Indirect Enzyme-Linked Immunosorbent Assay for Measurement of Human Immunoglobulins E and G to Purified Cow's Milk Proteins: Application in Diagnosis of Cow's Milk Allergy

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An indirect double-antibody enzyme-linked immunosorbent assay (ELISA) was developed for the measurement of human immunoglobulin E (IgE) and IgG to the cow's milk proteins (CMP) α -casein, α -lactalbumin, and β -lactoglobulin. Human serum albumin was used as the negative-antigen control. Rabbit anti-human IgE or IgG served as the primary antibody, and horseradish peroxidase-conjugated swine anti-rabbit immunoglobulin served as the secondary antibody. Positive control sera were obtained from patients with well-documented histories of cow's milk allergy, while negative control sera were obtained from cord bloods of healthy full-term infants and from normal adult volunteers without known milk allergy. Test sera were obtained from 41 children (ages, 3 months to 13 years; average age, 2.6 years) with suspected cow's milk allergy and clinical manifestations that included wheezing, rhinitis, atopic dermatitis, urticaria, or gastrointestinal disturbances. The patients were simultaneously evaluated by prick skin testing with scratch test antigen to whole CMP. Although only 13 (32%) of the 41 patients were positive by the prick skin test, 25 (61%) were positive by the IgE ELISA. Of the 25 IgE ELISA-positive patients, 20 were also positive by the IgG ELISA. There was concordance of positive results between skin testing and the IgE ELISA in only 9 patients (22%), and there was concordance of negative results in 12 patients (29%). Discordant results were observed in 20 patients (49%). These results indicate that the ELISA is more sensitive than prick skin testing in the identification of individuals with elevated levels of IgE to CMP.

Cow's milk supplementation in infant nutrition is now commonplace and is reflected in increased incidences of cow's milk protein (CMP)-induced adverse reactions which have diverse clinical manifestations (1, 13, 14). The lack of practical, efficient, and definitive diagnostic tests has restricted the identification of the allergy in these patients.

Until recently, the techniques for the evaluation of suspected immediate-type hypersensitivity to CMP have been limited to skin testing, the radioallergosorbent test (RAST), and elimination-challenge tests, all of which are either impractical or lacking in both sensitivity and specificity. Enzyme-linked immunosorbent assays (ELISAs) are equal to RAST in sensitivity in the detection of allergen-specific immunoglobulin (IgE). ELISAs have several major advantages over RAST, including a long shelf life of reagents, nonradioisotopic probes, and inexpensive instrumentation requirements. These advantages make possible allergen-specific IgE testing in even the most modestly equipped laboratories.

We have developed an indirect double-antibody ELISA for the detection of CMP-specific human IgE and IgG. All reagents used in the assay are commercially available, which simplifies their use in routine clinical laboratories. The procedure uses purified CMPs, i.e., α -casein, α -lactalbumin, and β -lactoglobulin, which have been previously described

as important allergens in cow's milk allergy (CMA) (10). The clinical usefulness of the ELISA was compared with that of prick skin testing in the evaluation of 41 children suspected of having CMA.

MATERIALS AND METHODS

Reagents. Purified bovine α -casein, α -lactalbumin, β -lactoglobulin, and cyanogen bromide-activated Sepharose 4B were obtained from Sigma Chemical Co., St. Louis, Mo. Purified human serum albumin (HSA) was obtained from Behring Diagnostics, San Diego, Calif. Hydrogen peroxide was obtained from J. T. Baker Chemical Co., Phillipsburg, N.J. Rabbit anti-human IgE and IgG and swine anti-rabbit immunoglobulin conjugated with horseradish peroxidase were obtained from Accurate Chemical and Scientific Corp., Westbury, N.Y. *ortho*-Phenylenediamine (OPD) was obtained from Fisher Scientific Co., King of Prussia, Pa. Flat-bottom 96-well polyvinyl chloride microtiter plates were obtained from Dynatech Laboratories, Inc., Alexandria, Va. Normal equine serum (NES) was obtained from Life Technologies, Inc., Chagrin Falls, Ohio. Whole-cow's-milk scratch test antigen (1/20 [vol/vol]) was obtained from Hollister-Stier, Elkhart, Ind.

