

The role of eosinophils and eosinophil cationic protein in monitoring oral challenge tests in children with food-sensitive atopic dermatitis

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To investigate the role of peripheral blood eosinophils and eosinophil cationic protein as parameters in monitoring oral food challenges, we monitored 25 infants and children with atopic dermatitis for up to 48 hours after 47 placebo-controlled oral food challenges with cow's milk, hen's egg, cow's milk and hen's egg, or placebo for up to 48 hours. Six healthy young nonatopic adult volunteers served as control subjects. Compared with baseline values, peripheral blood eosinophils decreased significantly immediately after clinical reaction in positive challenges ($p < 0.0004$), independent of the kind of reaction. Eosinophil cationic protein increased significantly 8 hours after provocation, with a maximum at 24 hours ($p < 0.03$). This increase was predominantly related to eczematous reactions ($p < 0.005$). Blood sampling immediately after clinical reaction (for eosinophils) and at 24 hours (for eosinophil cationic protein) seems to be useful in monitoring oral food challenges in children with atopic dermatitis. (J ALLERGY CLIN IMMUNOL 1994;94:963-71.)

Key words: Atopic dermatitis, challenge, children, ECP, eosinophil, food, provocation

Atopic dermatitis (AD) is one of the most common and troublesome chronic diseases in childhood. There is some controversy concerning the contribution of food allergy toward the degree of skin inflammation.^{1, 2} In the literature the frequency of a clinically important food allergy (according to double-blind, placebo-controlled food challenges) in children with AD has been suggested to be 33%.³ Even fatal and near-fatal anaphylactic reactions to food in children with asthma have been reported.⁴

So far, oral food challenges represent the "gold standard" for diagnosis of food allergy in these children.^{5, 6} Early (within 1 hour) and late-phase (after 1 hour) reactions after single oral provocations are well known.⁷⁻¹⁰ Although immediate reactions can quite easily be related to the sus-

Abbreviations used

AD: Atopic dermatitis
ECP: Eosinophil cationic protein
EOS: Peripheral blood eosinophil count
PCS: Pharmacia CAP System

pected food in most cases, late-phase reactions are very difficult to identify clinically.¹¹ Reliable objective parameters for identifying positive oral provocations are still lacking in most cases. This is especially true for eczematous late-phase reactions but also applies to early reactions, such as vomiting or diarrhea. The long interval between application of the provocative allergen and clinical reactions and the variable course of the disease make it difficult to prove causality. Nonsubjective monitoring parameters are required.

Mediators of allergic inflammation derive from various effector cells (e.g., mast cells, basophils, and eosinophils) and can be measured in biologic fluids. In AD the eosinophils and their granule proteins may play a role in the pathogenesis of the disease.¹²⁻¹⁴ We were therefore interested in peripheral blood eosinophils and eosinophil cationic protein (ECP) as suitable in vivo parameters for monitoring oral food challenges up to 48 hours

