

Cutaneous late-phase response in food-allergic children and adolescents with atopic dermatitis

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Summary

Food hypersensitivities contribute to disease exacerbation in a sub-group of children with atopic dermatitis (AD). It has been shown that only selected foods are capable of causing clinical reactions when ingested, whereas other foods, to which the patient is equally sensitive by skin-prick testing, may be tolerated. The purpose of this study was to examine the cutaneous late-phase response (LPR) to food antigens in food-allergic patients with AD and to determine if the skin reacted differently to 'relevant foods' (foods eliciting positive skin-prick tests and positive oral challenges) than to 'non-relevant foods' (foods eliciting positive skin tests but negative oral challenges). Using blister chambers adfixed to the skin, six children with AD were challenged epicutaneously with foods to which they had previously been shown to be sensitive. Histamine and PGD₂ were measured hourly for 10–12 hr in parallel with quantitation of the cellular traffic. There appeared to be no difference in any of the measured parameters between relevant foods and non-relevant foods, and the patterns of the LPR cells and mediators were similar to those previously described with aero-allergens in patients with respiratory allergy. Histamine rose to 13.0 ± 24 ng/ml ($P < 0.005$) during the first hours, declined to < 1 ng/ml by the fifth hour, and then rose a second time to 6.72 ± 3.4 ng/ml ($P < 0.05$) during the 12th hour. PGD₂ rose to an average of 312 pg/ml ($P < 0.05$) during the first 3 hr followed by a decline to baseline. The cellular traffic was similar to that observed during the LPR in atopic adults without AD. Neutrophils peaked at $11.2 \pm 6.8 \times 10^4$ cells but did not reach significance because of background traffic in the control chambers. Eosinophils were significantly increased ($P < 0.05$) and rose to $2.52 \pm 1.7 \times 10^4$ cells. Mononuclear cells ($P < 0.05$) and basophils ($P < 0.38$) were also increased but less than either neutrophils or eosinophils. These studies suggest that selectivity in gastrointestinal antigen absorption or differential antigen processing, transport and/or clearing may explain the differences in clinical reactivity to ingested food allergens.

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Introduction

Atopic dermatitis (AD) is a chronic eczematous dermatitis which is characterized by typical distribution, pruritus and a chronic or relapsing course in atopic individuals. The prevalence of AD in the general population is between 7 and 24 per 1000 and represents in excess of 10%

of the paediatric population [1–4]. It is associated with high serum IgE antibodies and an increased propensity for the development of respiratory allergy. Although the lack of specific diagnostic criteria for AD has contributed to the confusion in the literature regarding its pathogenesis, the diagnostic criteria of Hanifin and Rajka are now universally accepted; however, the pathogenesis of AD remains enigmatic [5].

The aetiology of AD is clearly multifactorial, including both genetic and environmental factors. Recent studies

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have shown a clear association between observed exacerbations of childhood AD and the ingestion of selected foods against which the individual has specific IgE antibodies [6–8]. Although children with AD may have multiple positive immediate skin tests or multiple positive radio-allergosorbent tests (RAST) to foods, only a few selected foods will provoke their clinical symptoms [6–8]. The reproduction of clinical symptoms by food challenges parallels a rise in plasma histamine, either from mast cells or peripheral blood basophils [9]. By using a skin chamber model, we have recently shown biphasic histamine release following a single challenge with ragweed antigen in allergic subjects [10, 11]. This late-phase response (LPR) to antigen is associated with an influx of neutrophils, eosinophils and basophils and the late release of histamine is significantly correlated with an increase in basophils which infiltrate the dermis [10, 11].

The purpose of this study was to examine the cutaneous LPR to food allergens in food-allergic paediatric patients with AD and to determine if the skin reacted differently to a relevant food (foods eliciting positive skin-prick tests and positive oral challenges) than to a non-relevant food (foods eliciting positive skin tests but negative oral challenges). The subjects' skin was challenged with both relevant and non-relevant foods. From fluids collected in hollow chambers adfixed to the skin, histamine and PGD₂ were measured hourly for 10–12 hr in parallel with quantitation of the cellular traffic. We wished to establish if these children displayed a pattern of mediator release and cellular traffic similar to that which we had previously observed in ragweed-allergic adults without dermatitis and whether the skin of food-allergic AD subjects was able to selectively discriminate in reactivity between relevant and non-relevant food antigens.