Sera. Negative control sera were obtained from cord bloods of healthy full-term infants and from normal adult volunteers without histories of allergies. Positive control sera were obtained from patients with well-documented histories of CMP allergy. Test sera were obtained from children undergoing evaluation for suspected CMP allergy. To evaluate the specificity of the primary rabbit antisera (gamma versus epsilon chain), a CMP-positive control serum

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was depleted of IgG by using an immunoabsorbent (rabbit anti-human IgG conjugated to Sepharose 4B) which was prepared as previously described (12).

Patient population. Forty-one patients were referred to the allergy clinic of the Children's Hospital of Philadelphia for evaluation of suspected CMA. They ranged in age from 3 months to 13 years (average age, 2 to 6 years), with a male/female ratio of 1.05 (21:20). The clinical symptoms included wheezing, rhinitis, dermatitis or urticaria, or gastrointestinal disturbances.

ELISA. The ELISA used was a modification of the assay previously described (5). The optimal protein concentration for adsorption of antigens onto the plastic of flat-bottom 96-well polyvinyl chloride microtiter plates was determined by the serial dilution of antigens (1 to 1,000 $\mu\text{g/ml}$) in 0.025 M EDTA (pH 9.3). The antigen diluent was prepared by mixing 50 ml of 0.1 M EDTA (disodium salt) with 70 ml of 0.1 M NaOH and 80 ml of distilled water. Initially, the antigens were evaluated individually, and later, they were evaluated in combination with all three CMP antigens. HSA was evaluated in parallel at the same protein concentrations. Aliquots (0.1 ml) of the serially diluted antigens were added to the appropriate wells, which were then individually sealed with Parafilm and incubated at 4°C for 24 h. The wells were then washed three times with calcium- and magnesium-free phosphate-buffered saline. Initially, the antigen-coated wells were not blocked with irrelevant protein (20% NES for 30 min at 37°C) before human serum was added. Comparative studies to evaluate the influence of various blocking procedures on nonspecific binding were performed subsequently and are described below. Both CMP-positive and -negative control sera were initially diluted in 0.025 M EDTA (pH 7.3). The serum and antiserum diluents were prepared by mixing 50 ml of 0.1 M EDTA with 46 ml of 0.1 M NaOH and 104 ml of distilled water. Initially, the diluent contained 20% (vol/vol) NES to prevent nonspecific binding. Comparative studies of serum diluents which contained different concentrations of NES were later evaluated and are described below. Aliquots (0.1 ml) of the serially diluted human sera were added in duplicate to the positive- and negative-antigen-coated wells, which were then sealed with Parafilm and incubated at 4°C for 24 h. The wells were then washed three times with phosphate-buffered saline. The washing was followed by the addition of 0.1 ml of rabbit anti-human IgE or IgG appropriately diluted in 0.025 M EDTA (pH 7.3) containing 20% NES. The wells were sealed with Parafilm and incubated at 37°C for 1 h. After the wells were washed three times with phosphate-buffered saline, 0.1 ml of swine anti-rabbit immunoglobulin conjugated to horseradish peroxidase, appropriately diluted in 0.025 M EDTA (pH 7.3) containing 20% NES, was added to the wells and incubated at 37°C for 1 h. The wells were then washed six times with phosphate-buffered saline, and 0.1 ml of enzyme substrate was added. The enzyme substrate was prepared immediately before it was used and consisted (per 100 ml) of 0.013 M OPD (137.5 mg), 0.2 M Tris hydrochloride (3.2 g), 0.15 M NaCl (pH 6.0; 0.9 g), and 0.003 M H_2O_2 (30 μl of 30% H_2O_2). The OPD was previously weighed and placed in a 150-ml translucent bottle. The Tris hydrochloride saline buffer (100 ml) was prepared separately and adjusted to pH 6.0 with 50 μl of 0.1 M NaOH just before it was added to the bottle containing the OPD. The OPD was then dissolved in Tris hydrochloride saline by vigorous shaking for 2 to 3 min. Finally, 30 μl of 30% (wt/wt) H_2O_2 was added to the enzyme substrate solution and mixed thoroughly, and 0.1 ml was added to each well of the microtiter plate. The plates were

FIG. 1. Influence of protein concentration of β -lactoglobulin- and HSA-coated wells on ELISA human serum IgE binding. The wells were coated for 24 h at 4°C with either β -lactoglobulin or HSA at concentrations of 1,000 (A), 100 (B), 10 (C), or 1 (D) $\mu\text{g/ml}$. The absorbance values of a serially diluted CMP-positive serum on wells coated with β -lactoglobulin (—) and HSA (---) are illustrated. S.D., Standard deviation; O.D.₄₉₀, OD at 490 nm.

incubated for 10 min at room temperature, and the enzyme reactions were stopped by adding 50 μl of 4 N H_2SO_4 (53 ml, 36 N qs 500 ml). The optical density (OD) of each well was then determined spectrophotometrically with a Dynatech MR 580 autoreader set at 490 nm. Net absorbance values were calculated by the following formula: net OD = (mean OD of CMP antigen - 2 SD) - (mean OD of HSA antigen + 2 SD), where SD is the standard deviation.

