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Detection of IgE Antibodies Specific for Allergens in Cow Milk and Cow Dander

Abstract

Sera from patients with cow milk protein allergy ($n = 6$) and cow dander allergy ($n = 5$) were analyzed for reactivity of IgE antibodies specific for allergens derived from milk and cow dander. The cow milk- and cow dander-allergic patients exhibited elevated specific IgE levels to milk and to cow dander, respectively, as determined by RAST. IgE immunoblot analysis revealed that cow milk-allergic patients exhibited IgE binding to the milk allergens casein, β -lactoglobulin, α -lactalbumin and bovine serum albumin. Four of six cow milk-reactive patients also exhibited IgE binding to cow dander proteins with molecular weights of 20, 22, 36, 50 and > 200 kD. IgE from the sera of cow dander-allergic patients reacted with the major allergens of cow dander (20 and 22 kD) and with other proteins with molecular weights of 24, 36, 42, 50 and 70 kD. Only one out of five of these sera displayed IgE reactivity with cow milk proteins with molecular weights of 69, 92 and > 200 kD. Inhibition studies revealed the cross-reactive nature of the IgE antibodies. Preincubation of cow milk-positive sera with cow milk and subsequent immunoblotting led to complete blocking of IgE binding to cow dander proteins and to cow milk proteins. Preincubation of cow milk-positive sera with cow dander extract led to blocking of IgE binding only to defined cow milk proteins: these could be identified in 2 cases as casein and in 1 case as β -lactoglobulin. ELISA inhibition assays confirmed the results and provided quantitation. The results demonstrate the existence of cross-reactive IgE antibodies reacting with similar or identical cow milk and cow dander allergens, which may cause clinical manifestations.

Key Words

Allergens
Cow milk
Dander, cow
IgE reactivity

Introduction

It is well documented that patients suffering from type-I allergy to tree pollen (in particular to birch pollen) frequently show adverse reactions to fruits and vegetables [1–3]. More recently, cross-reactions between avian (inhaled) allergens and egg (food) allergens have been described based on the reports of several cases [4, 5].

Cow milk (CM) and cow dander (CD) proteins are known to elicit symptoms (IgE-mediated and non-IgE-mediated) in some allergic infants and adults [6–11]. Although many studies were dealing with the characterization of the immunogenic components of these two sources (CM and CD), only little information is available regarding putative cross-reactive allergens in these 2 extracts [12–15]. Using polyacrylamide gel electrophoresis and gel

Table 1. Patients' serological data

Patient No.	Age years	Sex	Positive prick test	Positive RAST	Total IgE U/ml
1	31	m	milk	f2:3.3	297
2	8	f	milk, horse, mite, cat, egg, grass pollen, rye	f2:4.7,f1:4.7,d1:5,g3:4.5,e1:5,e4:2.3	> 1,000
3	8	m	milk, egg, dog, mite, birch and hazel pollen	f2:3.8,f1:3.9,e4:3.1,e5:3.4,g3:3.2,d1:3.7	> 1,000
4	36	m	milk, cow, birch and grass pollen, rye, peanut	f2:3.6,t3:4.4,w6:3.5,e4:3.2	> 1,000
5	51	m	milk, salmon, eel, mite	f2:5,f3:2.1,f78:5,d1:2.9,e4:2.4	> 1,000
6	8	f	milk, egg, grass and birch pollen, cat, soybean, lemon, rye, mugwort	f2:4.4,f1:3.7,d1:5,g3:4.8,f1:4.2.3	> 1,000
7	30	f	cow, sheep, horse, mite, swine, bird feather	e4:4.3,e83:2.3	210
8	45	f	cow, mugwort	e4:5,e83:3.4,f2:2.5	236
9	4	m	cow, milk	e4:4.8,f2:2.0	> 1,000
10	5	m	cow	e4:4,f2:2.2	120
11	22	m	cow	e4:5,e83:2.5	74

information regarding skin prick tests and RASTs of various allergens as well as total IgE of patients shown in figures 2-5.