Materials and methods

Six children and adolescents were selected (three male, three females), 9–19 years of age (average age 14 years), who fulfilled the diagnostic criteria for AD as outlined by Hanifin and Rajka [5]. All of the children had been studied previously to determine skin test positivity (and/or RAST sensitivity) and had been challenged orally in double-blind placebo-controlled food challenges as described previously [6–8]. All study subjects had clinical sensitivities to the relevant food at the time of the study. Studies were approved by the Johns Hopkins University School of Medicine Institutional Review Board and written consent was obtained from all parents and patients.

Skin tests

Shortly after admission, all children underwent skin testing to a battery of 28 different food antigens. Standard

glycerinated extracts (1:20 w/v; Greer Laboratories, Lenoir, NC, U.S.A.) were applied by the puncture technique using a Wyeth two-pronged needle.

Oral challenge

Foods for testing were placed in size '0' tartrazine-free opaque (Eli Lilly & Co., Indianapolis, IN, U.S.A.) with 400–500 mg of dehydrated powdered food per capsule or were disguised in a broth or juice. Dehydrated foods were obtained from camping stores (Richmoor, Van Nuys, CA, U.S.A.) or local health food stores. Up to 8 g of dehydrated food was administered in capsules (up to 18 capsules per challenge) or up to 10 g was administered in a broth or juice (up to 150 ml per challenge) over a 1-hr period.

Study design

Four subjects were challenged at three separate sites: a control site and two food antigen challenge sites corresponding to both relevant and non-relevant food antigens. The epicutaneous food antigen challenge was done blinded with the investigator directing the chamber study not being aware of which solution represented the relevant antigen and which represented the non-relevant antigen. One investigator (H.A.S.) calculated the food antigen concentration placed into the chambers based on biologically equivalent concentrations of relevant and non-relevant antigens which caused the same size mean weal diameter with puncture skin testing. After completing the above four subjects, it was determined that there was no detectable difference between the relevant and non-relevant food antigens. Therefore, the last two subjects were challenged with a relevant antigen and placebo substituted for non-relevant antigen in order to avoid the occasional cross-stimulation of the control chamber by the adjacent antigen chamber.

Blisters were induced on the volar aspect of each forearm by continuous suction (60 mmHg) applied for 90–120 min until a complete 5 mm blister was formed. All blisters were formed 12 hr prior to the placement of the skin chambers to allow any inflammatory mediators related to trauma to return to baseline levels. All subjects were treated on clinically uninvolved skin which had not previously been treated with topical corticosteroids.

Skin chamber collection

On the morning of the study, the blisters were aseptically unroofed. Sterile aluminium chambers with entry and exit ports were placed over the unroofed blister bases and taped into place. To avoid skin irritation induced by the

skin adhesive, the area surrounding the blister bases was covered by Saran WrapTM with a precise hole exposing the blister. A cutaneous adhesive, Skin Bond®, was then applied to secure an adequate seal with the wrap. Five hundred microlitres of the commercial food extracts to be tested were placed into the challenge chambers and Ringers' Lactate with 250 µg/ml of heparin (RLH) was placed in the control chamber. The chamber contents were evacuated hourly for up to 12 hr and replaced with 500 µl of RLH. The blister fluid from each sample was centrifuged at 1000 *g* in microcentrifuge tubes for 2 min and the supernatant saved for measurement of PGD₂ and histamine. The cell pellet was gently resuspended in 500 µl of RLH and 10 µl of cell suspension were placed on a Neubauer haemocytometer (American Optical, Buffalo, NY, U.S.A.) for cell counting. Three of the studies extended for 10 hr rather than the usual 12 hr either because the chambers became dislodged or the patient became markedly fatigued.