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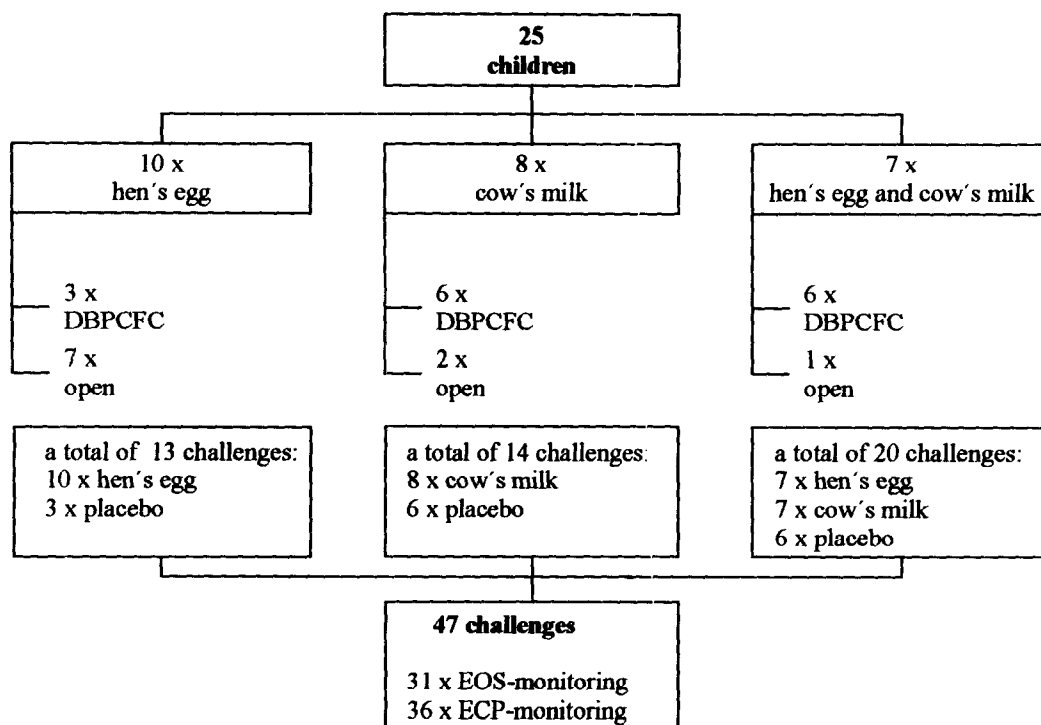


FIG. 1. Overview of challenge data. DBPCFC, Double-blind, placebo-controlled food challenge.

after provocation in children with food-sensitive AD.

METHODS

Patients and control subjects

Twenty-five children (13 boys and 12 girls) aged 7 months to 19 years (mean age, 3.6 years) were selected for the study. All patients had AD as defined by the criteria of Sampson¹⁵ and Seymour et al.,¹⁶ modified from the criteria of Hanifin and Rajka.¹⁷ Severity was scored according to the method of Costa et al.¹⁸ Costa score comprises assessment of 10 "intensity" criteria (extent of erythema, edema, vesicles, crusts, excoriations, scales, lichenification, pigmentation, pruritus, and loss of sleep—0 to 7 points) and "topography" items (extent of 10 skin areas—0 to 3 points). In our patients Costa scores before provocation ranged from 1 to 35 (mean = 19). Another inclusion criterion was the parents' suspicion of food intolerance.

Six healthy young adult volunteers (2 women and 4 men) aged 24 to 34 years (mean age, 28 years) served as control subjects. All were nonatopic and had no history of food intolerance.

Study design

The 25 children were subjected to a total of 47 oral food challenges with hen's egg, cow's milk, hen's egg and cow's milk, or placebo as shown in Fig. 1. Choice of allergen resulted from their history. Six healthy control

subjects were given hen's egg in the same way as the patients. Positive challenge results were compared with negative results, placebo challenges, and challenges from healthy control subjects.

Because of practical problems (e.g., insufficient veins in some small infants) complete monitoring of all time points could only be obtained in 8 of 15 placebo challenges; in the cases of allergen challenges with cow's milk and hen's egg, data were calculated and depicted for $n = 23$ for peripheral blood eosinophil count (EOS) (Fig. 2) and $n = 28$ for ECP (Fig. 3) for the same reasons (blood samples were not available from all time points).

For at least 5 days before provocation, patients and control subjects received a milk-free, egg-free, or milk- and egg-free diet. Antihistamines were withdrawn at least 3 days before challenge, and no child received astemizole. Topical corticosteroids were allowed twice a day at a concentration 0.01% betamethasone.

Successive doses of native hen's egg were 0.1, 0.3, 1.0, 3.0, 10.0, and 30.0 ml (which represents one full egg); successive doses were the same for native cow's milk but extended to 100.0 ml and 200.0 ml. The time interval between each dose was 30 minutes.

Casein hydrolysate with added banana flavor was used to mask the allergen (1:1 mixture). Placebo challenges were performed by administering the same volume of 100% casein hydrolysate-banana flavor solution.