diffusion. Osváth et al. [8, 9] stated that cross-sensitization between CM and CD might occur. Their speculation was based on the presence of bovine serum albumin (BSA) and serum globulin in CM. Moreover, RAST experiments suggested the existence of common allergenic proteins or epitopes in both sources [8]. According to these results, 50% of children with CM allergy who never came in contact with CD exhibited IgE against CD proteins [8]. However, it is not known whether major or minor allergens of CM or CD are involved in putative cross-reactions. Therefore the aim of the present study was to compare IgE-binding components in CM and CD extracts in order to identify cross-reacting allergens or common allergens present in both sources. IgE immunoblotting and ELISA were applied for this purpose.

Materials and Methods

Sera

Sera from patients with CM allergy ($n = 6$, 2 females and 4 males, ages ranging from 7 to 50 years, median 23 years) and CD allergy ($n = 5$, 2 females and 3 males, ages ranging from 4 to 45 years, median 21 years) were selected according to case history, positive skin prick tests and positive RAST (> 2.0) to CM or CD, respectively. The clinical manifestations ranged from urticaria, perioral swelling, pruritus and asthma bronchiale in the CM-allergic group, whereas symptoms of CD-allergic patients were rhinitis, asthma bronchiale and atopic dermatitis. Detailed serological data on the patients shown in figures 2-5 is given in table 1. Sera collected from 10 individuals with negative case history concerning type-I allergy, negative skin prick test and negative RAST to CM and CD extracts served as control group.

Protein Extracts

CD was purchased from Allergon AB (Engelholm, Sweden) and extracted according to Ipsen and Loewenstein [16] with slight modifications. Freshly dried raw material was extracted in 10 mmol/l potassium dihydrogen phosphate (pH 7.0), 2 mmol/l of ethylenediaminetetraacetic acid disodium salt, 10 mmol/l of sodium diethyldithiocarbamate, and 3 mmol/l sodium azide under continuous shaking for 24 h at 4 °C. To the extraction mixture 2% (w/v) suspended solid polyvinylpyrrolidone were added to avoid possible interactions of protein material in the extracts with aromatic compounds possibly present in CD extract. Particles were removed by centrifugation (40,000 g for 1 h at room temperature). The supernatant was extensively (72 h) dialyzed against 50 mmol/l of potassium dihydrogen phosphate (pH 7.0) containing 1 mmol/l of sodium azide, lyophilized and stored at -20 °C.

Soluble CM extract (skin prick test solution) was purchased from ALK (Copenhagen, Denmark). Before use for electrophoresis, extracts were dissolved in distilled water and diluted in concentrated Laemmli sample buffer [17].

The protein concentrations of the protein preparations were measured according to Bradford [18] using BSA as standard (Protein Assay, Bio-Rad, Munich, FRG).

SDS-PAGE and Immunoblotting

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out as described previously [1]. The separated proteins were transferred onto nitrocellulose (pore size 0.45 μ m, Schleicher & Schuell, Dassel, FRG) at 300 mA for 60 min at 4 °C [19]. IgE blots were performed essentially as described [1]. Briefly, strips were saturated for 30 min with either phosphate-buffered saline (PBS) BSA Tween (50 mmol sodium phosphate, 0.5% [wt/vol] BSA, 0.5% [vol/vol] Tween 20) as described by Ebner et al. [1] or in PBS/Tween (50 mmol Tris, 150 mmol NaCl, 0.5% Tween 20) without BSA. After preincubation, the strips were incubated at 4 °C overnight with patients' sera diluted 1:10 in one of the above-mentioned buffers. After several washings with buffer, bound IgE was detected with the Phadebas RAST kit-supplied ^{125} I-rabbit anti-human IgE. Blots were investigated by autoradiography at -70 °C, using hyperfilm MP (RPN 6, Amersham) in exposure cassettes (X-omatic, Kodak).