Cell counts

Cell counts were done on at least 100 nucleated cells in nine large squares. The cell suspension was then placed in a Cytospin centrifuge (Shandon Southern Instruments Inc., Sewichley, PA, U.S.A.) and centrifuged at 600 r.p.m. for 6 min. Paired slides were stained with Diff-QuikTM, a modified Wright-Giemsa stain, and with 0.5% alcian blue (pH 1.0) for 60 min. Following a distilled water rinse they were counter-stained with a 0.1% Safranin in 1% acetic acid. For Wright-Giemsa-stained cells, 200–300 cells were counted. For alcian blue-stained cells, either the total number of cells on the slide or 1000 cells were counted, whichever was the less [12].

Measurement of mediators

For measurement of PGD₂, 200 µl of blister fluid were precipitated in 1600 µl of HPLC grade ethyl alcohol. The tubes were centrifuged at 5000 r.p.m. for 5 min and the supernatants containing the prostanoids transferred to separate tubes to be evaporated in an evaporating centrifuge for 3 hr. The dried specimens were then stored at –70°C until assayed. A competitive radioimmunoassay with a sensitivity of 40 pg/ml, as described previously, was used for PGD₂ measurement [13–15].

Untreated blister fluid for histamine assay was frozen immediately after collection and stored at –70°C. The histamine assay is an enzymatic assay which has previously been described [13] and has a sensitivity of 0.10 ng/ml. This radioenzymatic assay is based on the conversion of histamine to (³H)-methylhistamine in the presence of histamine-*N*-methyltransferase and (³H)-*S*-adenosyl-L-methionine [13].

Statistical analysis

Statistical analysis was done using a paired *t*-test utilizing the Statview program; the *P*-values represent two-tailed values.

Results

Table 1 shows the demographics of the study population. All patients had discontinued antihistamines for at least 7 days prior to the study and were currently being treated intensively with hydration, topical emollients and topical corticosteroids. All but one patient were on diets which eliminated the food(s) previously shown by double-blind challenges to trigger an allergic response for at least 2 years. Four of six patients had no evidence of active eczematous lesions. During the food challenges, patients developed a pruritic, erythematous macular or morbilliform rash in areas of previously involved skin, which did not appear active at the time of challenge. Lesions developed within 15–90 min of the challenge and generally cleared within 2–3 hr.

Figure 1 shows the skin chamber histamine and PGD₂ concentrations in response to an epicutaneous challenge with the food antigen to which the subject was skin test-positive. The chamber mediators were followed hourly for 10 hr in four subjects and for 12 hr in another two subjects (*n* = 6). All reported results represent net mediator release (control value subtracted from the representative antigen value). As noted in Fig. 1(a) net histamine concentrations peaked at 13.8 ± 2.4 ng/ml (*P* < 0.005) during the first hour followed by a decline to 0.93 ng/ml (*P* < 0.05) during the fifth hour. After reaching a nadir during the fifth hour the histamine collected in the antigen-stimulated chambers again rose to 6.98 ± 4.2 ng/ml and 6.72 ± 3.4 ng/ml for the 11th and 12th hours, respectively. Figure 1(b) depicts the net PGD₂ production for the 12 hr study period. PGD₂ peaked during the first 3 hr averaging 312 ± 104 pg/ml and then declined to a baseline of 72 ± 9.0 pg/ml during the last 4 hr of the study. Of interest was the rise of histamine during the first hour which is much more acute when compared with the slower time kinetics observed during the later hours, thus suggesting that the sources of the histamine may be different in the early and late phases (i.e. mast cells 'early' and basophils 'late'). It should also be noted that the production of PGD₂ appears confined to the early phase of the food antigen challenge (Fig. 1(b)), a pattern of release characteristic of the skin mast cell and not that of the basophil which is incapable of producing this prostanoid [13]. Of further interest, the quick rise of PGD₂ during the first hours in these patients was more rapid than that seen in similar studies performed on patients with respiratory allergy [10]. When the histamine release