Children and control subjects were observed for up to 48 hours after each allergen or placebo challenge to

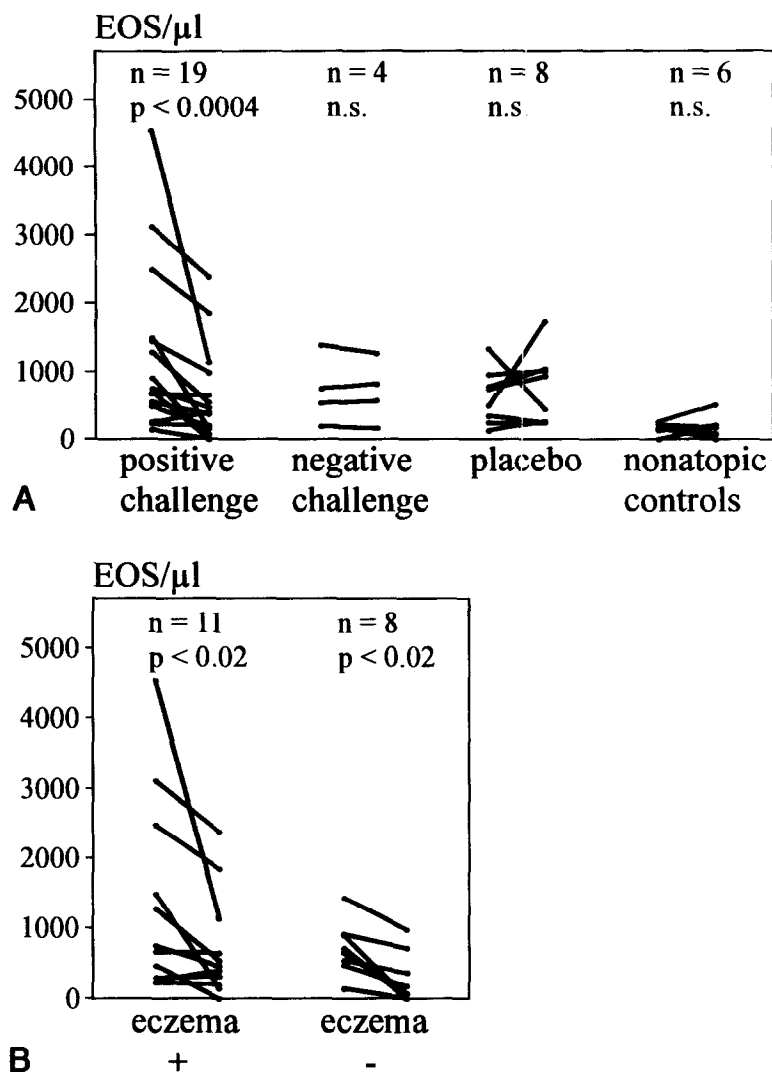


FIG. 2. Eosinophils before challenge and immediately after clinical reaction in positive, negative, and control challenges (A) and according to eczematous skin reactions (B).

evaluate late-phase reactions to food provocation, which we defined as any reaction that occurred more than 1 hour after the highest dose was given.

Procedures

Blood samples were drawn from a venous catheter at the time of admission (for baseline values in comparison with clinical severity) and at the time challenges were started (baseline values), followed at 2-hour intervals up to clinical reaction or until 8 hours after starting challenge. Thereafter, blood was taken immediately after an observed clinical reaction and at 1, 2, 3, and 8 hours later. In any case, additional blood was taken at 24, 36, and 48 hours after provocation was started.

Serum samples were prepared by allowing blood to clot at room temperature for 60 minutes, followed by

centrifugation twice at 1350 g for 10 minutes, according to recently published guidelines.¹⁹ Serum samples were stored at -20°C until they were analyzed.

Leukocyte counts were determined from peripheral blood in ethylenediaminetetraacetic acid with a Coulter counter (Coulter Electronics, Krefeld, Germany). Eosinophil counts were calculated by differential blood cell count.

Eosinophil cationic protein (ECP) from sera was determined by a radioimmunoassay (Pharmacia, Uppsala, Sweden).²⁰ All assays were run in duplicate. The intraassay coefficient of variation was 3.9%, and the interassay coefficient of variation was 2.1%.