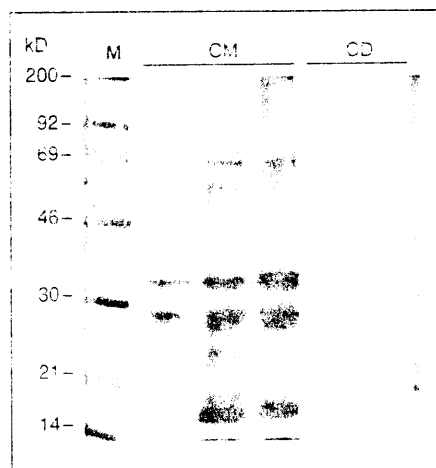


Fig. 1. Coomassie brilliant blue R stained 15% SDS-PAGE shows the separation of CM and CD extract in various concentrations. CM: 14 kD α -lactalbumin, 18 kD β -lactoglobulin, 29–36 kD casein fractions, 69 kD BSA. Lane M = Molecular weight marker (Amersham International, U.K.).

IgE ELISA and ELISA Inhibition

IgE ELISA measurement was performed according to Ylonin et al. [11]. For the ELISA inhibition experiments, patients' sera were preincubated overnight at 4°C with 10, 3, 1, 0.3, 0.1, 0.03, 0.01, 0.003 and 0 mg/ml of either CM or CD extract and subsequently applied on ELISA plates (Nunc-Immuno Plate I, Denmark) coated with 5 μ g/ml of protein in coating buffer (0.05 mol carbonate buffer, pH 9.5) [11].

IgE Immunoblot Inhibition Experiments

One milliliter of CM-allergic patients' sera diluted 1:10 in a previously described buffer [1] was preincubated under continuous shaking at 4°C overnight with 0.5 mg/ml of α -lactalbumin, β -lactoglobulin, casein and BSA (Sigma, Munich, FRG) or whole milk extract, respectively. The high protein concentrations were used to achieve maximal inhibition results. Protein titration was used for ELISA inhibition assays. Thereafter immunoblotting was performed on a CD extract-coated strip. The same sera were preincubated with CD extract (0.5 mg/ml) and tested in the same way with CM extract-coated strips.

Results

SDS-PAGE

Coomassie brilliant blue R stained 15% SDS-PAGE-separated proteins of CM and CD extract revealing representative protein fractions ranging from 14 to 69 kD in case of CM and 20 to 25 kD in case of CD, respectively (fig. 1).

IgE Immunoblots

IgE Reactivity of Sera from Individuals with CM Allergy to CM Proteins. CM-allergic patients displayed IgE binding to the milk allergens casein, β -lactoglobulin, α -lactalbumin and BSA (fig. 2a). The IgE binding was comparable

