy.[12,16,25-27] Here we found that food sIgE levels were detected commonly in sera from the EoE cohort (76%), but interestingly, 36% of control children also had detectable sIgE to milk (Table I). Although mostly of low titer (0.10-2.0 IU/mL), values were greater in children with EoE than control children. We additionally performed sIgE assays for the milk-specific components Bos d 4, Bos d 5, and Bos d 8 in sera from the 71 patients with EoE and 210 control subjects. Consistent with the results using CM extract, the frequency of detectable sIgE and levels of sIgE to all 3 milk components were greater in children with EoE than control children (Table III).

Using the generally accepted value of 1 IU = 2.42 ng of IgE, we converted units of sIgE to micrograms per milliliter[22] and calculated sIgG$_4$/sIgE ratios for Bos d 4, Bos d 5, and Bos d 8 in subjects with detectable levels of both sIgG$_4$ and sIgE. Not unexpectedly, the prevalence of coexisting sIgG$_4$ and sIgE responses to Bos d 4, Bos d 5, and Bos d 8 was significantly greater among patients with EoE (each *P* < .001; Table IV). Absolute values for sIgG$_4$ to CM proteins were commonly 10,000-fold greater than the IgE response to the same protein in sera from both children with EoE and control children.

Next, we determined the strength of the association for sIgG$_4$ to CM proteins with EoE. There were positive odds ratios (ORs) for all 3 CM proteins with moderate- to high-titer (≥1.0 µg/mL) and

high-titer (≥10 µg/mL) sIgG$_4$ (Table V). The strongest association was for high-titer sIgG$_4$ to Bos d 8 (OR, 8.4; 95%,CI 4.3-15), but notably, these values were not significantly different than the ORs based on the presence of sIgE to CM proteins.

CM sIgG$_4$ levels adjusted for age, sex, and dairy consumption

Compared with control children, the EoE cohort had greater heterogeneity in age and diet and a higher percentage of boys (Table I). Thus we assessed sIgG$_4$ responses to CM in subgroups of the EoE and control populations. In addition, we carried out regression analysis accounting for differences in age, sex, and CM consumption. First, we focused on children with EoE 10 to 18 years of age (median, 14.0 years) because these were more closely matched with children from the Project Viva cohort (median, 12.9 years; Fig 3). Of the 29 boys and 15 girls in this EoE subgroup, most were consuming dairy products (86% and 80%, respectively). There was no clear difference in levels of sIgG$_4$ to CM proteins in those that consumed or avoided dairy, although this analysis was limited by the small number of children avoiding dairy in this age group. Titers of sIgG$_4$ to Bos d 4, Bos d 5, and Bos d 8 were greater in boys with EoE than control boys and also in girls with EoE than control girls (Fig 3). Interestingly, there was a trend toward higher sIgG$_4$ levels to CM proteins in boys with

TABLE III. Prevalence and levels of sIgE to CM proteins in patients with EoE and control subjects

	Prevalence of sensitization, no. (%)*			sIgE titer (IU/mL), GM (95% CI)‡		
	Patients with EoE (n = 71)	Control subjects (n = 210)	P value†	Patients with EoE (n = 71)	Control subjects (n = 210)	P value§
Bos d 4	39 (55)	26 (12)	<.001	0.87 (0.54-1.40)	0.25 (0.19-0.32)	<.001
Bos d 5	42 (59)	38 (18)	<.001	0.73 (0.46-1.14)	0.29 (0.24-0.36)	<.01
Bos d 8	38 (54)	32 (15)	<.001	0.60 (0.34-1.07)	0.20 (0.17-0.24)	<.01

GM, Geometric mean.
*Detectable sIgE was defined as values of 0.1 IU/mL or greater.
†*P* values were calculated by using the Fisher exact test.
‡GM (95% CI) was calculated by using positive values.
§*P* values were calculated by using the Mann-Whitney test.

TABLE IV. Analysis of sIgG$_4$ to sIgE ratios

	Prevalence of detectable sIgG$_4$ and sIgE, no. (%)*			sIgG$_4$/sIgE ratio, GM [95% CI]‡	
CM protein	Patients with EoE (n = 71)	Control subjects (n = 210)	P value†	Patients with EoE	Control subjects
Bos d 4	38 (54)	21 (10)	<.001	18,800 (1,820-46,300)	14,800 (2,910-63,700)
Bos d 5	39 (55)	34 (16)	<.001	23,200 (4,340-62,000)	12,900 (6,690-29,300)
Bos d 8	37 (52)	32 (15)	<.001	25,000 (5,170-72,300)	12,791 (4,380-31,600)

GM, Geometric mean.
*Detectable sIgG$_4$ was defined as values of 0.07 μg/mL or greater and detectable sIgE as values of 0.1 IU/mL or greater.
†*P* values were calculated by using the Fisher exact test.
‡sIgG4/sIgE ratios were only calculated for sera with detectable sIgG4 and sIgE to the relevant CM protein. These values were calculated by normalizing units of sIgE (in international units per milliliter) to units of sIgG4 (micrograms per milliliter) with the following conversion factor: 1 IU = 2.42 × 10^{-3} μg.