Specific IgE was determined by using a solid-phase immunoassay, the Pharmacia CAP System (PCS) (Kabi-Pharmacia, Uppsala, Sweden).²¹

For statistical analysis, the Wilcoxon signed-rank test

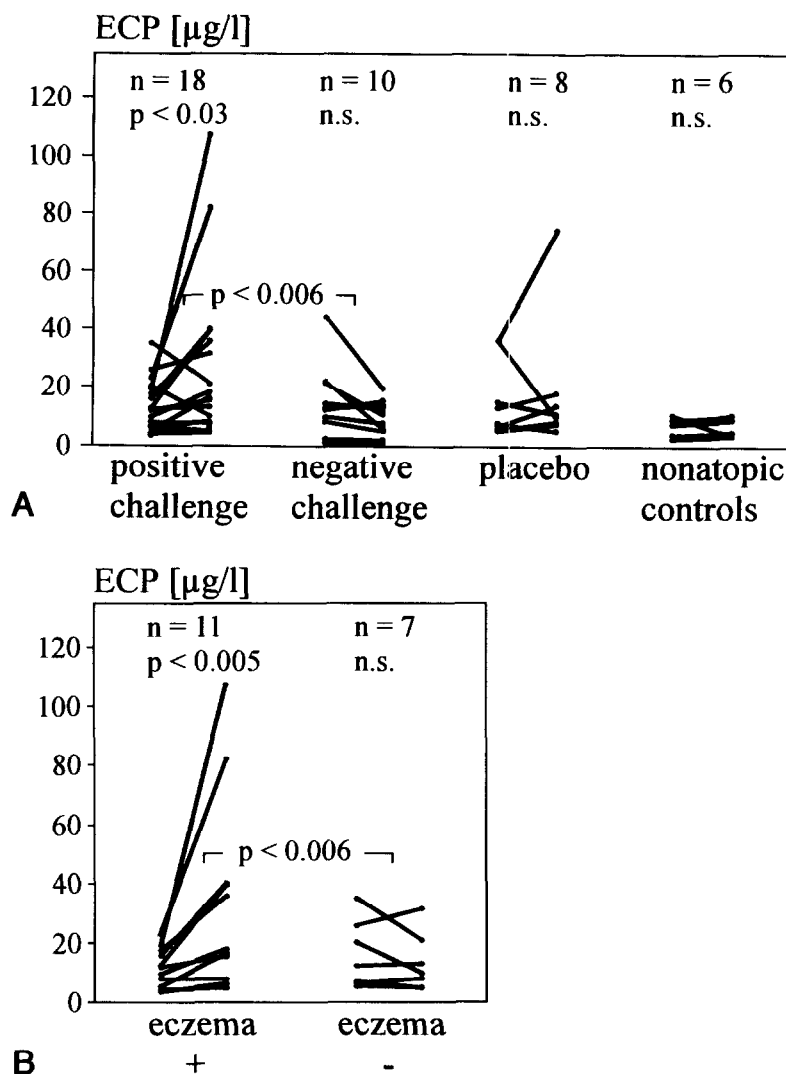


FIG. 3. Serum ECP concentration before and 24 hours after food challenge in patients and control subjects (A) and according to type of clinical reaction (B).

was used for comparison of EOS and ECP in each group and for comparison of the disease severity in Costa score groups. Mann-Whitney U tests and linear rank correlation tests were used for comparison between groups.

RESULTS

Total IgE of patients ranged from 39 to 8740 kU/L (mean = 1360 kU/L). Specific IgE (stated in PCS classes) for cow's milk ranged from less than 0.35 kU/L (PCS class 0) to greater than 100.0 (PCS class 5) with a mean of 15.2 kU/L (PCS class 3). For hen's egg, specific IgE ranged from less than 0.35 kU/L (PCS class 1) to 79.9 kU/L (PCS class 5) with a mean of 27.5 kU/L (PCS class 4). Among control subjects, total IgE was less

than 100 kU/L, and specific IgE against cow's milk and hen's egg was negative in all cases (<0.35 kU/L, PCS class 0).

