

Determination of food specific IgE levels over time can predict the development of tolerance in cow's milk and hen's egg allergy

Lynette P. C. Shek, MBBS, MRCP,^a Lars Soderstrom, MSc, FRSS,^b
Staffan Ahlstedt, PhD,^{b,c} Kirsten Beyer, MD,^a and Hugh A. Sampson, MD^a
New York, NY, and Uppsala and Stockholm, Sweden

Background: The majority of children with cow's milk and hen's egg allergy develop clinical tolerance with time. However, there are no good indices to predict when and in whom this occurs.

Objective: The aim of this study was to determine if monitoring food specific IgE levels over time could be used as a predictor for determining when patients develop clinical tolerance.

Methods: Eighty-eight patients with hen's egg and 49 patients with cow's milk allergy who underwent repeated double-blind, placebo-controlled food challenges were included in the study. Using the Pharmacia CAP-System FEIA, specific IgE (sIgE) levels to cow's milk and hen's egg were retrospectively determined from stored serum samples obtained at the time of the food challenges. Logistic regression was used to evaluate the relationship between tolerance development and the decrease in sIgE levels over a specific time period between the two challenges.

Results: Twenty-eight of the 66 egg-allergic and 16 of the 33 milk-allergic patients lost their allergy over time. For egg, the decrease in sIgE levels ($P = .0014$) was significantly related to the probability of developing clinical tolerance, with the duration between challenges having an influence ($P = .06$). For milk there also was a significant relationship between the decrease in sIgE levels ($P = .0175$) and the probability of developing tolerance to milk but no significant contribution with regard to time. Stratification into 2 age groups, those below 4 years of age and those above 4 years of age at time of first challenge, had an effect, with the younger age group being more likely to develop clinical tolerance in relation to the rate

Abbreviations used

DBPCFC: Double-blind, placebo-controlled food challenge
sIgE: Specific IgE

of decrease in sIgE. The median food sIgE level at diagnosis was significantly less for the group developing "tolerance" to egg ($P < .001$), and a similar trend was seen for milk allergy ($P = .06$). Using these results, we developed a model for predicting the likelihood of developing tolerance in milk and egg allergy based on the decrease in food sIgE over time. **Conclusion:** We found that the rate of decrease in food sIgE levels over time was predictive for the likelihood of developing tolerance in milk and egg allergy. Using the likelihood estimates from this study could aid clinicians in providing prognostic information and in timing subsequent food challenges, thereby decreasing the number of premature and unnecessary double-blind, placebo-controlled food challenges. (*J Allergy Clin Immunol* 2004;114:387-91.)

Key words: Food allergy, egg allergy, milk allergy, CAP System FEIA, quantitative IgE, diagnostic test, natural history

Food allergy affects 6% to 8% of children under the age of 3 years, and the incidence appears to be rising.¹ Fortunately, the majority of children develop tolerance within the first 3 to 5 years of life. The 2 foods responsible for most of these reactions are cow's milk and hen's egg. About 80% of children with cow's milk allergy become clinically tolerant by the third year of life² and about 50% do so for egg.³ To date, there are no good indices to predict when and in whom this occurs.

Several studies have looked at various factors that could be of prognostic value in food allergy. For egg allergy, 1 study found that the main predictors were symptoms at initial exposure and size of prick skin tests reactions.³ In the case of cow's milk allergy, James and Sampson found that neither of these was predictive of tolerance. Instead, the initial casein and β -lactoglobulin specific IgE were lower and decreased significantly in patients who became tolerant.⁴ A later study confirmed the finding that the concentration of milk specific IgE (sIgE) was lower in the group of children who became tolerant compared to those with lasting allergy.⁵ Among peanut-allergic children, the initial clinical reaction as well as the presence of concomitant allergy to other foods were reported to be

From ^athe Division of Pediatric Allergy & Immunology and Jaffe Institute for Food Allergy, The Mount Sinai School of Medicine; ^bPharmacia AB Diagnostics; and ^cthe Institute of Environmental Medicine and Center of Allergy, Karolinska Institute.

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Reprint requests: Hugh A. Sampson, MD, Department of Pediatrics, Box 1198, The Mount Sinai School of Medicine, One Gustave L. Levy Place, New York, NY 10029-6574. E-mail: hugh.sampson@mssm.edu.