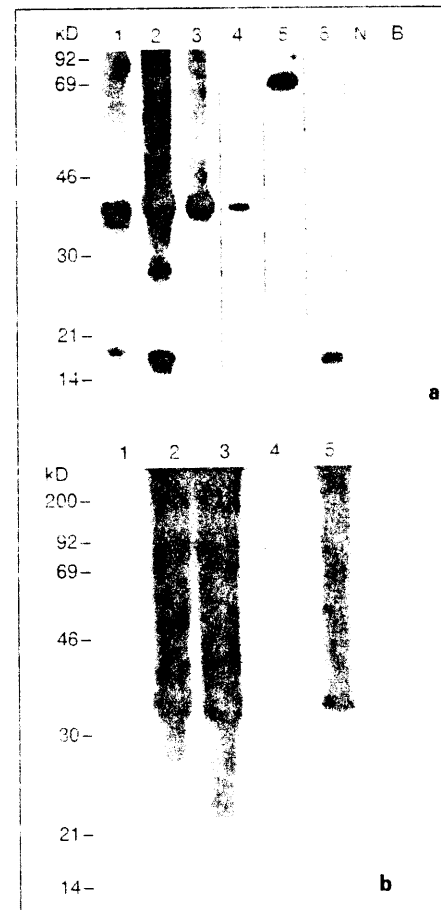


Fig. 2. a IgE immunoblots with sera from 6 CM-allergic patients on CM extract-coated strips. Lane 1–6 = Patients with CM allergy; lane N = Normal human serum pool; lane B = buffer control. **b** IgE immunoblots with sera from 5 cow milk allergic patients on cow dander extract-coated strips. Patients 2, 3, 5: IgE binding to minor allergens of cow dander extract. Patient 4: weak IgE binding to the major allergens of cow dander (20, 22 kD).

with RAST results obtained when RAST strips coated with α -lactalbumin, β -lactoglobulin and casein were used. Patient 4 reacted exclusively with casein (fig. 2a). With the exception of serum from patient 5, none of the sera tested reacted with BSA in both buffer systems, with or without BSA. The remaining IgE reactivity to BSA in patient 5 in the presence of 0.5% BSA in the buffer might be due to the combination of a high concentration of BSA-specific IgE and a short delay between mixing of serum with buffer and applying onto nitrocellulose strips.

IgE Reactivity of CM-Allergic Patients with CD Proteins. Four of six patients also demonstrated IgE binding to additional CD proteins with molecular weights of 20, 22, 36,

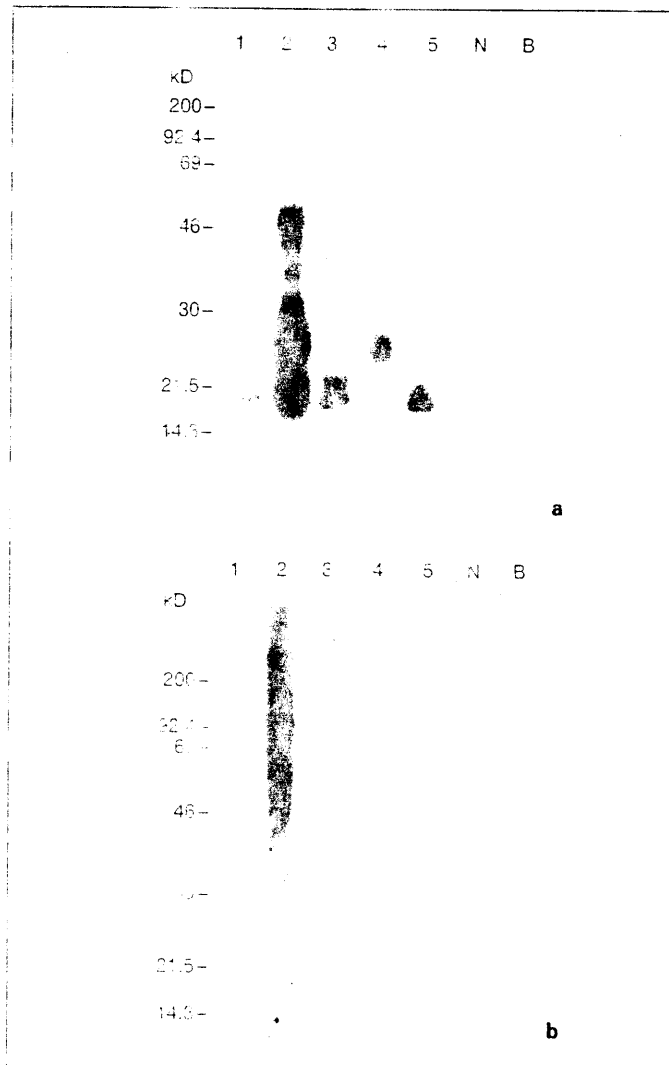


Fig. 3. **a** IgE immunoblots with sera from 5 CD-allergic patients on CD extract-coated strips. All patients IgE reacted with the major allergens of CD of 20 and 22 kD. Patients 2, 4 and 5 reacted additionally with minor allergens (24, 38, 42, 50, 70 and >200 kD). **b** IgE immunoblots with sera from 5 CD-allergic patients on CM extract-coated strips. Only patient 2 reacted with CM allergens of 68, 90 and >200 kD.