TABLE V. Analysis of the relationship between EoE status and sIgE and sIgG$_4$ titers to CM proteins

		Prevalence of sIgE or sIgG$_4$, no. (%)			
		Patients with EoE (n = 71)	Control subjects (n = 210)	P value*	OR (95% CI)
CM protein	Assay result				
Bos d 4	sIgE ≥0.10 IU/mL	39 (55)	26 (12)	<.001	8.6 (4.6-16)
	sIgG$_4$ ≥0.07 μg/mL	65 (92)	146 (70)	<.001	4.7 (2.0-12)
	sIgG$_4$ ≥1.00 μg/mL	55 (77)	87 (41)	<.001	4.9 (2.6-9.0)
	sIgG$_4$ ≥10.0 μg/mL	31 (44)	21 (10)	<.001	7.0 (3.6-13)
Bos d 5	sIgE ≥0.10 IU/mL	42 (59)	38 (18)	<.001	6.6 (3.6-12)
	sIgG$_4$ ≥0.07 μg/mL	68 (96)	184 (88)	.07	3.2 (0.94-11)
	sIgG$_4$ ≥1.00 μg/mL	62 (87)	135 (64)	<.001	3.8 (1.8-8.1)
	sIgG$_4$ ≥10.0 μg/mL	40 (56)	40 (19)	<.001	5.5 (3.1-9.8)
Bos d 8	sIgE ≥0.10 IU/mL	38 (54)	32 (15)	<.001	6.4 (3.4-12)
	sIgG$_4$ ≥0.07 μg/mL	68 (96)	204 (97)	.70	0.7 (0.2-2.7)
	sIgG$_4$ ≥1.00 μg/mL	61 (86)	114 (54)	<.001	5.1 (2.5-11)
	sIgG$_4$ ≥10.0 μg/mL	36 (51)	23 (11)	<.001	8.4 (4.3-15)

P values were calculated by using the χ^2 or Fisher exact test.

EoE compared with girls with EoE, but the difference was only significant for Bos d 5 (*P* = .009). Conversely, in the control population there was a trend in the opposite direction between boys and girls. In keeping with this, logistic regression accounting for age and dairy consumption suggested a stronger association between high-titer sIgG$_4$ and EoE among boys than girls. For example, the adjusted ORs were greater than 20 for all 3 CM proteins in boys (Table VI). In contrast, the ORs for girls were less than 10 to all 3 CM proteins, and in the case of Bos d 5, the OR was not statistically significant. However, in the combined model sex did not achieve significance as an interaction term.

The group of younger children with EoE (2-9 years old) consisted of 20 boys and 7 girls. Of these, 37% were avoiding all dairy, and sIgG$_4$ titers to Bos d 5 and Bos d 8 were significantly lower in those avoiding dairy (and trended lower for Bos d 4; see Fig E4 in this article's Online Repository). Notably, however, sIgG$_4$ titers to Bos d 5 and Bos d 8 were detectable in all 10 subjects avoiding dairy. Although the control population in the current study is not age matched to young children, this is a greater prevalence than a recent report that investigated sIgG$_4$ responses in a birth cohort at 2 years of age.[28] Total IgG$_4$ levels were not influenced significantly by sex, age, or CM consumption.

Because our initial experiments revealed that gluten sIgG$_4$ levels are often high in children with EoE, we also evaluated the strength of this association (see Fig E5 in this article's Online Repository). The OR for boys with EoE (adjusted for age and
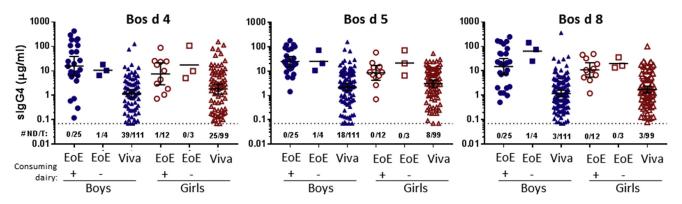
FIG 3. sIgG$_4$ levels (geometric mean [95% CI]) to Bos d 4, Bos d 5, and Bos d 8 in 10- to 18-year-old patients with EoE stratified by sex and dairy consumption compared with sex-matched control children. *Numbers below the dotted line* indicate the number not detectable out of the total in the group *(#ND/T).*

TABLE VI. Relationship between EoE status and high-titer sIgG$_4$ ($\geq$10 μg/mL) to CM proteins

CM protein	Group	Unadjusted*		Adjusted†	
		P value	OR (95% CI)	*P* value	OR (95% CI)
Bos d 4	All	<.001	7.0 (3.6-13)	<.001	11.7 (5.3-26)
	Boys	<.001	12.5 (4.6-34)	<.001	23.9 (7.6-76)
	Girls	.001	5.1 (1.9-13.8)	.004	5.6 (1.8-18)
Bos d 5	All	<.001	5.5 (3.1-10)	<.001	9.2 (4.5-19)
	Boys	<.001	8.3 (3.8-18)	<.001	23.7 (8.3-67)
	Girls	.01	3.4 (3.1-9.8)	.07	2.7 (0.92-8)
Bos d 8	All	<.001	8.4 (4.4-16)	<.001	14.8 (6.7-32)
	Boys	<.001	10.0 (4.0-25)	<.001	21.2 (7.2-63)
	Girls	<.001	10.5 (3.8-29)	<.001	9.3 (2.9-30)