Baseline peripheral values of blood eosinophils and baseline serum ECP levels were significantly higher in children with AD than in healthy control subjects in comparison to clinical severity (Costa score) (Fig. 4, A and B).

According to clinical criteria, 21 of 32 allergen provocation results were assessed as positive and 11 of 32 as negative. Fourteen of 15 placebo challenges were negative. In 6 of 21 positive provocations, clinical reaction involved one organ, whereas two or more organs were involved in 15 of 21 oral challenges (Table I). Twelve of 21 positive provocations were judged to be single

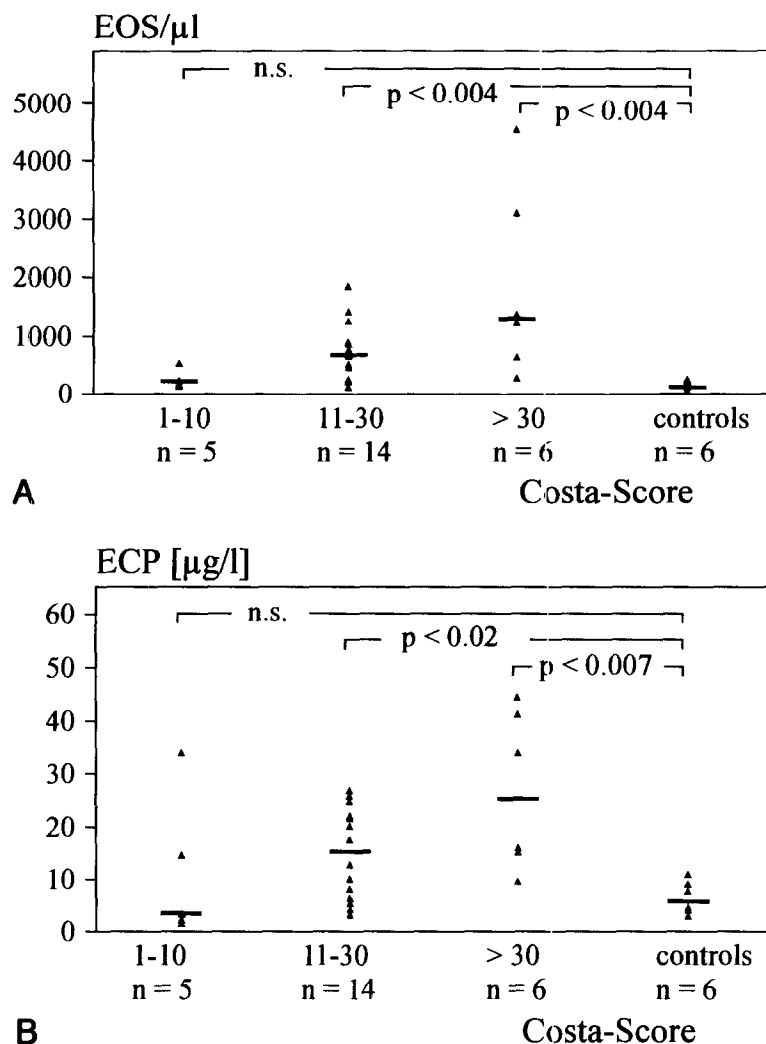


FIG. 4. Baseline values of peripheral blood eosinophils (**A**) and ECP serum concentration (**B**) in comparison with clinical severity and with control subjects (medians).

early reactions, and 7 of 21 as early plus late reactions. Only two single late reactions were noted (Table I). Skin inflammation (eczematous lesions), as rated by the Costa score, increased to a statistically significant extent in children with a positive skin reaction, from a mean of 26 to a mean of 35 ($p < 0.003$). The six healthy control subjects did not show any clinical reaction to hen's egg during the 48-hour observation period.

Fig. 5 shows a representative time course for EOS and ECP of a single patient (T.D.) with an eczematous reaction after provocation and the time course of a patient with a negative challenge outcome (F.H.).