50 and >90 kD. IgE immunoblot of patients 1–5 are shown in figure 2b. Patient 4 reacted exclusively with the major allergens of CD (20 and 22 kD) although in a very weak form. Patients 2, 3 and 5 displayed strong IgE reactivity with a 36-kD protein.

IgE Reactivity of Sera from Individuals with CD Allergy to CD Proteins. IgE from all CD-allergic patients' sera (5 of 5) reacted with the major allergens of CD (20 and 22 kD) and with other proteins with molecular weights of 25, 36, 50 and 70 kD (fig. 3a).

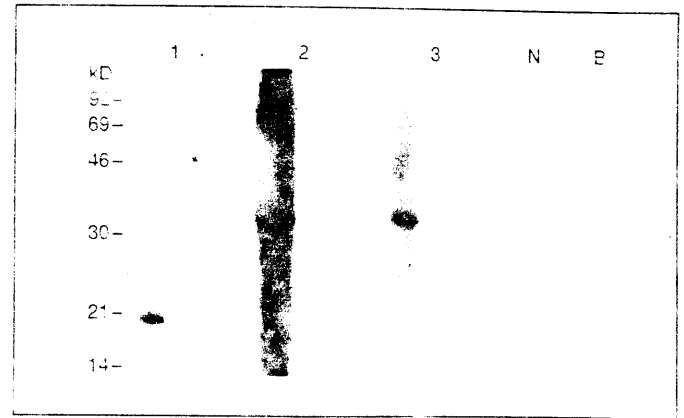


Fig. 4. Inhibition experiment I. Inhibition was performed by preincubation of milk-allergic patients' sera with whole milk extract before testing on CD extract-coated strips. Lanes 1–3 = IgE binding before inhibition (left part) and after inhibition (right part).

IgE Reactivity of CD-Allergic Patients with CM Proteins. Only one of these sera showed IgE-reactivity with CM proteins (69, 92 and >200 kD; fig. 3b).

The sera from the control group showed no IgE reactivity either on CM extract or on CD extract by immunoblotting analyses (fig. 2a, 3a).

Immunoblot Inhibition Experiments

Inhibition with CM Proteins. Figure 4 illustrates inhibition experiments on 3 CM-allergic patients' sera (patients 2, 3 and 5; fig. 2b.) In all 3 cases, IgE binding to the 36-kD protein of CD was completely abolished after preincubation with CM. In lane 1 IgE binding to the 20-kD protein was also blocked. Preincubation of CM-allergic patients' sera with defined CM proteins (α -lactalbumin, β -lactoglobulin, casein and BSA) or whole milk extract and subsequent testing on CD extract-coated strips led to individual inhibition patterns. In 2 of 6 patients, preincubation with casein prevented IgE binding to the above-mentioned CD proteins (data not shown). Testing on milk strips as control confirmed the specific inhibition of the respective proteins.

Inhibition with CD Extract. Preincubation of CM-allergic patients' sera with 0.5 mg/ml of CD extract led to a reduction in IgE binding to CM allergens (fig. 5). The high antigen concentration used for preincubation was chosen to achieve a maximum of inhibition. Quantitative analysis was performed by ELISA inhibition as discussed later. In patients 1, 4 and 6, IgE binding to casein (36 kD) and β -lactoglobulin (18 kD) could be inhibited or strongly reduced by preincubation with CD extract, respectively (fig. 5).