Skin tests. Prick skin tests with whole-cow's-milk antigen (1/20 [vol/vol]; Hollister-Stier) were done on all patients and compared with saline and histamine controls (1.8 U/ml). Diameters of both erythema and induration were measured. Reactions with erythema and induration greater than or equal to the histamine control were considered positive.

RESULTS

Antigen binding to wells of ELISA plates. The optimal conditions for coating ELISA plates were evaluated by determining the influence of antigen protein concentrations on the detection of CMP-specific IgE. Figure 1 illustrates the binding of IgE by a serially diluted CMP-positive control serum to β -lactoglobulin- and HSA-coated wells. Optimal specific and nonspecific binding of IgE was observed at protein concentrations of 1 to 10 $\mu\text{g/ml}$. Similar titrations were obtained with α -casein and α -lactalbumin. Therefore, in experiments which compared all three CMP antigens

together relative to individual antigens coated on wells, each antigen was used at a concentration of 5 $\mu\text{g/ml}$ (total, 15 $\mu\text{g/ml}$), with the HSA control antigen at 15 $\mu\text{g/ml}$. CMP antigens which were evaluated individually were supplemented with HSA at a concentration of 10 $\mu\text{g/ml}$ to equalize the total protein concentration.

Influence of blocking proteins on ELISA nonspecific IgE binding. Various concentrations of NES in the human serum diluent were evaluated for the ability to reduce nonspecific reactions. Significant nonspecific binding of IgE to both β -lactoglobulin- and HSA-coated wells occurred with diluents lacking blocking proteins (Fig. 2A). Significant reductions of nonspecific binding were observed with diluents containing up to 20% NES, without significant reductions in specific binding. Diluents containing 20% NES reproducibly resulted in lower nonspecific binding of IgE to HSA-coated wells and was therefore used for all subsequent experiments.

Influence of total serum IgG on ELISA human serum IgG binding to β -lactoglobulin- and HSA-coated wells. The importance of including a negative-antigen control in the evaluation of the sera of the patients is demonstrated by a longitudinal study of a patient with polyclonal-B-cell activation whose total serum IgG fluctuated markedly over 9 months. The amount of nonspecific IgG binding was directly propor-

FIG. 2. Influence of blocking protein (NES) in human serum diluent on ELISA human serum IgE binding. The wells were coated for 24 h at 4°C with 1 μg of β -lactoglobulin or HSA per ml. A CMP-positive serum was serially diluted in diluent containing no NES (A), 5% NES (B), 10% NES (C), or 20% NES (D). The absorbance values for a serially diluted CMP-positive serum on wells coated with β -lactoglobulin (—) and HSA (---) are illustrated. S.D., Standard deviation; O.D.₄₉₀, OD at 490 nm.

FIG. 3. Influence of total serum IgG on ELISA human serum IgG binding to β -lactoglobulin- and HSA-coated wells. CMP-positive serum samples which varied markedly in total IgG were obtained from a patient with polyclonal-B-cell activation. The absorbance values obtained for four samples (1/400 dilution) collected at 2- to 4-month intervals over 9 months are illustrated. S.D., Standard deviation; O.D.₄₉₀, OD at 490 nm.

tional to the total serum IgG level (Fig. 3). Without the use of the negative-antigen control, the amount of CMP-specific IgG would have been inaccurately estimated. Although the example illustrated in Fig. 3 represents an extreme, the normal variable levels of IgG which occur in children and are age dependent require the use of negative-antigen controls in addition to negative-serum controls to monitor this intrinsic variable.

Evaluation of heavy-chain specificity for the primary ELISA antibody. A CMP-positive serum was depleted of IgG and compared with the untreated serum in the binding of IgG and IgE to both β -lactoglobulin- and HSA-coated wells. IgG depletion of the serum had no significant effect on IgE binding (Fig. 4). There were no significant cross-reacting specificities present in the rabbit anti-human IgE antiserum. The depletion was considered complete in that IgG binding to β -lactoglobulin-coated wells with the IgG-depleted serum was reduced to absorbance levels obtained with HSA-coated wells.