Peripheral blood eosinophil numbers decreased significantly from baseline values immediately after the clinical reaction ($p < 0.0004$) (Fig. 2, A).

They were still significantly decreased after 1 hour ($p < 0.008$), 3 hours ($p < 0.009$), 24 hours ($p < 0.02$), and 48 hours ($p < 0.03$) in those provocations with a positive clinical reaction. This decrease was independent of mode of reaction (eczema vs no eczema, early vs late reaction) (Fig. 2, B). Challenges of healthy control subjects, negative challenges and placebo challenges, showed no significant variations in eosinophils (Fig. 2).

In contrast to peripheral blood eosinophils, the serum ECP concentration remained unchanged at the time of clinical reaction. However, 8 hours after clinical reaction ($p < 0.05$), there was a significant increase of ECP in patients with a positive clinical provocation, which was even more pronounced at 24 hours after provocation ($p < 0.03$) (Fig. 3, A). The increase of ECP at 24 hours

TABLE 1. Clinical data for patients and control subjects

	Age (mo)	Total IgE (kU/L)	Specific IgE (kU/L)		Clinical reaction		Costa score (before → highest)	Clinical reaction		Costa score (before → highest)	Clinical reaction		Costa score (before → highest)
					early	late		early	late		early	late	
			Hen's egg	Cow's milk	Hen's egg			Cow's milk			Placebo		
			Patients										
K.H.	55	5530	79.9	>100	AD, GI	AD	35 → 40	AD, UR	AD	24 → 45	—	—	31 → 30
D.K.	26	2000	2	66.6	—	—	31 → 32	AD, GI	—	27 → 38	—	—	28 → 27
M.F.	7	59	10	2	UR, GI	—	16 → 20	UR, GI	—	14 → 18	—	—	12 → 12
P.K.	8	88	20	1	UR, GI	—	10 → 30	—	—	13 → 13	—	—	11 → 13
J.O.	18	1013	11	0.5	AD	AD	25 → 29	—	AD	19 → 25	—	—	22 → 20
S.S.	9	559	57.7	13	UR, GI	—	16 → 30	AD	AD	21 → 30	—	—	15 → 15
N.M.	31	1532	28	24	UR, GI	—	14 → 22	UR, GI	—	21 → 24			
D.P.	17	547	5		—	—	27 → 25				—	AD	25 → 30
L.K.	8	312	21		GI	—	10 → 10				—	—	12 → 12
B.B.	236	666	36.1		AD, GI	—	27 → 32				—	—	28 → 27
T.D.	22	439	10		AD, GI	—	23 → 29						
M.T.	22	1571	20		AD, GI	AD	26 → 33						
M.L.	134	5630	78.5		GI	—	18 → 18						
U.H.	81	1552	0.6		—	—	20 → 22						
F.H.	30	2000	11		—	—	19 → 21						
N.F.	50	697	5		AD, RC	AD	22 → 34						
R.K.	8	100	74.7		AD, UR	AD	34 → 43						
T.G.	23	39		<0.35				—	—	2 → 2	—	—	2 → 2
K.B.	49	8740		2				—	—	35 → 37	—	—	35 → 36
V.B.	108	100		0.5				—	—	1 → 1	—	—	2 → 1
D.B.	12	102		10				—	—	8 → 7	—	—	7 → 8
H.H.	27	88		2				—	—	12 → 13	—	—	13 → 14
S.P.	66	240		<0.35				—	—	27 → 28	—	—	28 → 27
A.V.	10	125		4				UR, GI	—	14 → 27			
C.W.	17	271		2				—	AD	32 → 39			
Mean	43	1360	28	15									
Control subjects													
H.V.	408	76	<0.35	<0.35	—	—	0 → 0						
W.S.	339	47	<0.35	<0.35	—	—	0 → 0						
G.O.	372	15	<0.35	<0.35	—	—	0 → 0						
M.K.	292	32	<0.35	<0.35	—	—	0 → 0						
K.B.	302	5	<0.35	<0.35	—	—	0 → 0						
T.S.	299	5	<0.35	<0.35	—	—	0 → 0						
Mean	335	30	<0.35	<0.35									

GI, Gastrointestinal reactions; UR, urticaria; RC, rhinoconjunctivitis.

was predominantly due to those patients with an eczematous reaction ($p < 0.005$) (Fig. 3, B). ECP decreased significantly from 24 to 48 hours ($p < 0.02$) and was not statistically different from baseline values at 48 hours. In 8 of 11 children in the eczema group, the clinical reaction was a late-phase reaction. ECP did not show any significant variation in the placebo challenges or in healthy control subjects.