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prognostic factors.⁶ In that study, the initial peanut sIgE level was not significantly different between those who developed tolerance and those who did not. However, peanut sIgE measured at the time of passing oral peanut challenge was significantly lower in those who passed the challenge. Recently, it has been shown that the presence of IgE to certain sequential epitopes on different cow's milk proteins is associated with persistent cow's milk allergy.⁷ However, it does not predict the time point of tolerance development.

Unfortunately, no single factor has been consistently found to be predictive of tolerance in all the various studies. Also, different criteria were used to determine who was food-allergic, with none of the studies relying on positive double-blind, placebo-controlled food challenges (DBPCFCs) as the inclusion criteria in all cases. The DBPCFC is the "gold standard" for diagnosis of food allergy, and up to 60% of cases that are thought to be food-allergic prove otherwise when subjected to the DBPCFC.⁸ Another inconsistency between the studies is the different durations of follow-up.

The measurement of food sIgE in children is widely available, easily performed, and reproducible. The Pharmacia CAP-System FEIA (Pharmacia Diagnostics AB, Uppsala, Sweden) is an accurate and standardized way of measuring allergen sIgE. The aim of our study was to monitor food sIgE levels over time and to determine whether this could be used as a predictor for determining when patients develop clinical tolerance.

METHODS

Study population

Eighty-eight patients with hen's egg and 49 patients with cow's milk allergy who had undergone 2 or more DBPCFCs to cow's milk or hen's egg at our research unit were included in the study. Patients were divided into 2 groups; (1) patients with at least 2 positive DBPCFCs representing those who remained allergic, and (2) patients with an initial positive DBPCFC followed by a negative one representing those who became tolerant. DBPCFCs were performed under physician supervision as previously described.⁹ As most of these patients were recruited before the 95% predictive decision points for clinical reactivity were established,¹⁰ challenges were performed yearly for up to 10 years or until a final clinical decision could be established as either "tolerant" or "persistent allergy." Informed consent was obtained and the study was approved by the Institutional Review Board.

sIgE measurement

sIgE to cow's milk and hen's egg were measured retrospectively from stored serum samples using the Pharmacia CAP-System FEIA, with a range of detection from <0.35 to >100 kU/L. Serum was obtained at the time of the food challenge and stored at -80°C until analyzed. It has been shown that IgE remains stable for decades when stored frozen.¹¹

Statistical methods

The primary outcome was "tolerance" or "persistent allergy" as determined by DBPCFC to egg or milk. For comparison between groups, we used the Mann-Whitney test. Logistic regression was used

to evaluate the relationship with the decrease in sIgE levels between the first and last challenge and also the time period between these two challenges. The relative change in sIgE levels between the first and last challenge was defined as $X = \ln(\text{sIgE last challenge}) - \ln(\text{sIgE first challenge})$ and the time between these occasions was defined as $Z = \ln(\text{age at last challenge} - \text{age at first challenge})$, measured in months. The model was then formulated as the probability of becoming tolerant as a function of the relative change, X , and the time, Z :

$$\text{logit}(\text{Outgrown}) = \alpha + \beta X + \gamma Z$$

Odds ratios were estimated using the model and appropriate tests and confidence intervals were generated according to Wald, using a P value of .05 as significant. Computerized statistical analyses were performed using SAS System V8.01.

RESULTS

Twenty-eight patients who had become tolerant to hen's egg and 60 who had not were identified; for milk this was 16 who had become tolerant and 33 who had not. Table I shows the patient characteristics. Most of the patients had atopic dermatitis as their first sign of food allergy, with these symptoms usually presenting in infancy. However, the diagnosis was confirmed with DBPCFC much later, as seen from the median age of first challenge. Five of the patients in the "persistent" egg allergy group had never knowingly ingested egg prior to the challenge; they had avoided egg because of a strong family history of food allergy. This was also true for one patient from the "tolerant" milk allergy group. There was no significant difference in the number of patients who had concomitant allergy to another food or the age of first and last challenge. The sIgE at first challenge was significantly less in the "tolerant" group when compared to the "persistent" group for egg allergy, with a similar trend seen for milk allergy ($P < .001$ and $P = .06$, respectively; Table I).