*Unadjusted results of logistic regression.
†Adjusted for age, milk consumption, and sex; when stratified by sex, adjustment was for age and milk consumption.

dairy consumption) was lower for gluten (OR, 7.4; 95% CI, 3.0-18) than for CM proteins, and the association was not significant in girls (OR, 2.7; 95% CI, 0.8-9). We also sought to assess whether the association of sIgE responses to CM proteins was modified when the cohort was stratified by sex and adjusted for age and dairy consumption. Interestingly, and in contrast to the results for sIgG$_4$, the association of sIgE responses to CM proteins (as well as extract) and EoE was not strengthened when accounting for age and dairy consumption, and evidence for a sex effect was minimal (see Fig E6 and Table E4 in this article's Online Repository).

Changes in sIgG$_4$ levels to CM proteins during a CM elimination diet

Finally, we monitored sIgG$_4$ levels to CM proteins to evaluate changes in titers when CM was removed from the diets of 13 children with EoE.[15] sIgG$_4$ levels to Bos d 4, Bos d 5, and Bos d 8 decreased by an average of approximately 40% to 60% after 6 to 8 weeks of the CM-free diet (Fig 4 and see Fig E7 in this article's Online Repository). Levels decreased in all subjects, including both those who experienced remission during the diet (n = 8) and those who did not experience remission (n = 5). Despite the decrease associated with the avoidance diet, mean

levels were still greater in children with EoE than in control children.

DISCUSSION

Although sIgE levels to proteins from CM and other foods are common in patients with EoE, the titers are generally low, and the existing evidence does not support a causal role for IgE.[12,16,26,27] The data presented here demonstrate that high titers of sIgG$_4$ to CM proteins are much more common in children with EoE than in early adolescent children from a birth cohort. To our knowledge, sIgG$_4$ levels to food allergens in patients with EoE are some of the highest, if not the highest, levels of sIgG$_4$ to any antigen reported in children. High IgG$_4$ levels relative to IgE is a serologic pattern often observed with allergen tolerance, including the response to allergen immunotherapy, receiving multiple bee stings, or living with a cat.[29-33] Thus EoE challenges the traditional view of IgG$_4$ because the high titers of sIgG$_4$ are clearly not associated with esophageal tolerance to CM.

In nonallergic subjects IgG$_4$ contributes a small proportion of serum IgG; however, the normal range for serum IgG$_4$ levels (0.01-2.00 mg/mL) implies greater variability than that seen with other IgG isotypes.[34] Using assays with different mAbs to specific heavy chain epitopes on IgG$_4$, we provided confirmation
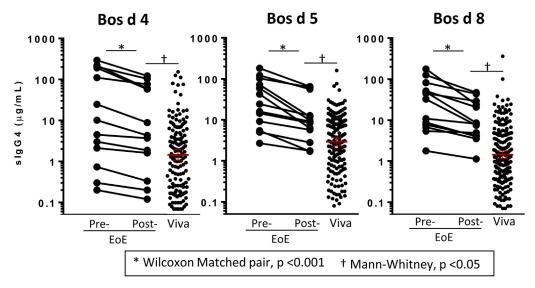
FIG 4. sIgG$_4$ levels to Bos d 4, Bos d 5, and Bos d 8 in patients with EoE before and after a 6- to 8-week CM elimination diet (n = 13) compared with baseline levels in control subjects (n = 210). Statistical analysis was performed with the Wilcoxon matched pair test and the Mann-Whitney test.

of sIgG$_4$ and total IgG$_4$ quantitation.[35] Additionally, we found that sIgG$_4$ to CM proteins can represent a significant proportion of total IgG$_4$, and it was clear that the sIgG$_4$/sIgE ratios for Bos d 4, Bos d 5, and Bos d 8 were often 10,000:1 or greater. In contrast, food allergies with anaphylaxis are associated with high-titer sIgE levels, often in the presence of low levels of sIgG$_4$.[36-38] Biologically, IgG$_4$ has several features in keeping with its role in allergen tolerance, including functional monovalency caused by the Fab arm exchange, inability to activate complement, and low affinity for most Fcγ receptors.[17,39-41] Thus the initial report demonstrating high levels of food sIgG$_4$ in the serum and esophagus of adults with EoE was intriguing.[13] Although subsequent investigations have confirmed those observations,[19,42] the assays for sIgG$_4$ used whole food extracts rather than purified proteins, were only semiquantitative, and were not compared with sIgE levels to the same foods.