Comparison of multiple- versus individual-CMP antigen coating on ELISA IgE binding. Individuals have variable IgE specificities to the heterogeneous population of proteins that make up cow's milk. Three of these proteins, α -casein, α -lactalbumin, and β -lactoglobulin, have been shown to be particularly important in CMP intolerance (10). Therefore, these three antigens were evaluated individually and in combination. The use of multiple-antigen specificities enhanced the detection of CMP-specific IgE but was not additive for the individual antigens (Fig. 5). Further, in all sera tested with individual antigens, most of the IgE binding occurred with α -casein.

Comparison of methods for reducing nonspecific IgE binding in ELISA with multiple-antigen-coated wells. The influence of irrelevant-protein (20% NES) blocking of antigen-coated wells before the addition of human sera is illustrated in Fig. 6. Wells which were not blocked with NES and received human serum lacking NES (bar A) produced the

FIG. 4. Influence of IgG depletion of a CMP-positive human serum on ELISA IgE binding to β -lactoglobulin- and HSA-coated wells. The wells were coated for 24 h at 4°C with 1 μ g of β -lactoglobulin or HSA per ml. Dilutions (1/50 for IgE ELISA and 1/1,000 for IgG ELISA) of untreated and IgG-depleted CMP-positive sera were added to antigen-coated wells. The absorbance values for untreated and IgG-depleted sera on β -lactoglobulin- and HSA-coated wells are illustrated. S.D., Standard deviation; O.D.₄₉₀, OD at 490 nm.

highest level of nonspecific IgE binding. Furthermore, blocking alone (bar C), without the addition of NES to the human serum diluent, was inadequate in reducing nonspecific reactions. Blocking wells with NES and the addition of NES to the human serum diluent (bar D) provided the highest level of net absorbance (specific IgE binding) and was used in subsequent studies for the evaluation of the sera from controls and patients.

ELISA intraplate variance for measuring human IgE and IgG binding to CMP and HSA antigen-coated wells. Conditions which were found to be optimal for performance of the assay include the following: (i) coating of the wells with 5 μ g of each of the CMP antigens per ml together (total protein, 15 μ g/ml) and 15 μ g of HSA per ml for 24 h at 4°C; (ii) blocking of the antigen-coated wells with 20% NES in 0.025 M EDTA (pH 7.3) for 30 min at 37°C before the addition of human sera (after the blocking step, the plates were emptied without washing); (iii) dilution of the human sera in 0.025 M EDTA (pH 7.3) containing 20% NES (the control and patient sera were diluted 1/5 and 1/1,000 for the IgE and IgG assays, respectively); and (iv) dilution of the primary and secondary antisera in 0.025 M EDTA (pH 7.3)–20% NES. The intraplate variance for replicate samples of CMP-positive and -negative sera are illustrated in Table 1. High-absorbance replicates yielded lower percent coefficients of variance (4.5 to 6.6%), whereas low-absorbance replicates had higher but acceptable percent coefficients of variance (4.9 to 12.1%).

ELISA evaluation of sera from negative and positive control groups. Table 2 summarizes data obtained from the application of the ELISA to the detection of CMP-specific IgE and

IgG in sera from three groups, i.e., cord bloods from healthy full-term deliveries, normal adult peripheral blood, and peripheral blood from documented CMP-intolerant children. Of the five sera from CMP-intolerant children, one had an IgE net absorbance value (OD, 0.107) which fell within the 95% confidence limit for cord blood (OD, 0.114) and normal adult peripheral blood (OD, 0.136). The remaining sera were well outside the 95% confidence limit of both negative control groups. Two of the sera from CMP-intolerant children had IgG net absorbance values (ODs, 0.173 and 0.326) which fell within the 95% confidence limit for cord bloods (OD, 0.345) and normal adult controls (OD, 0.451). For the evaluation of test sera from patients, the highest IgE (OD, 0.269) and IgG (OD, 0.339) net absorbance values obtained with cord bloods were arbitrarily used as the limits for denoting negative reactivity.

