

IgE-binding peptides coupled to a commercial matrix as a diagnostic instrument for persistent cow's milk allergy

To the Editor:

Cow's milk allergy (CMA) is the most common cause of food allergy in the first years of life, affecting approximately 2.5% of children.¹ The majority of children outgrow their CMA by 3 to 4 years of age; however, about 15% of children retain their allergy into the second decade.^{2,3} The mechanisms behind this development of clinical tolerance to food proteins remain poorly understood.

In our previous studies, we characterized the sequential IgE-binding epitopes for the major cow's milk allergens.⁴⁻⁷ Observed differences between younger children who are likely to outgrow their allergy and older patients with persistent cow's milk allergy suggested that epitope specificity of IgE antibody responses might predict the clinical outcome of CMA.⁴ Using SPOTs-membrane technology and sera from 10 patients with persistent and 10 patients with transient CMA, we identified informative IgE-binding epitopes that were not recognized by any of the patients with transient CMA but showed binding by most of the patients with persistent allergy.⁸ The current study was performed to confirm the relevance of these informative epitopes in a larger study population.

Therefore, 74 patients with challenge-proven CMA were included in the study. Twenty-nine patients subsequently outgrew their allergy, 15 of those at a median age of 3 years (range, 1-4 years; group C) and 14 at a median age of 8 years (range, 6-18 years; group B). The remaining 45 children followed until a median age of 8 years (range, 5-22 years; group A) retained their allergy, and were assumed to have persistent CMA.

Five peptides, 10 amino acids in length, representing the IgE-binding epitopes on α s1-casein, α s2-casein, and κ -casein, were synthesized and coupled to ImmunoCAP (Pharmacia Diagnostics, Uppsala, Sweden). These peptides were chosen from previously identified peptides because they were recognized solely by patients with persistent CMA.⁸ The decapeptides were coupled to the cyanogen bromide-activated cellulose solid phase matrix, ImmunoCAP, at optimal conditions: for α s1-casein, AAs 69-78 (EEIVPNSVEQ) and AAs 123-132 (MKEGI-HAQK); for α s2-casein, AAs 33-42 (ENLCSTFCKE) and AAs 171-180 (YQKFALPQYL); and for κ -casein, AAs 155-164 (SPPEINTVQV). Peptide-coupled ImmunoCAPs and reagents were kindly provided by Pharmacia Diagnostics. Levels of cow's milk and peptide-specific IgE were determined using the Pharmacia UniCAP system. Banked sera obtained at the time of clinical reactivity and stored at -80°C were used for analyses. Thirteen of the 74 patient sera used in the current study were also used in our previous study on informative epitopes.⁸

Milk peptide-specific IgE was significantly higher in patients with persistent CMA at a median age of 8 years

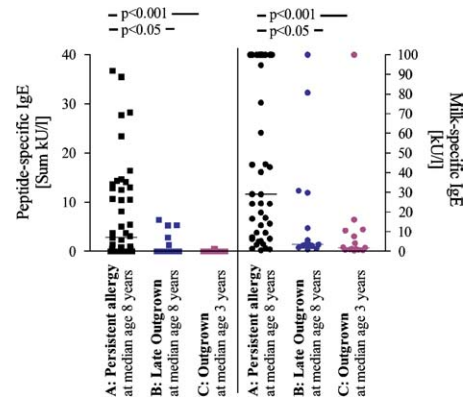


FIG 1. Milk and milk peptide-specific IgE levels in patients with persistent CMA (group A) compared with children with subsequent late (group B) or regular tolerance development (group C) at time of clinical reactivity.

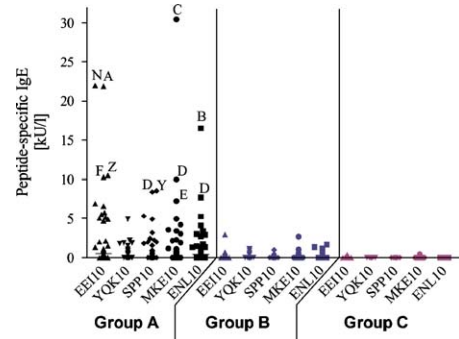


FIG 2. Peptide-specific IgE for individual peptides at time of clinically relevant allergy in 3 groups of patients: (A) with persistent CMA, and (B) with late and (C) with regular tolerance development. Letters highlight the patients with the highest levels for the individual peptides.