Choosing control groups for studies on allergic disease is challenging because allergic sensitization is common in the community. Therefore, choosing only "nonallergic" or "nonsensitized" control subjects might exclude much of the population. Selecting control subjects who underwent esophagoscopy and did not meet the criteria for EoE also has pitfalls.[13,19,42] The control group used here was 210 early adolescent children from a cohort that was enrolled from before birth, and allergic history played no role in the recruitment.[20] The case-control data made it possible to assess the risk of EoE diagnosis associated with given titers of food sIgG$_4$ and sIgE. For example, high-titer (≥10 μg/mL) sIgG$_4$ to Bos d 8 had an OR of 8.4 (95% CI, 4.3-15) for EoE status. Intriguingly, when adjusted for age and CM consumption, there was a trend for a stronger association between IgG$_4$ levels and EoE for boys than girls. This latter finding is particularly interesting in light of the fact that EoE is more common in male subjects of all ages.[2]

The significant ORs between IgG$_4$ responses to CM and EoE suggest that sIgG$_4$ could be associated with the inflammation and fibrosis seen in patients with EoE. This is not to say that the IgG$_4$ itself is causal but rather that it is almost certainly a component of the immune response that drives EoE. A real possibility is that CM sIgG$_4$ is an epiphenomenon in patients with EoE, perhaps related to an aberrant T$_H$2 or regulatory T-cell response.[43,44] Accordingly, the fact that sIgG$_4$ is not sufficient to cause EoE is supported by the fact that 10% of control subjects also had high-titer sIgG$_4$ to CM proteins. This number is far greater than the number of cases of EoE that would be expected in an unselected population based on the national prevalence of the disease.[2] However, a pathogenic role for IgG$_4$ cannot be dismissed entirely. For example, EoE has features in common with IgG$_4$-related disease.[13,42,45,46] Not only are both diseases male dominant and steroid responsive, but biomarkers of IgG$_4$-related disease, including IgG$_4$-producing plasma cells and granular IgG$_4$ deposition, are present in the esophagus in patients with EoE.[13,42] One possible scenario by which IgG$_4$ could be pathogenic would involve formation of extracellular immune complexes, which would be favored in a situation in which sIgG$_4$ antibody levels were largely restricted to a single allergen (group) and high concentrations of allergen were present in the tissue before Fab arm exchange occurred. This is consistent with our observation that sIgG$_4$ levels to CM proteins contributed more than 10% of total IgG$_4$ in 35% of our patients with EoE. Taken together, the question of whether high-titer sIgG$_4$ is an epiphenomenon or is mechanistically involved in the inflammation seen in patients with EoE is an important area for future inquiry.

Regardless of the exact role of IgG$_4$ in patients with EoE, the high titers of this antibody subclass might provide insight into reasons for failed tolerance. B-cell class-switch recombination (CSR) to IgG$_4$ shares elements that are involved in CSR to IgE, such as IL-4 and IL-13. However, IgG$_4$ has also been associated with high levels of IL-10 secreted from regulatory B and T cells.[32,47,48] Patients with EoE have increased levels of TGF-β1 localized to eosinophils and mast cells in the esophagus.[49,50] TGF-β1 can suppress IgE CSR and also promote mast cell accumulation and eosinophil survival. T cells play a central role in

CSR; thus upstream defects in T-cell activation or development could contribute to both the pathology and IgG_4 production in patients with EoE.

High serum levels of $sIgG_4$ to CM proteins in patients with EoE highlight a few practical issues related to *in vitro* diagnostics. One is the question of whether $sIgG_4$ levels to CM could be useful as a biomarker for the diagnosis or monitoring of EoE. Although clearly this would require prospective investigation, the difference in ORs between girls and boys reported here suggests that this question should be addressed with consideration to sex. Additionally, the findings here might shed light on why multiple studies using ISAC have provided results that conflict with results from ImmunoCAP in regard to the relationship between $sIgE$ levels to food and EoE. For example, 2 groups recently showed little evidence for $sIgE$ to CM proteins by using ISAC to study the sera of adults with EoE.[14,26] Previously, we reported this phenomenon in adult and pediatric patients with EoE but found that many of those sera produced positive results with ImmunoCAP.[16] Compared with ISAC, ImmunoCAP accommodates approximately 10^6-fold more allergen.[51] Suppression of ISAC signals has been reported in adults with grass allergy receiving grass pollen subcutaneous immunotherapy.[29] The very high $sIgG_4/sIgE$ ratios for CM proteins seen here provide a simple explanation for the negative results using $sIgE$ microarrays in patients with EoE.[14,15,26,52]

A limitation to our study is that many allergens of other foods have not been characterized and therefore are not available for use in commercial assays. Moreover, our data show IgG_4 responses to proteins from other foods that are included in the 6-food elimination diet. This is particularly true for wheat. We found high levels of $sIgG_4$ to gluten but not to Tri a 19 or other gliadins. Wheat is a complex source material containing potentially many candidate EoE-relevant allergens. In addition, wheat allergens can be cross-reactive with grasses and other foods.[53-55] Although we recognize the presence of high levels of $sIgG_4$ to gluten in patients with EoE, these results are currently difficult to interpret on a molecular level, as we were able to do for CM. Moreover, the association of $sIgG_4$ levels to gluten was not as strong as the association with $sIgG_4$ levels to CM proteins.

Another limitation is that gastrointestinal symptoms were not evaluated in the control subjects because there were no relevant questions included in the questionnaire.[20] Additionally, performing esophagoscopy with biopsy on control children is not feasible in this birth cohort. It is possible that some children included in the control group could have EoE, but we believe this is unlikely given the prevalence of this disease.[2] Based on a national prevalence estimated to approach 100/100,000 subjects, it is unlikely that there would be more than 1 or 2 cases (if any) in the control cohort.