Comparison of CMP ELISA and prick skin tests in the evaluation of 41 children with suspected CMA. The 41 children with suspected CMA ranged in age from 3 months to 13 years (average age, 2.6 years) with a male/female ratio of 1.05 (21:20). Their clinical symptoms included wheezing (53.6%, or 22 of 41), rhinitis (31.7%, or 13 of 41), skin involvement such as dermatitis or urticaria (29.2%, or 12 of 41), and gastrointestinal disturbances (7.3%, or 3 of 41). Some patients had more than one clinical symptom. Of 41 patients, 13 (32%) had positive prick skin tests, while 25 patients (61%) were IgE ELISA positive (Table 3). Of the 25 IgE ELISA-positive sera, 20 were also IgG positive. There was concordance of positive results between skin testing and

FIG. 5. Influence of multiple-CMP and individual-CMP antigen coating on ELISA IgE binding. The wells were coated for 24 h at 4°C with 5 μ g each of β -lactoglobulin, α -casein, and α -lactalbumin per ml (A); 5 μ g of α -casein and 10 μ g HSA per ml (B); 5 μ g of α -lactalbumin and 10 μ g of HSA per ml (C); 5 μ g of β -lactoglobulin and 10 μ g of HSA per ml (D); and 15 μ g of HSA per ml (E). Absorbance values obtained with a 1/5 dilution of pooled CMP-positive human sera on each of the antigen formulations are illustrated. S.D., Standard deviation; O.D.₄₉₀, OD at 490 nm.

FIG. 6. Comparison of methods for reducing nonspecific ELISA human serum IgE binding. The IgE ELISAs were done as follows. Antigen-coated wells were not blocked before the addition of human serum in diluent lacking NES (A) or containing 20% NES (B). Antigen-coated wells were blocked with 20% NES before the addition of human serum in diluent lacking NES (C) or containing 20% NES (D). Absorbance values obtained with a 1/5 dilution of pooled CMP-positive human sera on CMP- and HSA-coated wells are illustrated. S.D., Standard deviation; O.D.₄₉₀, OD at 490 nm.

the IgE ELISA in only 9 patients (22%), and there was concordance of negative results in 12 patients (25%). Discordant results were observed in 20 patients (49%).

DISCUSSION

Cow's milk contains more than 25 protein components that may induce specific-antibody production. The most frequently observed allergenic components of cow's milk are β -lactoglobulin, α -casein, and α -lactalbumin (1, 10). CMA may involve one or more of the four classic types of hypersensitivity reactions described by Gell and Coombs (1, 14, 15). Further, CMP-specific IgG has been associated with late reactions to CMP (4).

TABLE 1. Intraplate variance for the measurement of human IgE and IgG binding to CMP and HSA antigen-coated wells

Antigen-antibody reaction ^a (n = 12)	Mean OD	SD	CV (%) ^b	Net OD ^c
CMP-positive IgE	0.760	0.050	6.6	0.504
HSA-positive IgE	0.136	0.010	7.4	
CMP-positive IgG	1.028	0.050	4.5	0.704
HSA-positive IgG	0.204	0.010	4.9	
CMP-negative IgE	0.165	0.020	12.1	-0.028
HSA-negative IgE	0.133	0.010	7.5	
CMP-negative IgG	0.233	0.02	8.6	0.001
HSA-negative IgG	0.172	0.01	5.8	

^a Reaction mixtures were prepared with CMP-positive and -negative reference sera which were evaluated at a dilution of 1/5 for IgE and 1/1,000 for IgG.

^b Coefficient of variance.

^c Derived from the formula given in Materials and Methods.

TABLE 2. ELISA CMP-IgE and -IgG net absorbance values obtained with the sera of two negative control groups and patients with documented CMP intolerance

Source of serum (n)	Immunoglobulin	ELISA CMP-antigen net absorbance ^a (range)
Cord blood (10)	E	0.038 $\pm$ 0.076 (-0.008-0.269)
	G	0.117 $\pm$ 0.228 (-0.133-0.339)
Normal adult (10)	E	0.064 $\pm$ 0.172 (-0.015-0.240)
	G	0.155 $\pm$ 0.296 (-0.118-0.430)
CMP-intolerant children (5)	E	0.481 $\pm$ 0.540 (0.107-0.737)
	G	0.586 $\pm$ 0.760 (0.173-1.089)

^a Arithmetic mean $\pm$ two standard deviations.