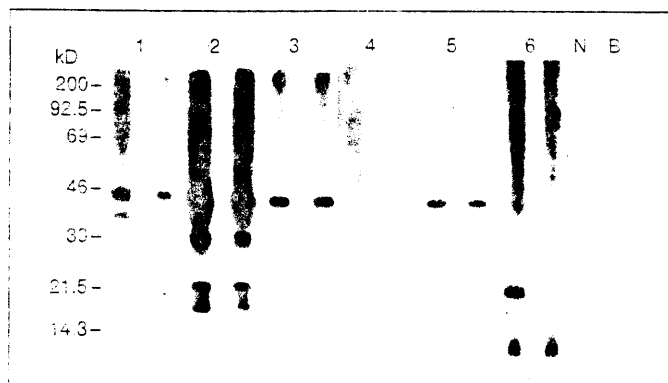


Fig. 5. Inhibition experiment II. Inhibition was performed by preincubation of milk-allergic patients sera with CD extract before testing on CM extract-coated strips. Lanes 1–6 = IgE binding before inhibition (left part) and after inhibition (right part).

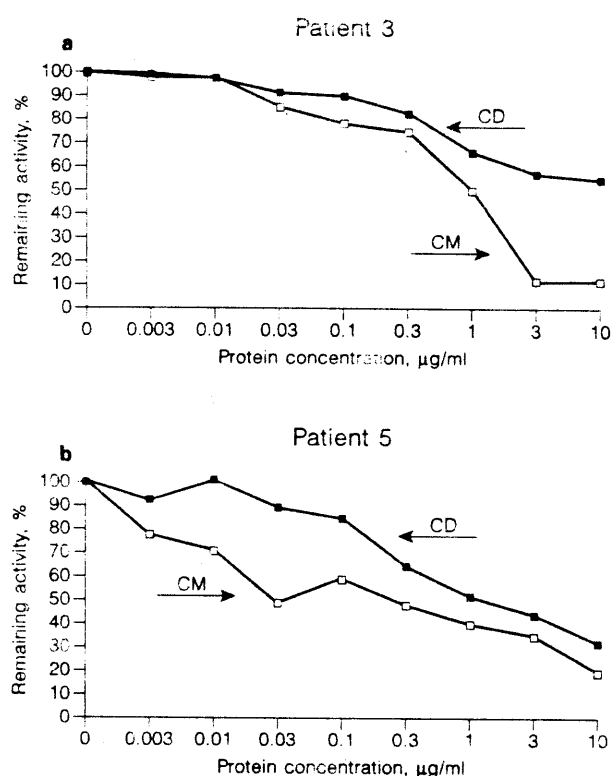


Fig. 6. ELISA inhibition experiments. 100 μl of sera from patient 3 (a) and patient 5 (b) were incubated with 100 μl of CM and CD extract containing 10, 3, 1, 0.3, 0.1, 0.03, 0.01, 0.003 and 0 μg total protein. Incubation was performed at 4 °C overnight. Samples only incubated with PBS were used as controls.

Therefore, our findings suggest either the presence of these proteins (β-lactoglobulin and casein) in CD or the presence of epitopes shared by both extract sources.

ELISA Inhibition

Figure 6 illustrates the quantitative IgE-binding inhibition performed on patients 3 and 5. Patients' sera preincubated with increasing concentrations of CD extract revealed a 45 and 68% inhibition when the microtiter well coated and inhibiting protein concentration was equal (5 μg/ml). When preincubation was performed under the same conditions with CM, a 90 and 80% inhibition could be achieved, revealing a complete block of CD-reacting IgE by the applied milk proteins. The stronger inhibition potency of milk proteins might be explained by the presence of a higher percentage of inhibiting proteins in comparable amounts of extract.

Discussion

Allergens have been identified in different cow-derived extracts like hair, dander, saliva, urine, amniotic fluid, meat and milk [15]. Since cross-reactivity between food allergens and inhaled allergens are frequently responsible for clinical symptoms in allergic children and adults [6], we compared the IgE binding components in CM and CD extracts. Patients' IgEs were used to identify cross-reacting allergens.