Measuring antibodies to specific proteins has already provided important information about the IgE response to foods. Using the same approach, we report here that titers of $sIgG_4$ to CM proteins are greater in children with EoE than in children from an unselected birth cohort. Interestingly, the results also suggest that the relative difference in the magnitude of the $sIgG_4$ response to CM between children with EoE and control children might be greater in boys than girls. Further investigation into the nature of food $sIgG_4$ antibodies should inform efforts to unravel the immune response that is causal in patients with EoE, as well as the development of noninvasive biomarkers of disease.

Key messages

- IgG_4 antibodies to CM proteins are common in children with EoE and also in children from an unselected birth cohort.
- High-titer IgG_4 antibodies to the major CM proteins (Bos d 4, Bos d 5, and Bos d 8) are strongly associated with EoE.
- EoE is a male-dominated disease, and in this cohort the ORs for high-titer IgG_4 antibodies to CM proteins were very high for EoE in boys.

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METHODS

Inclusion criteria and enrollment details for control subjects and groups for secondary analyses

Between 1999 and 2002, women were recruited into Project Viva in early pregnancy from 8 obstetric offices of Atrius Harvard Vanguard Medical Associates, a multispecialty group practice in eastern Massachusetts.[E1] Exclusion criteria for the mothers included multiple gestation, gestational age of 22 weeks or greater at recruitment, inability to answer questions in English, and plans to move away from the study area before delivery. There were no exclusion criteria related to disease status for the control subjects (children of the mothers). Of 2128 live singleton births, 1038 children attended an in-person visit in their early teenage years (median age, 12.9 years; interquartile range, 12.5-13.4 years), of whom 773 provided blood and 647 had sufficient serum for the IgE assays. We randomly selected 210 of these 647 samples for assay.

Of the patients with EoE from Nationwide Children's Hospital, 13 children were additionally enrolled in a study evaluating the effectiveness of CM elimination diet over 6 to 8 weeks.[E2] Sera were collected before and after the diet as part of the protocol and assayed for sIgG$_4$ to CM proteins for secondary analyses. For comparison, we measured sIgG$_4$ titers to CM proteins in sera from 10 children with CM allergy, which was defined as having an sIgE titer to CM of 0.35 IU/mL or greater along with a recent history of anaphylactic reactions within an hour of consuming CM. These sera were collected with informed consent and banked at the University of Virginia.

IgG$_4$ absorption assays

IgG$_4$ absorption assays were carried out by coupling 10 mg of nBos d 4 and nBos d 5 (Sigma-Aldrich) to 1 g of cyanogen bromide–activated Sepharose 4B (GE Healthcare, Uppsala, Sweden). The suspension was brought up in PBS to make a 50% suspension. For control experiments, mock beads were created by coupling human serum albumin to Sepharose. An equal volume (approximately 0.5 mL) of serum from 3 patients with EoE (in the present study) and each of the bead suspensions in separate tubes were incubated on a vertical rotator overnight at 4°C. Afterward, beads were removed with centrifugation, and sera were assayed for sIgG$_4$ levels to Bos d 4, Bos d 5, and Bos d 8. The remaining IgG$_4$ signal after the assay was calculated as the percentage difference of values from absorption with Bos d 4 and Bos d 5 and those from mock absorption.

The Bos d 4 absorption selectively removed sIgG$_4$ to Bos d 4 but not sIgG$_4$ to Bos d 5 and Bos d 8. Similarly, the Bos d 5 absorption resulted in selective removal of sIgG$_4$ to Bos d 5 (Fig E1, A). These results demonstrated that ImmunoCAP IgG$_4$ assays for Bos d 4, Bos d 5, and Bos d 8 are specific.

Solid-phase RIAs for specific IgG using molecular allergens

Solid-phase RIAs were performed to measure specific IgG, specific IgG$_1$, and sIgG$_4$ levels to nBos d 4 and nBos d 5 (Sigma-Aldrich).[E3] The allergens were radiolabeled with iodine 125 with chloramine-T. Recombinant Protein G–Sepharose 4B conjugate was obtained from Invitrogen (Camarillo, Calif) for measuring IgG levels. For IgG$_4$ assays, 10 mg of the anti-human IgG$_4$ mAb clone RJ4 (Abingdon Health, York, United Kingdom), MH164-4 (Sanquin Blood Supply, Amsterdam, The Netherlands), or HP-6023 (Sigma-Aldrich) was coupled to 1 g of cyanogen bromide–activated Sepharose 4B. For IgG$_1$, anti-human IgG$_1$ mAb clone HP-6001 (Sigma-Aldrich) was used.

We added 0.25 mL of a 50% suspension of the prepared Sepharose media in PBS with 0.3% human serum albumin, 0.5 mL of buffer (0.01 mol/L EDTA, 0.3% human serum albumin, 0.05% NaN$_3$, and 0.2% Tween-20 in PBS), and 10 µL of serum to 2-mL polystyrene tubes. The mixture was incubated at room temperature on a vertical rotator for 4 hours. The suspension was centrifuged and washed (in PBS with 0.1% Tween-20) 5 times before 0.5 mL of buffer and 50 µL of radiolabeled allergen (in PBS with 0.3% human serum albumin) were added. The suspension was rotated for 4 hours and washed again. The tubes were measured with a gamma counter, and a standard curve was generated for quantitation. Results were expressed in units per milliliter, with a background of 5 U/mL.