Table 1. Demographic data on the six study patients and information related to the design of the study

Patient	Age (year)	Sex	Elimination diet (months)	Food tested		Duration of study (hr)
				Relevant	Non-relevant	
BP	10.5	M	Egg, milk, peanut, soy, oat (100)	Egg (7/7)	Rye (5/5)	10
ES	18	F	Beef (48)	Beef (6/29)	Soy (6/17)	10
RB	15.5	M	Soy (30)	Soy (5/5)	Rye (4/4)	10
TP	9.9	M	Milk, peanut (3)	Peanut (9/12)	Soy (6/14)	12
MB	19	F	Egg (72)	Egg (9/11)	n.t.	12
CW	11	F	Shrimp (48)	Shrimp (13/23)	n.t.	12

Three patients were studied for 10 hr and three patients for 12 hr. The results of skin-prick tests for the relevant and non-relevant foods are given as mean diameters of weal and flare in mm (weal/erythematous flare)

provoked by challenges with relevant antigens and non-relevant antigens were compared there was no significant difference; histamine release secondary to relevant food antigens peaked at 16.4 ± 4.48 ng/ml and to non-relevant antigens at 13.5 ± 3.84 , $P < 0.375$.

Figure 2 illustrates the similarity between the relevant and the non-relevant food challenges in a single patient. Figure 2(a) shows the patient released 18 ng/ml of histamine in response to peanut (1:5), a food which was known to provoke a generalized pruritic, erythematous, cutaneous eruption when ingested by this patient. In contrast, when the same patient was challenged epicutaneously with soy (1:1), a non-relevant food, he released 19.2 ng/ml of histamine. Similarly, the average net histamine for the last 3 hr of the relevant peanut antigen (4.23 ng/ml) and the non-relevant soy antigen (5.0 ng/ml) was essentially the same.

The cutaneous cellular traffic at the site of the food antigen challenge was examined by collecting and quantitating the cells that migrated into the adfixed skin chambers. The cellular traffic expressed graphically in Fig. 3 represents the net cell counts with the number of cells collected in the control chamber subtracted from the respective antigen site for each hour. As seen in Fig. 3a, neutrophils were first detected during the sixth hour and increased 5.3-fold by the last hour. Some neutrophils, albeit less, were recovered from the control chambers in response to the non-specific trauma of the blister and, for this reason, statistical significance was lost even for hours 11 and 12. Eosinophils peaked at $2.62 \pm 1.74 \times 10^4$ (Fig. 3(b)) while the neutrophils peaked at $11.2 \pm 6.8 \times 10^4$. Eosinophils were usually not recovered from the control site, thus suggesting that eosinophils display a specificity for the food antigen-challenged site. The numbers of mononuclear cells (Fig. 3c) and basophils (Fig. 3d) were considerably less than the other two cells. The mono-

nuclear cells were increased at all time points from 8 to 12 hr but only significantly at hours 10 and 11. Basophils increased during the last 3–4 hr of the study coincident with the second rise of histamine. The magnitude of basophil increase, however, did not reach statistical significance. Our previous studies examining the skin response to aero-allergen challenge did show a statistically significant increase in basophils which strongly correlated with the late rise in histamine [10]. Our inability to obtain significance in the present study is probably related to termination of the study at 10 hr in one-half of our subjects since the greatest number of basophils usually appear during hours 11 and 12. Nevertheless, the rise in late-phase histamine appears to correlate with basophil influx in patients with AD when challenged with a food antigen.