(group A) compared with children who subsequently outgrew their allergy in the first few years of life at a median age of 3 years (group C) or patients who lost their allergy at a median age of 8 years (group B; $P < .001$ and $P < .05$, respectively; Fig 1). Specifically, only 2 of 15 patients in group C and 5 of 14 patients in group B recognized any of the selected peptides at the time of clinically relevant milk allergy, whereas 30 of 45 patients in group A did so. Median milk-specific IgE levels were also significantly different among the 3 patient groups (Fig 1). However, the range was from <1.0 to >100 kU/L in all 3 groups, resulting in an overlap not observed in this way for the milk peptide-specific IgE. Fig 2 shows the level of peptide-specific IgE for the individual peptides in all 3 groups of patients. The antibody pattern was heterogeneous in that the highest levels for the individual peptides were found in different patient sera.

In summary, our current study confirmed that IgE antibodies to distinct allergenic epitopes of cow's milk

proteins can be used as a marker of persistent CMA. The successful linkage of these peptides to a commercial matrix should enable the conduct of large prospective studies and simplify future diagnostic approaches.

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Topical viscous budesonide suspension for treatment of eosinophilic esophagitis

To the Editor:

Eosinophilic esophagitis (EE) is a clinical entity composed of marked diffuse esophageal eosinophilia and basal zone hyperplasia thought to be provoked, in at least a subset of patients, by food and/or aeroallergen exposure.^{1,2} In parallel with other atopic disorders, the incidence of EE appears to be increasing.^{3,4} Current treatment options for children are limited, and if steroid therapy is initiated for treatment, we routinely use a fluticasone metered dose inhaler (MDI) puffed into the oropharynx and swallowed as topical esophageal therapy.⁵ However, we have encountered 2 patients who failed to respond to elemental formula and were unable, because of developmental problems, to perform the puff and swallow technique. We report here treatment of these 2 patients with an oral suspension of budesonide.

Patient 1 was a 6-year-old boy with a history of abdominal pain, failure to thrive, and vomiting since infancy, and developmental delay with isolated EE diagnosed in 2001. His allergic history was significant for allergic rhinitis secondary to cat, dog, and dust mite exposure as well as positive UniCAP radioallergosorbent testing (Pharmacia, Kalamazoo, Mich) for fish and milk. After upper gastrointestinal endoscopy (EGD) with esophageal biopsies demonstrating a maximum eosinophil count of 60 per high-powered field (HPF at 400 $\times$ magnification), he was diagnosed with EE and treated with oral corticosteroid (prednisone 0.5 mg/kg daily) for 1 month. This resulted in resolution of esophageal eosinophilia with a maximum of 2 eosinophils per HPF on repeat EGD with biopsy. He was subsequently treated with omeprazole (Prilosec, AstraZeneca, Wilmington, Del) 10 mg twice daily and fluticasone (Flovent, GlaxoSmithKline, Research Triangle Park, NC) 220 mcg twice daily as topical esophageal therapy, but repeat EGD with biopsy after 6 months demonstrated a maximum eosinophil count of 50 per HPF. His therapeutic regimen was changed to elemental formula and fluticasone, but repeat EGD with biopsy demonstrated persistent esophageal eosinophils with a maximum eosinophil count of 74 per HPF accompanied by basal zone hyperplasia. He was referred to the Children's Hospital Eosinophilic Gastrointestinal Disorders clinic for further management of EE recalcitrant to usual therapy. At the time of his referral, he continued to have vomiting and poor weight gain, and his mother reported significant difficulty with the puff and swallow technique. He was placed on a regimen of twice-daily oral budesonide suspension (Pulmicort, AstraZeneca) 500 μ g mixed with sucralose (Splenda, McNeils Nutritionals, Washington, Pa), a nonabsorbed sugar, to increase fluid viscosity, to be swallowed twice daily. After 3 months of therapy, repeat EGD showed a normal esophagus, and biopsies demonstrated complete resolution of both esophageal eosinophils and basal zone hyperplasia. In addition, his abdominal pain and vomiting resolved, resulting in increased appetite with documented