We compared the quantitative accuracy of ImmunoCAP IgG$_4$ assays and the solid-phase IgG$_4$ RIA for Bos d 4 and Bos d 5 by using sera from 16 patients with EoE in the current studies (Fig E1, B). There were close quantitative correlations between the 2 methods for sIgG$_4$ to Bos d 4 ($r_S = 0.97$, $P < .001$) by using the mAb clone HP-6023 and also for sIgG$_4$ to Bos d 5 by using 3 different mAb clones: HP-6023 ($r_S = 0.92$, $P < .001$), MH164-4 ($r_S = 0.89$, $P < .001$), and RJ4 ($r_S = 0.86$, $P < .001$). By using RIAs, detectable sIgG$_4$ to Bos d 4 was found in 11 of 16 patients, and detectable sIgG$_4$ to Bos d 5 was found in all patients for HP-6023 and MH164-4 and in 14 of 16 for RJ4. In addition to sIgG$_4$ to Bos d 4 and Bos d 5, we also measured specific IgG and IgG$_1$ antibodies. With these measurements, we determined the contribution of the IgG$_1$ and IgG$_4$ isotype to the IgG response to Bos d 4 and Bos d 5 (Table E2). The IgG response to these CM proteins appeared to be IgG$_4$ dominant in the 16 patients with EoE analyzed.

Total IgG$_4$ assays

The ImmunoCAP total IgG$_4$ assay (IgA/IgG Calibrator ImmunoCAP) technique (off-label use) is based on use of anti-κ and anti-λ light chain mAbs on solid phase to capture antibodies from serum. Bound IgG$_4$ is then detected with a fluorescence-labeled anti-human IgG$_4$ mAb. Before running these assays, sera were diluted to 1:50 to 1:100 (or more if necessary) in specific IgA/IgG sample diluent. Of note, the ImmunoCAP instrument does an additional 1:100 dilution, thus the final dilution is 5,000-10,000-fold.

Assay validation experiments demonstrated good dilution linearity (Fig E2, A) and also a solid-phase binding capacity that is sufficient to handle at least a 128-fold excess of IgG$_1$ antibodies over IgG$_4$ antibodies without a decrease in quantitation (Fig E2, B). Additionally, we measured total IgG$_4$ levels in sera from 53 subjects in the study using both nephelometry and ImmunoCAP assays and found a very good correlation between the methods ($r_s = 0.98$ [0.97-0.99], $P < .001$; Fig E2, C). The mean percentage coefficient of variation for the ImmunoCAP total IgG$_4$ assay was 3.4%.

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E2. Kruszewski PG, Russo JM, Franciosi JP, Varni JW, Platts-Mills TA, Erwin EA. Prospective, comparative effectiveness trial of cow's milk elimination and swallowed fluticasone for pediatric eosinophilic esophagitis. Dis Esophagus 2016; 29:377-84.
E3. Krop EJ, Matsui EC, Sharrow SD, Stone MJ, Gerber P, van der Zee JS, et al. Recombinant major urinary proteins of the mouse in specific IgE and IgG testing. Int Arch Allergy Immunol 2007;144:296-304.