Discussion

The role of IgE-mediated hypersensitivity in the pathogenesis of AD has remained an enigma and is widely debated by both dermatologists and allergists. Over half of children with AD will develop allergic rhinitis and/or allergic asthma and over 80% of children with AD will have elevated serum IgE [16–18]. Routine histological examination of involved skin of patients with AD has not suggested an immediate hypersensitivity reaction and the histology simply mirrors the spectrum of acute, subacute or chronic dermatitides seen with a variety of other inflammatory skin disorders. Despite the rarity of eosinophils in histological sections of AD, definitive proof that eosinophils play a role in the pathogenesis of AD has been established [19–21]. Further studies by Sampson *et al.* [22] have shown that basophils from food-allergic AD children display a marked increase in spontaneous basophil histamine release which decreases to normal levels after

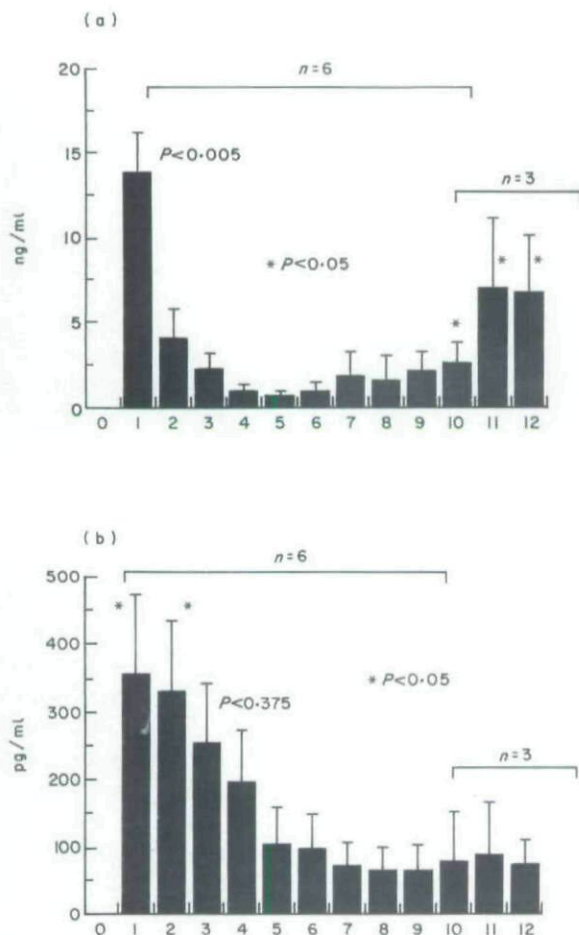


Fig. 1. Hourly skin chamber histamine release (a) and PGD2 production (b) for the 12 hr duration of the study. Error bars indicate the s.e. of the mean. As described in the text, four subjects were studied with both the relevant and non-relevant food antigens. Another two subjects were studied with the relevant food antigen only.

the patient's adherence to specific food allergen elimination diets. They also showed that mononuclear cells from these patients spontaneously produced a cytokine (histamine-releasing factor) which provokes basophil histamine release by interacting with cell-bound IgE. This indirect evidence has suggested that eosinophils and basophils (in addition to mononuclear cells) may play roles in the pathogenesis of AD, supporting findings similar to those of the LPR to aero-allergens in adult subjects [10].

The purpose of the present study was to examine the cutaneous LPR to food allergens in children with AD and food allergy and to determine if the skin reacted differently to a relevant food (positive skin test and positive oral food challenge) compared with a non-relevant food (positive skin test and negative oral challenge). Utilizing