Application of the logistic model

The logistic model was applied separately for egg and milk. For both, the relative change was negative; that is, the sIgE levels decreased over time. For egg there was a significant relationship between the decrease in sIgE levels ($P = .0014$) and also an influence of the time between the first and last challenge ($P = .06$) to the probability of developing tolerance. For milk there was also a significant relationship between the decrease in sIgE levels ($P = .0175$) and the probability of developing tolerance but no significant contribution with regard to time (Table II). As can be seen from Table II and Figures 1 and 2, the slope of the relative change in sIgE levels is steeper for egg compared to milk; ie, a greater decrease in the sIgE concentration between the first and last challenge for egg has a greater effect on the probability of becoming clinically tolerant. The effect of the duration between the first and last challenge almost reached statistical significance for egg ($P = .06$) but not for milk ($P = .27$).

TABLE I. Characteristics of egg- and milk-allergic patients

	Egg allergy (n = 88)		Milk allergy (n = 49)	
	"Persistent" group (n = 60)	"Tolerant" group (n = 28)	"Persistent" group (n = 33)	"Tolerant" group (n = 16)
Male sex (n)	33 (55.0%)	18 (64.3%)	25 (75.8%)	11 (68.8%)
Onset of food-induced symptoms in infancy (n)	53 (88.3%)	24 (85.7%)	30 (90.9%)	13 (81.3%)
	(5 avoided from infancy)			(1 avoided from infancy)
AD as initial symptom (n)	42 (70.0%)	22 (78.6%)	24 (72.7%)	8 (50.0%)
Median age at 1st challenge (months, range)	42.6 (6.1-211.3)	35.0 (5.8-138.6)	34.7 (10.8-106.9)	34.2 (4.0-135.3)
Median age at last challenge (months, range)	82.7 (21.3-266.3)	80.9 (31.9-220.9)	78.2 (35.6-177.6)	82.6 (26.0-183.1)
Presence of other food allergies (n)	42 (70.0%)	20 (71.4%)	28 (84.8%)	11 (68.8%)
Initial food sIgE (kU/L, range)	16.25 (<0.35->100.0)*	2.41 (<0.35->100.0)	29.1 (0.54->100.0)	4.63 (0.60->100.0)

AD, Atopic dermatitis; sIgE, specific immunoglobulin E.

There were no statistically significant differences between the "persistent" and "tolerant" groups for either egg and milk allergy except for initial sIgE in the egg allergy group.

* $P < .001$.

TABLE II. Estimated coefficients from logistic regression for egg and milk, with stratification by age at first challenge

	Estimate	SE	χ^2	P value	Total (n)	"Tolerant" (n)
Egg, all ages					88	28
Intercept	1.06114	1.3059	0.66	.4165		
ln(Time)	-0.75515	0.4040	3.49	.0616		
ln(Change)	-0.78567	0.2461	10.19	.0014		
Milk, all ages					49	16
Intercept	0.675711	1.7663	0.15	.7021		
ln(Time)	-0.54553	0.4945	1.22	.2700		
ln(Change)	-0.60485	0.2546	5.64	.0175		
Egg, <4 years					55	21
Intercept	1.599274	1.6357	0.96	.3282		
ln(Time)	-0.81333	0.4979	2.67	.1024		
ln(Change)	-0.73404	0.2807	6.84	.0089		
Egg, >4 years					33	7
Intercept	0.577023	2.4038	0.06	.8103		
ln(Time)	-0.79303	0.7502	1.12	.2905		
ln(Change)	-0.88888	0.5214	2.91	.0882		
Milk, <4 years					37	11
Intercept	-0.79262	2.2959	0.12	.7299		
ln(Time)	-0.26435	0.6226	0.18	.6711		
ln(Change)	-0.91243	0.3924	5.41	.0200		
Milk, >4 years					12	5
Intercept	3.302088	3.2565	1.03	.3106		
ln(Time)	-1.06514	0.9325	1.30	.2534		
ln(Change)	-0.15053	0.4150	0.13	.7168		

SE, standard error.

Comparison between children <4 years and >4 years old at time of first challenge

The total group of children was then poststratified into two age groups, those below and those above 4 years of age at their first visit. The logistic model was applied to each strata. For egg, the significant relationship with the

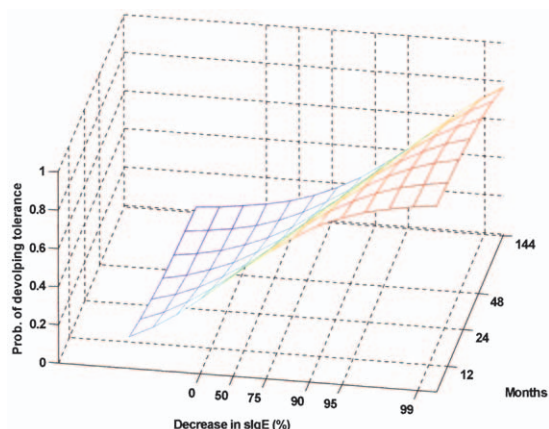


FIG 1. The relationship between developing tolerance in egg allergy and the relative change in sIgE antibody concentration and time.