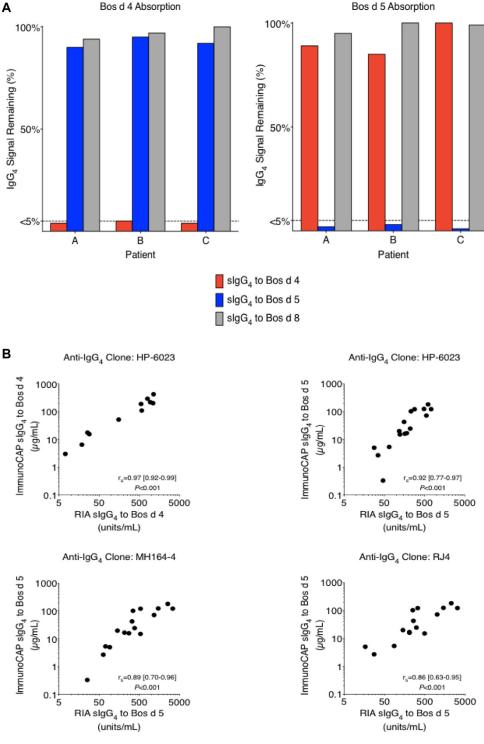FIG E1. **A,** Bos d 4 and Bos d 5 absorption assays in 3 pediatric patients with EoE included in these studies. **B,** Quantitative correlations between ImmunoCAP assays for sIgG$_4$ to Bos d 4 and Bos d 5 and solid-phase IgG$_4$ RIA by using 3 different anti-human IgG$_4$ mAbs (HP-6023, MH164-4, and RJ4) and sera from 16 pediatric patients with EoE in the current studies.
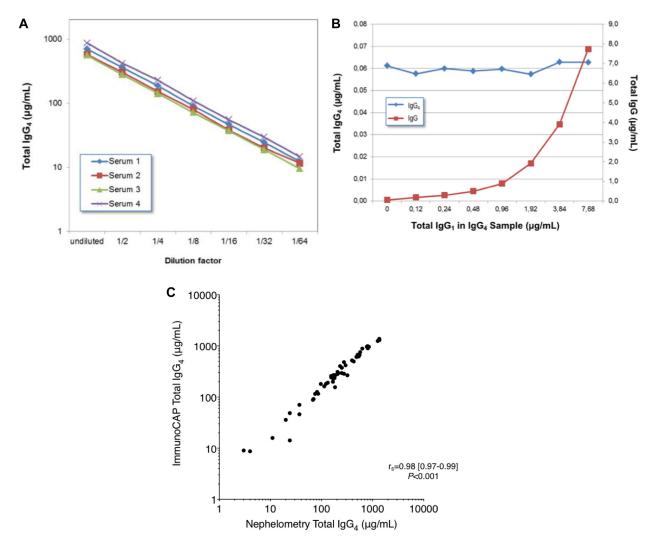
FIG E2. A, Total IgG$_4$ assay validation experiments: dilution linearity (2-fold series) of the total IgG$_4$ assay in 3 random serum samples. **B,** Total IgG$_4$ measurements in the presence of increasing IgG$_1$ concentrations and **C,** quantitative correlation (Spearman rank-order test) between the ImmunoCAP total IgG$_4$ assay and nephelometry for IgG$_4$ in 53 subjects.
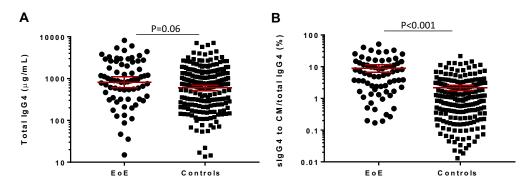
FIG E3. Titer of total IgG$_4$ (geometric mean [95% CI]; **A**) and sIgG$_4$ to CM (sIgG$_4$ to Bos d 4 plus Bos d 5 plus Bos d 8) as a percentage of total IgG$_4$ (geometric mean [95% CI]; **B**) in 71 pediatric patients with EoE and 210 control subjects (2 patients with EoE and 3 control subjects without positive sIgG$_4$ results to CM were excluded). Comparison between groups was done with the Mann-Whitney test.
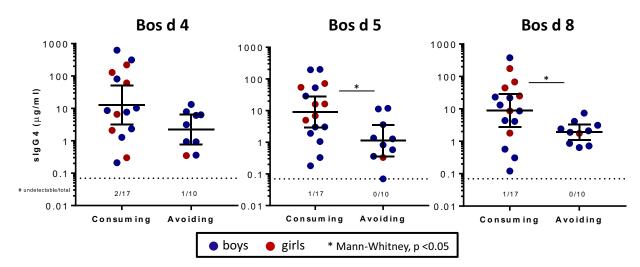
FIG E4. CM sIgG$_4$ levels in boys and girls less than 10 years old with EoE stratified by consumption or avoidance of dairy products. Comparison was done with the Mann-Whitney test.
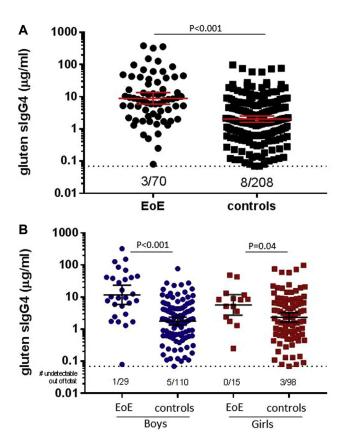
FIG E5. A, sIgG$_4$ levels (geometric mean [95% CI]) to gluten in 71 patients with EoE and 210 control subjects. **B,** sIgG$_4$ levels to gluten in 10- to 18-year-old patients with EoE stratified by sex compared with sex-matched control children. Statistical analysis was performed by using the Mann-Whitney test. *Values below the dotted line* indicate numbers of negatives out of the total in the group.
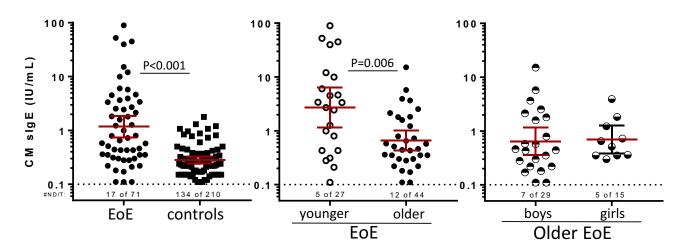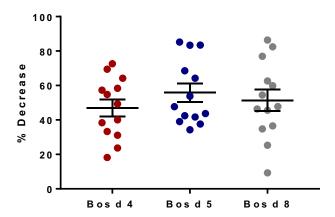
FIG E6. sIgE levels to CM extract in children with EoE and control children. Of the EoE cohort, 2- to 9-year-olds were classified as younger, and 10- to 18-year-olds were classified as older. Geometric means with 95% CIs exclude undetectable (≤0.1 IU/mL) values. Comparison was with the Mann-Whitney test.