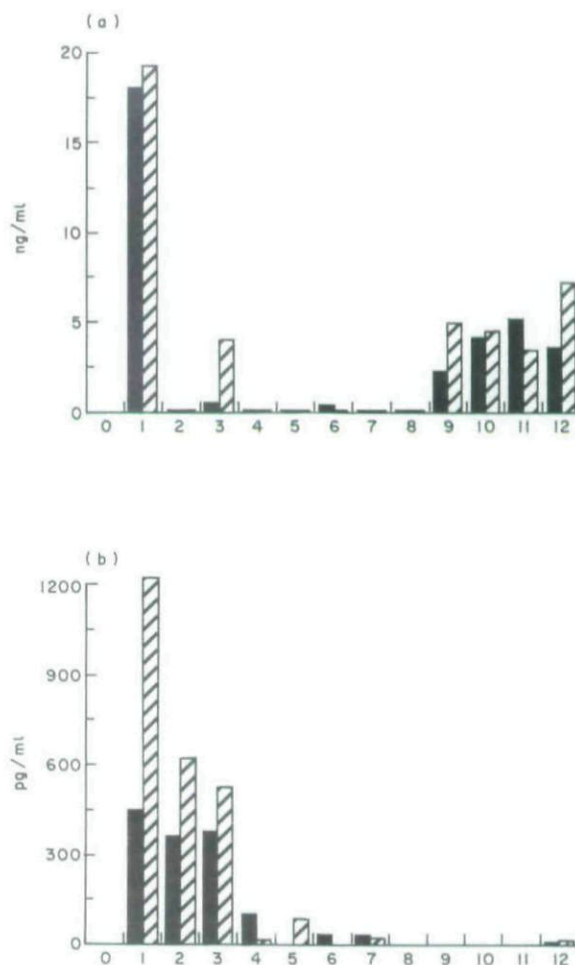


Fig. 2. Hourly histamine release (a) and PGD2 production (b) for the 12 hr study period in a representative patient (TP). ■, Egg (relevant food); ▨, soy (non-relevant food).

adfixed chambers, the skin was challenged with extracts of relevant and non-relevant foods. Chamber fluid histamine and PGD2 content were measured hourly for up to 12 hr and the inflammatory cell infiltration was quantitated. The LPR has been shown to be IgE-mediated and available evidence suggests that the inflammatory events surrounding the LPR may play major roles in the pathogenesis of allergic airway disease [23-24]. The findings in this study add support to the belief that the mediators, cells and cytokines involved in the LPR may also be relevant to the pathogenesis of AD in children with food hypersensitivities [25]. This hypothesis is further supported by our observation of clinical improvement in AD and the loss of clinical hyper-reactivity following the institution of an appropriate elimination diet [22]. The findings in this study also argue in favour of a role for the peripheral blood basophil as the source of the late-phase histamine, evidence which is corroborated

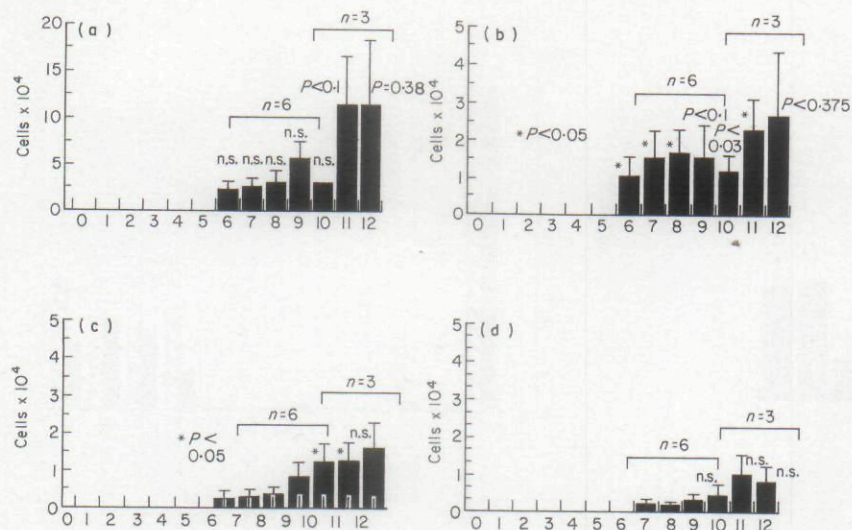


Fig. 3. Absolute number of cells migrating into the adfixed skin chamber for the duration of the study. Net cell numbers are shown with control values subtracted from antigen for each hour challenged sites. (a) Neutrophils; (b) eosinophils; (c) mononuclear cells and (d) basophils. Error bars indicate the s.e. of the mean ($n=6$).

by the observed absence in the LPR of PGD2, a mediator characteristic of the mast cell and not of the basophil. However, it seems likely that the late events, including the pruritus and cutaneous inflammation, are more likely the result of chemical mediators and toxic products released by eosinophils and basophils [21].