decrease in sIgE antibody levels remained ($P = .0089$) for the age group below 4 years, although for the children above 4 years the relationship became weaker ($P = .088$). The relationship with the time between challenges became weaker for both age groups (Table II). For milk, the slope for the relative change of sIgE became steeper for the children below 4 years of age ($P = .02$) compared to the results for all children (Fig 3, b). For the children older than 4 years, no relationship was seen with the decrease in sIgE or with the time between the challenges (Table II). This was probably due to the fact that only 12 patients were in that group and out of those only 5 became tolerant.

Using the logistic models, we developed estimates for the probability of a child developing tolerance based on the decrease in sIgE levels and time. For a child with egg allergy below the age of 4 years at the first challenge, the probability of the child developing tolerance was .52 for a decrease of 50%, .65 for a decrease of 75%, .78 for

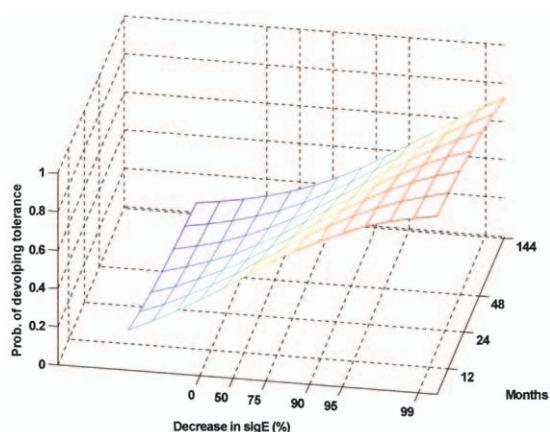


FIG 2. The relationship between developing tolerance in milk allergy and the relative change in sIgE antibody concentration and time.

a decrease of 90%, and .95 for a decrease of 99% in sIgE levels over 12 months (Fig 3; Table III). However, if a decrease of 90% in egg sIgE did not occur over a 12-month period, but over 5-year period, the probability of developing tolerance was less (.49; Fig 3). Using the same assumptions for milk, the probability of developing tolerance was .31 for a decrease of 50%, .45 for a decrease of 70%, .66 for a decrease of 90%, and .94 for a decrease of 99% in sIgE levels over 12 months. In the same way as for hen's egg, a decrease of 90% in milk sIgE over a 5-year period gave a lower probability for developing clinical tolerance (.56; Fig 3).

DISCUSSION

In this study, we have shown that there is a relationship between the degree of decrease in food sIgE antibody concentrations over time and the likelihood of developing tolerance. A greater decrease in sIgE levels over a shorter period of time was indicative of a greater likelihood of developing tolerance. Of greater practical relevance to the clinician, we were able to develop estimates for the likelihood of developing tolerance based on the degree of decrease in food sIgE over a 12-month period (Table III). Levels of food sIgE that could predict clinical reactivity to egg and milk with greater than 95% certainty have been previously established and confirmed prospectively.^{10,12} Our estimates used in conjunction with these decision levels could reduce the number of DBPCFCs performed. For example, a patient below the age of 4 years with egg allergy could have an initial egg sIgE level of 10 kU/L, the 95% positive predictive value for clinical reactivity being 7 kU/L. If a repeated measurement a year later fell to 1 kU/L (a decrease of 90%), the clinician could be confident (based on our model) that the patient had at least a 78% chance of passing the food challenge at that point. If the repeat measurement was 5 kU/L (a decrease of 50%), the likelihood of passing the challenge would fall to 52%. The clinician may then recommend delaying a repeat food