CMA usually appears within the first 2 or 3 months of life and often disappears by the age of 2 or 3 years (1). However, well-documented cases of CMA have been described for teenagers and adults (15). The gastrointestinal tract is the organ most frequently involved, followed by the respiratory tract and skin.

A number of methods have been used to support the diagnosis of CMA. Skin testing by pricking or intradermal injection is the most widely used diagnostic procedure in current allergy practice (9). Unfortunately, both false-positive and false-negative reactions occur frequently, a fact which can be attributed to variables intrinsic to the procedure, including the variability of responsiveness of the test site (3, 10, 11, 13). Evaluation of histological changes in intestinal biopsy samples after cow's milk challenge has been described in a number of studies of patients with gastrointestinal symptoms (17-19). The measurement of antibodies to CMPs by hemagglutination, precipitation, and RAST has been used extensively in the diagnosis of CMA (2, 16). ELISAs have been applied to the measurement of IgG, IgA, and IgM to CMP (7, 8). ELISAs have been shown to be as sensitive as RAST for the detection of allergen-specific IgE (2, 6). Unfortunately, apart from elimination and challenge, none of the routinely available laboratory tests have a degree of reliability sufficient for definitive diagnosis. The disparities between these procedures are attributable, at least in part, to considerable qualitative and quantitative variations in the reagents used in these assays. In addition to reagent variability, skin testing is subject to many other intrinsic variables, including subjective interpretation and variation of responsiveness of the test site. RAST, although sensitive, continues to yield unacceptably high levels of negative results in patients who are exquisitely sensitive to the offending allergen (2).

We have developed an indirect ELISA for the detection of CMP-specific human IgE and IgG. The assay utilizes all commercially available reagents, including purified CMP antigens, which allows for the normalization of assay conditions on a longitudinal basis. Although the population of

TABLE 3. Comparison of prick skin test and ELISA results for CMP-specific IgE in 41 children evaluated for CMA

Skin test result	No. of patients with ELISA net OD values of:		Total no. of patients
	>0.27	$\leq$ 0.27	
Positive	9	4	13
Negative	16	12	28
Total	25	16	

patients evaluated in this study was small, the data indicate that this ELISA is more sensitive than prick skin testing in the identification of individuals with elevated levels of IgE to CMP. The observed performance differences between the two procedures cannot be totally related to qualitative differences in the reagents used in the two assays. However, a comparison (on a microgram-per-microgram basis) by ELISA of the antigenicity of the material used for skin testing and the CMP formulation used in this study revealed that the skin test antigen (net OD, 0.196) was less antigenic than the formulation of the three purified CMPs (net OD, >1.4) routinely used for the ELISA. These data indicate that the formulation of appropriate antigenic specificities is crucial in the development of in vitro procedures for the detection of CMP-specific IgE. The performance characteristics of the ELISA may also be improved by the use of primary and secondary probes which have much higher affinities than realized with antibody-antibody systems, such as biotin-conjugated antibodies and avidin-conjugated enzymes.

Although we have demonstrated considerable qualitative differences in the antigenicity of the skin-test antigen versus the antigen used for ELISA, the high level of discordant results between the two procedures (49%) cannot be attributed solely to differences in reagents. The results suggest that the IgE ELISA is much more sensitive in the detection of CMP-specific IgE in the vasculature than the classical skin test of mast cell-associated CMP-specific IgE is in the dermis. Further, the high percentage of patients with elevated levels of IgE to CMP, as determined by the IgE ELISA, suggests that CMP-specific IgE may be a significant integral component of humoral immune responses to CMP in the age group studied. This conclusion is supported by preliminary studies of CMP-specific IgE in paired cord versus 4-to-8-week-postpartum blood of cow milk-fed, full-term healthy infants. In these studies, a high percentage of seroconversion by the IgE ELISA has been observed (manuscript in preparation). These data suggest that critical levels of circulating allergen-specific IgE must be achieved before adequate sensitization of tissue mast cells in the respiratory tract, gastrointestinal tract, and skin can be established to effect the clinically observed manifestations of antigenic challenge. The application of the ELISA described to the longitudinal evaluation of patients during the critical time observed with CMA may provide important information regarding the kinetics and amplitude of IgE responses to CMP in normal versus atopic individuals. Such data should be helpful in establishing appropriate criteria for the accurate identification of those patients with abnormal humoral responses to CMP. However, until such data are available, the diagnosis of CMA is dependent on clinical considerations in conjunction with the in vitro diagnostic methods currently available.

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