Mononuclear cells were shown to infiltrate the skin during the LPR in children with food allergies and AD. When stimulated, these mononuclear cells are capable of releasing various cytokines which may have a direct effect on the pathogenesis of the events in the late phase in atopic dermatitis. We recently reported that late-phase blister chamber fluid contains a histamine-releasing factor as well as significant quantities of interleukin-1 [26–28] which augments basophil histamine release. One or both of these cytokines may explain the increased basophil releasability to anti-IgE and calcium ionophore A23187 reported by Marone *et al.* [29]. Furthermore, Sampson *et al.* [22] demonstrated that peripheral blood mononuclear cells from patients with food hypersensitivity spontaneously produced a histamine-releasing factor which acted on the basophils through surface-bound IgE. The net increase in mononuclear cells observed during the LPR in the food-allergic children may potentially contribute to the pathogenesis of AD. Lastly, it should be noted that histological demonstration of the mononuclear infiltrate during the late phase does not occur until 24–48 hr and our study focused on the events of the first 12 hr. It is possible, therefore, that the mononuclear cell infiltration and the associated cytokine products may be more important than can be appreciated by this study.

The clinical significance of the eosinophil and basophil influx is easier to appreciate than the relevance of the neutrophils which were shown to migrate into the cutaneous chambers. Leiferman *et al.* [30] have clearly

shown the presence of neutrophil-derived elastase in the skin during an IgE-mediated LPR but they were unable to demonstrate elastase deposition in lesional skin of atopic dermatitis despite an abundance of eosinophil granule major basic protein. They conclude that the difference in elastase deposition in AD and the LPR suggests that the pathogenesis of AD may be more complex than simply an ongoing LPR. It is conceivable that the neutrophilic infiltrate which we observed may be a non-specific response to inflammation and may have little to do with the pathogenesis of AD.

Data in this study indicate that the skin of food-allergic patients with AD reacts similarly when challenged directly with either a relevant food or a non-relevant food. However, these subjects only react clinically when orally challenged with relevant foods and not to other foods to which they appear equally sensitive by skin testing. Several possibilities might account for the differences in clinical responsiveness observed between relevant and non-relevant foods: the intestine may be more permeable to relevant foods (allowing them to be transported to distal target organs) or the intestine may process relevant and non-relevant foods differently (e.g. neutralizing allergenic epitopes in non-relevant foods [31]). Alternatively, both relevant and non-relevant antigens may cross the mucosal barrier but the non-relevant antigens may be cleared (e.g. via antigen–antibody complexing) and prevented from reacting with cutaneous mast cells. The latter seems probable since even normal individuals allow small amounts of intact food antigens to penetrate the gastrointestinal barrier. In addition, there is some evidence to suggest that children with AD have increased intestinal permeability [32–34]. It is also possible that infants and young children with AD may have a delay in gastrointestinal maturation thereby allowing intact food anti-

gens to penetrate further than their non-atopic counterparts [34,35]. Although these studies do not address selective intestinal permeability to food antigens, this is an area which needs to be more fully explored. The study does suggest that cutaneous reactivity to food antigens applied locally in food-allergic children will not necessarily reflect how they react clinically to ingested food allergens, a finding which has been observed clinically by many others.

In summary, data have been presented which establish that children with AD and food hypersensitivities develop a cutaneous LPR which appears similar to that described in individuals with respiratory allergy in response to skin challenges with aero-allergens. This LPR is characterized by a late release of histamine with the basophil being the most likely source of this mediator, which is consistent with other studies of the cutaneous LPR [10]. Our data further support a role for the eosinophil in addition to the neutrophil and the mononuclear cell. We were unable, however, to demonstrate any measurable difference in the cutaneous response to relevant (symptoms when challenged orally) and non-relevant (no symptoms with oral challenge) antigens. This suggests that the observed difference between relevant and non-relevant food antigens may be related to a differential gastrointestinal absorption, gastrointestinal processing or immunological clearing of food antigens.

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