Our Western blot experiments revealed that CM-allergic patients (4 of 6) exhibit IgE against milk proteins as well as CD proteins, whereas CD-allergic patients' IgEs mainly do not recognize CM proteins (1 of 5). According to our findings we were not able to define one allergen responsible for the cross-reactivity of IgE antibodies. In 2 patients anti-casein IgE antibodies and in 1 patient anti-β-lactoglobulin IgE antibodies were found to bind to CD proteins. In ELISA and ELISA inhibition experiments, a quantitation of the results was achieved.

Osváth et al. [8] outlined the presence of BSA and α-globulin in various CD preparations by means of immunoelectrophoresis. Loewenstein et al. [14] showed 10 of the antigens of bovine whey, including albumin, α₂-macroglobulin, transferrin, IgG and IgA to be in common with those of cow hair and CD using crossed radioimmuno-electrophoresis. Based on RAST experiments Osváth et al. [8] calculated that 50% of CM-allergic children also had IgE against CD, although these children never had contact with cow hair and CD. Our experiments confirm these results. 4 of 6 CM-allergic patients also reacted with

CD proteins. Therefore, we asked whether cross-reacting IgE allergens in CM and CD would be responsible for this phenomenon. CD-allergic patients did not have IgE against milk proteins, whereas nearly all milk-allergic patients did exhibit IgE to CD proteins. This finding supported our assumption that sensitization to CM allergens leads to production of IgE antibodies reacting with proteins present in CD extract. These IgE antibodies might either: (1) react with identical or similar proteins present in CDs or (2) they cross-react with proteins bearing common epitopes with milk proteins. The first hypothesis would presume that all patients reacting with a defined allergen in CM would also react with the same protein of identical molecular weight in the CD extract. This was not so for the sera tested in our investigation. Therefore the second hypothesis was taken into consideration.

Inhibition experiments demonstrated that the sera of milk-allergic patients, preincubated with milk, lost their reactivity to CD proteins (fig. 4). In order to identify a defined cross-reacting milk protein, we preincubated patients' sera with commercially available milk protein components (α -lactalbumin, β -lactoglobulin, casein and BSA) before testing on CD extract-coated strips (data not shown). In all 5 investigated patients, IgE reactivity with CD proteins could be abolished by preincubation with whole milk extract, but only in 2 of 5 patients could the IgE reactivity to CD be blunted by casein preincubation. The partial inhibition effect of casein in only 2 patients excluded the possibility of contamination of the commercially available casein with other milk proteins like BSA or globulins. On the other hand, the fact that casein was also able to block IgE binding to CD proteins enlarged the pro-

blems involved in the putative IgE cross-reactivity observed by us and former investigators [8–10]. These investigators outlined exclusively the importance of IgE against BSA and α -globulin.

Preincubation of CM-allergic patients' sera with CD extract and subsequent testing on CM extract-coated strips revealed blocking of IgE binding to casein in 2 patients and additionally to β -lactoglobulin in 1 of 2 patients, respectively (fig. 5). Herein we found evidence that anti- β -lactoglobulin IgE antibodies can also be trapped by CD proteins. Whether the 2 major allergens (20 and 22 kD) or BSA, α -globulin or other CD proteins are the target for these IgE antibodies could not be deduced from our experiments. The present data suggest that CD proteins have fewer immunogenic determinants than CM proteins with respect to IgE reactivity.

In summary, our results reveal the existence of cross-reactive IgE antibodies in milk-allergic patients to allergens in CD. IgE antibodies against β -lactoglobulin and casein were responsible for the observed cross-reactivity with proteins in CD. According to our study we underline the existence of cross-reacting allergens or common epitopes in CM and CD involving casein and β -lactoglobulin. Presuming the possibility that CD extract is contaminated with milk proteins (casein, β -lactoglobulin), e.g., through licking the fur by the animals, we would expect that the reactivity of milk-allergic patients is identical in dander and milk extract. This was not the fact.

Herein evidence of another cross-reaction between food allergens and aeroallergens could be shown and underlines the association between respiratory allergies and food hypersensitivities.

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IgE Reactivity to Milk and Cow Dander

Announcement

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