DISCUSSION

In our study population we could confirm the relationship between clinical severity of AD (Costa score) and blood EOS, as well as serum ECP levels (Fig. 4), which has previously been reported in other studies.^{12, 22-26} Because eosinophils, as important effector cells of skin inflammation, and their mediators, released in vivo, seem to reflect disease activity of AD,^{27, 28} they are

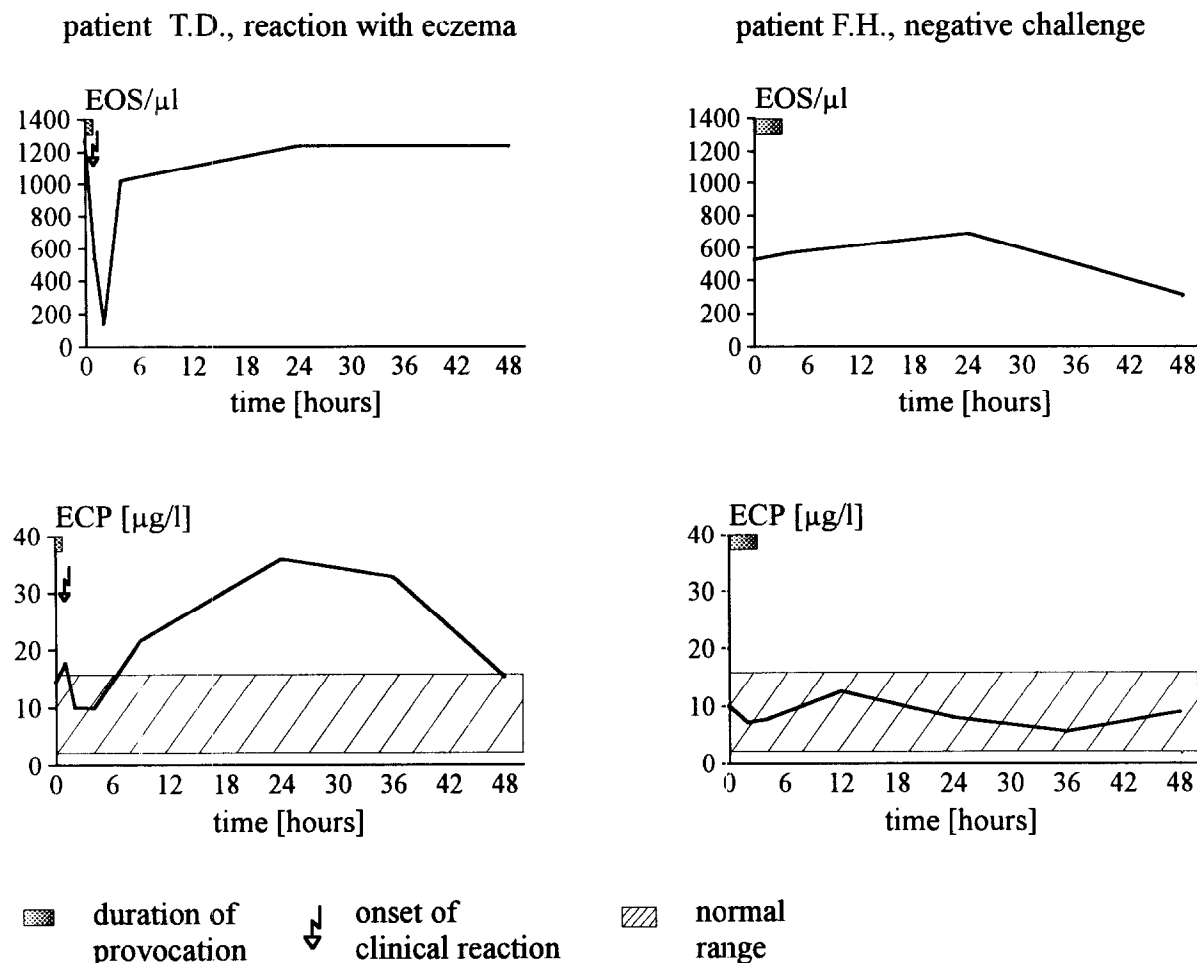


FIG. 5. Time course of EOS and ECP for a representative patient with a positive reaction (T.D.) and a patient with a negative reaction (F.H.).

candidates for clinical monitoring of food challenges in children with AD.

For studying both early and late-phase reactions after double-blind, placebo-controlled food challenges, blood-sampling at short intervals over 48 hours is necessary. Because children were infants, precluding extensive blood sampling for ethical reasons, we preferred the use of open food challenges in some of our younger patients in this study.

Clinical criteria for assessing the outcome of oral challenges were, in addition to the Costa score, objective symptoms of other organs (e.g., vomiting, diarrhea, urticaria). Subjective complaints, undetectable by objective parameters, such as headache, abdominal pain, or itching were not judged to be positive if they represented the sole symptoms. Itching, in particular, had been causing problems in assessing outcome of food challenges as positive. This lack of objective in-

formation demonstrates the need for monitoring food challenges by nonclinical criteria, such as measurements of mediators from effector cells involved in allergic inflammation.

Skin symptoms are the most common manifestation of oral food challenges.^{5, 29} In 19 of 21 positive oral food challenges (90%) skin symptoms were noted. Other studies reported similar values (84%).³⁰

The decrease in EOS after oral food challenges, which we observed in children, has previously been described in a Swedish study of adults with food intolerance.³¹ We suggest that the explanation for this observation is migration of eosinophils into affected tissues. More detailed studies are warranted to prove this hypothesis.

Our data indicate that EOS seems to be a useful parameter for monitoring oral food challenges in children with food-sensitive AD. A clinical reaction did not always involve worsening of