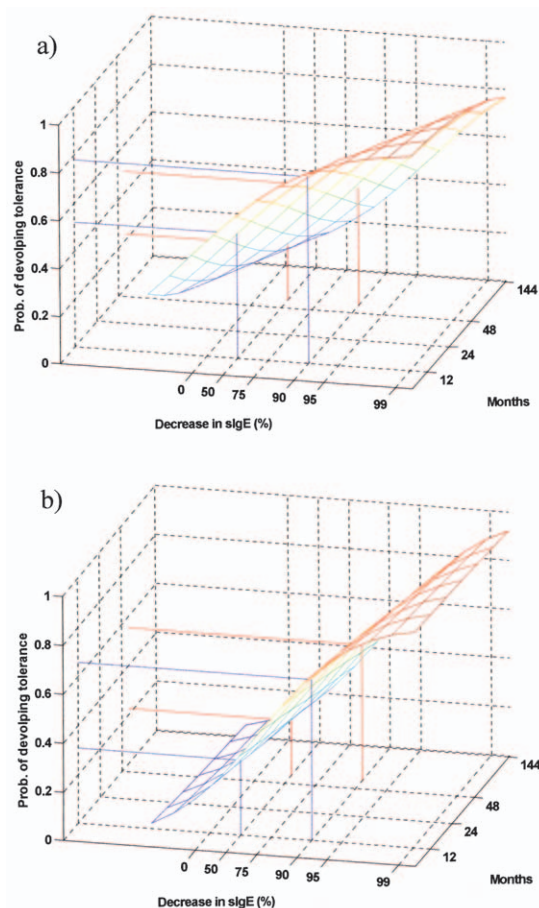


FIG 3. The relationship between developing tolerance and the relative change in sIgE antibody concentration and time for (a) hen's egg and age below 4 years and (b) cow's milk and age below 4 years at challenge.

TABLE III. Probability of developing tolerance for egg and milk allergy based on the decrease in respective food sIgE levels over 12 months

Decrease in sIgE over 12 months (%)	Probability of developing tolerance	
	Egg allergy	Milk allergy
50	.52	.31
75	.65	.45
90	.78	.66
99	.95	.94

sIgE, specific immunoglobulin E.

Data for children diagnosed before age 4 years.

challenge until the egg sIgE levels fell further, thereby eliminating the discomfort, expense, risks, and time wasted should the second challenge fail.

We also were able to show that poststratification into two age groups, greater and less than 4 years of age at the initial challenge, gave different predictive patterns for egg and milk. We chose the age of 4 years as the cutoff because

patients diagnosed below this age were more likely to develop clinical tolerance. It would be important to develop estimates for patients below and above this age as the outcome is different for each group. For children diagnosed before 4 years of age, the slope in our model was steeper; that is, a similar decrease in sIgE resulted in a greater likelihood of developing tolerance compared to those older than 4 years. However, it should be noted that we had fewer patients in the older age group for both egg and milk, and more patients would be needed to make the appropriate statistical estimates.

For food allergy, other factors have been reported to be of prognostic significance in many studies. These include age at presentation, symptoms at diagnosis, concomitant allergy to other foods, and initial food sIgE. Of these, none were significantly different in our study between the “persistent” and “tolerant” groups except for initial sIgE levels in egg-allergic patients. This has been seen in studies of cow’s milk allergy.^{4,13} However, reports in egg^{3,14,15} and peanut allergy are conflicting.⁶ For milk allergy, IgE binding to certain linear epitopes seem to predict persistence of allergy.¹⁶⁻¹⁸ Our laboratory is pursuing this further to see if it is predictive of persistence in a larger cohort of patients and in other foods. However, it cannot predict the time point when tolerance has developed.

In this study, we included only patients whose initial diagnosis and final evaluation of tolerance development was established by DBPCFC, eliminating the problem of uncertain diagnoses. Also, we were able to include the duration between challenges into our statistical model. This has been a shortcoming in previous studies, where the length of follow-up varied, making comparison between studies difficult. The rate of decline of food sIgE is also affected by time. In our model, we standardized our estimates to a 12-month period, which is the average length of time between measurements of sIgE in clinical practice. The clinician can measure sIgE levels over a 12-month period and apply our model to estimate the probability of the child developing tolerance. It was intriguing to note that the likelihood of developing tolerance to egg was influenced by time, but this was less so for milk. It is tempting to postulate that this could be due to differences in the level of maturity of the gastrointestinal tract or immune system in handling the two different antigens.

It should be noted that the model proposed applies to patients with IgE-mediated milk and egg allergy only. About 40% to 50% of milk allergy is non-IgE-mediated,¹⁹ and prognostic factors in these patients are poorly understood. Also, the majority of our patients had atopic dermatitis, a population in which IgE levels are greater compared to patients without atopic dermatitis. Further studies are needed to determine if our estimates apply to patients without atopic dermatitis. More importantly, prospective studies are required, both by us and other investigators, to see if our model will hold up to scrutiny in a larger cohort of patients.