FIG E7. Change in sIgG$_4$ levels in patients with EoE (n = 13) after a 6- to 8-week CM elimination diet.

TABLE E1. Summary of allergens used in the current study (adapted from www.allergen.org)

Source	Molecular allergen	ImmunoCAP assay	Biochemical name
CM *(Bos domesticus)*	Bos d 4	f76	α-Lactalbumin
	Bos d 5	f77	β-Lactoglobulin
	Bos d 6	Re204	Serum albumin
	Bos d 8*	f78	Mixture of caseins (α-S1-, α-S2-, β-, and κ-caseins)
	Bos d LF	Streptavidin	Lactoferrin (or lactotransferrin)
	α-gal	Streptavidin	α-gal
Wheat *(Triticum aestivum)*	Tri a 14	f433	Nonspecific lipid transfer protein (type 1)
	Tri a 19	f416	ω-5 Gliadin (seed storage protein)
	Gliadins*	f98	Mixture of gliadins (α-, β-, γ-, and ω-gliadins)
	Gluten*	f79	Mixture of gliadins and glutenins
Egg *(Gallus domesticus)*	Gal d 1	f233	Ovomucoid
	Gal d 2	f232	Ovalbumin
	Gal d 4	Rk208	Lysozyme C
Soy *(Glycine max)*	Gly m 4	f353	Pathogenesis-related protein (PR-10; Bet v 1 family member)
	Gly m 5	f431	β-conglycinin (vicilin, 7S globulin)
	Gly m 6	f432	Glycinin (legumin, 11S globulin)
Peanut *(Arachis hypogaea)*	Ara h 1	f422	Cupin (vicilin-type, 7S globulin)
	Ara h 2	f423	Conglutin (2S albumin)
	Ara h 3	f424	Cupin (legumin-type, 11S globulin, glycinin)
	Ara h 8	f352	Pathogenesis-related protein (PR-10; Bet v 1 family member)
	Ara h 9	f427	Nonspecific lipid transfer protein (type 1)

*Indicates a mixture of molecular allergens.

TABLE E2. CM-specific IgG$_1$ and IgG$_4$ levels as a percentage of sIgG in patients with EoE expressed as GM

CM protein	IgG$_1$, % (95% CI)*	IgG$_4$, % (95% CI)*
Bos d 4	29% (24-34)	69% (64-75)
Bos d 5	34% (15-45)	64% (54-74)

GM, Geometric mean.

*The GM (95% CI) was calculated by using the positive values (values >0% IgG).

TABLE E3. Demographics and characteristics of children with CM-induced anaphylaxis (n = 10)

Demographic/characteristic	CM-induced anaphylaxis (n = 10)
Age (y), median (range)	7 (1-18)
Female sex, no. (%)	5 (50.0)
Total IgE (IU/mL), GM (95% CI)	401 (105-1,532)
CM sensitization, no. (%)	10 (100)
sIgE to CM (IU/mL), GM (95% CI)	23.9 (5.94-96.6)
sIgG$_4$ to Bos d 4 (μg/mL), GM (95% CI)	0.78 (0.13-4.72)
sIgG$_4$ to Bos d 5 (μg/mL), GM (95% CI)	1.29 (0.45-3.69)
sIgG$_4$ to Bos d 8 (μg/mL), GM (95% CI)	1.10 (0.39-3.11)

The GM (95% CI) was calculated by using positive values (sIgE, ≥0.1 IU/mL; sIgG$_4$, ≥0.07 μg/mL).
GM, Geometric mean.

TABLE E4. Relationship between EoE status and sIgE levels to CM proteins in children stratified by sex

CM protein	Sex	Prevalence of detectable sIgE, no. (%)		Unadjusted		Adjusted*	
		Patients with EoE	Control subjects	*P* value	OR (95% CI)	*P* value	OR (95% CI)
CM extract	Boys	37/48 (77%)	38/111 (34%)	<.001	6.5 (3.0-14)	<.001	5.2 (2.2-12)
	Girls	17/23 (74%)	38/99 (38%)	.003	4.5 (1.7-13)	.037	3.3 (1.1-10)
Bos d 4	Boys	27/48 (56%)	11/110 (10%)	<.001	9.6 (4.3-22)	<.001	8.9 (3.6-22)
	Girls	12/23 (52%)	15/99 (15%)	<.001	7.1 (2.6-19)	.009	4.7 (1.5-15)
Bos d 5	Boys	29/48 (60%)	18/109 (17%)	<.001	7.7 (3.6-17)	<.001	6.9 (2.9-16)
	Girls	13/23 (57%)	20/99 (20%)	.001	5.1 (2.0-13)	.021	3.7 (1.2-11)
Bos d 8	Boys	25/48 (52%)	15/110 (14%)	<.001	6.9 (3.1-15)	<.001	5.4 (2.2-13)
	Girls	13/23 (57%)	17/99 (17%)	<.001	6.3 (2.4-17)	.027	3.6 (1.1-11)

*Adjusted for age and milk consumption.