underlying eczema in the children but comprised symptoms of other organs as well. EOS also decreased in the latter cases, and therefore decreases seem to occur independently of the presence or absence of AD.

Serum ECP concentration increased 8 hours after positive food challenges, with a maximum at 24 hours. The time lag between fall in EOS detected immediately after clinical reaction and rise in ECP at 8 to 24 hours after provocation can be explained by the time needed by eosinophils for adhesion, migration, activation, and degranulation of their granule proteins, which finally result in inflammatory skin alterations. Indeed, the increase of ECP was especially seen in provocations with deterioration of eczema and seems to reflect disease activity. We know of no other data in the literature concerning ECP serum concentrations in late eczematous reactions after food challenges. Further studies of placebo-controlled food challenges are warranted to prove that the rise in serum ECP concentration is not merely an epiphenomenon. One should bear in mind that ECP, although it is highly specific for eosinophils, is localized in neutrophils as well and therefore may be partially derived from neutrophils.³²

Furthermore, the late rise in serum ECP after food challenges in children with AD seems to represent proof of the occurrence of late-phase reactions after oral food challenges. From our data we cannot say whether ECP would rise in patients with positive late reactions who do not have underlying AD.

In conclusion, EOS and ECP in combination may be a useful parameter for monitoring oral food challenges in patients with food-sensitive AD. ECP seems primarily to reflect the state of skin activity, whereas a decrease in EOS was observed independently of skin reactions. Assessing the role of food allergy in test subjects with AD is extremely difficult, despite highly sophisticated procedures such as the double-blind, placebo-controlled food challenge. Blood sampling immediately after clinical reaction (for EOS) and at 24 hours (for ECP) might be helpful in assessing the outcome of oral food challenges in patients with AD. In the near future we will be monitoring more placebo-controlled food challenges in an attempt to back up our observations.

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