In summary, we have used a simple, standardized, reproducible and objective test, the measurement of food sIgE by Pharmacia CAP System FEIA, to construct a model for predicting the likelihood of developing clinical tolerance in individuals with milk and egg allergy. The confirmation of this model, and subsequent application in clinical practice, would aid clinicians in the timing of food challenges and in providing prognostic information for patients and their families.

REFERENCES

1. Sampson HA. Food allergy. Part 1: immunopathogenesis and clinical disorders. *J Allergy Clin Immunol* 1999;103:717-28.
2. Host A, Halken S, Jacobsen HP, Christensen AE, Herskind AM, Plesner K. Clinical course of cow’s milk protein allergy/intolerance and atopic diseases in childhood. *Pediatr Allergy Immunol* 2002;13(Suppl 15):23-8.
3. Boyano-Martinez T, Garcia-Ara C, Diaz-Pena JM, Martin-Esteban M. Prediction of tolerance on the basis of quantification of egg white-specific IgE antibodies in children with egg allergy. *J Allergy Clin Immunol* 2002;110:304-9.
4. James JM, Sampson HA. Immunologic changes associated with the development of tolerance in children with cow milk allergy. *J Pediatr* 1992;121:371-7.
5. Sicherer SH, Sampson HA. Cow’s milk protein-specific IgE concentrations in two age groups of milk-allergic children and in children achieving clinical tolerance. *Clin Exp Allergy* 1999;29:507-12.
6. Skolnick HS, Conover-Walker MK, Koerner CB, Sampson HA, Burks W, Wood RA. The natural history of peanut allergy. *J Allergy Clin Immunol* 2001;107:367-74.
7. Jarvinen KM, Beyer K, Vila L, Chatchatee P, Busse PJ, Sampson HA. B-cell epitopes as a screening instrument for persistent cow’s milk allergy. *J Allergy Clin Immunol* 2002;110:293-7.
8. Sampson HA. Food allergy. Part 2: diagnosis and management. *J Allergy Clin Immunol* 1999;103:981-9.
9. Sampson H. Diagnosing food allergy. In: Spector S, editor. *Provocation testing in clinical practice*. New York: Marcel Dekker, Inc; 1995. p. 623-46.
10. Sampson HA, Ho DG. Relationship between food-specific IgE concentrations and the risk of positive food challenges in children and adolescents. *J Allergy Clin Immunol* 1997;100:444-51.
11. Henderson CE, Ownby D, Klebanoff M, Levine RJ. Stability of immunoglobulin E (IgE) in stored obstetric sera. *J Immunol Methods* 1998;213:99-101.
12. Sampson HA. Utility of food-specific IgE concentrations in predicting symptomatic food allergy. *J Allergy Clin Immunol* 2001;107:891-6.
13. Hill DJ, Firer MA, Ball G, Hosking CS. Natural history of cows’ milk allergy in children: immunological outcome over 2 years. *Clin Exp Allergy* 1993;23:124-31.
14. Bemhisel-Broadbent J, Dintzis HM, Dintzis RZ, Sampson HA. Allergenicity and antigenicity of chicken egg ovomucoid (Gal d III) compared with ovalbumin (Gal d I) in children with egg allergy and in mice. *J Allergy Clin Immunol* 1994;93:1047-59.
15. Eggleston PA. Prospective studies in the natural history of food allergy. *Ann Allergy* 1987;59:179-82.
16. Chatchatee P, Jarvinen KM, Bardina L, Beyer K, Sampson HA. Identification of IgE- and IgG-binding epitopes on alpha(s1)-casein: differences in patients with persistent and transient cow’s milk allergy. *J Allergy Clin Immunol* 2001;107:379-83.
17. Chatchatee P, Jarvinen KM, Bardina L, Vila L, Beyer K, Sampson HA. Identification of IgE and IgG binding epitopes on beta- and kappa-casein in cow’s milk allergic patients. *Clin Exp Allergy* 2001;31:1256-62.
18. Vila L, Beyer K, Jarvinen KM, Chatchatee P, Bardina L, Sampson HA. Role of conformational and linear epitopes in the achievement of tolerance in cow’s milk allergy. *Clin Exp Allergy* 2001;31:1599-606.
19. Host A, Halken S. A prospective study of cow milk allergy in Danish infants during the first 3 years of life. Clinical course in relation to clinical and immunological type of hypersensitivity reaction. *Allergy* 1990;45:587-96.