Clinical and immunologic variables in skin of patients with atopic eczema and either positive or negative atopy patch test reactions

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Background: Epicutaneous application of aeroallergens induces a positive atopy patch test (APT) response in about 50% of patients with atopic eczema (AE) and sensitization for these allergens.
Objective: To elucidate the mechanisms determining the outcome of the APT, the following questions were addressed. Are there differences in clinical features between patients with AE who have positive versus negative APT responses? Is a macroscopically negative APT response also histologically negative, and if so, are there differences in clinically noninvolved skin between the two groups regarding (1) the sensitivity toward an irritant, (2) the composition of cellular infiltrate, (3) the presence of aeroallergen-specific T cells, and (4) the number of IgE$^+$ cells?
Methods: Punch biopsy specimens from both house dust mite patch tested and the clinically noninvolved skin of patients with AE who have positive APT responses (n = 10) and negative APT responses (n = 10) and those from the normal skin of atopic individuals without AE (n = 10) and nonatopic volunteers (n = 10) were analyzed by using immunohistochemistry with mAbs against eosinophil cationic protein, IgE, the high-affinity receptor for IgE, and CD3 and CD25 mAbs. Furthermore, T-cell lines were propagated from noninvolved skin of all patient and control groups. The T-cell lines were tested for house dust mite specificity.
Results: Negative APT sites were immunohistochemically similar to clinically noninvolved AE skin. There were no significant differences between patients with AE who had positive and negative APT results regarding either clinical features, the composition of cellular infiltrate, or the presence of allergen-specific T cells in clinically noninvolved skin. However, differences were observed regarding the presence of IgE on epidermal CD1a$^+$ cells.
Conclusion: Our results indicate that a positive APT reaction requires the presence of epidermal IgE$^+$ CD1a$^+$ cells in clinically noninvolved skin, but that also other, as yet unknown, discriminatory factors are involved. (J Allergy Clin Immunol 2000;105:1008-16.)

Key words: *Atopic eczema, atopy patch test, IgE, FcεRI, T cells, allergen*

Atopic eczema (AE) is a common chronic skin disorder that is associated with atopy in 80% of the cases.[1,2] Aeroallergens can act as a trigger for AE. This is demonstrated by the fact that in about 50% of patients with AE, eczematous skin reactions appear after epicutaneous application of aeroallergens in the so-called atopy patch test (APT).[3] The histopathology of the APT reaction, showing infiltration of CD4$^+$ T cells, dendritic cells, and eosinophils, has strong similarities with lesional AE skin and suggests a cell-mediated immune response.[4,5]

Part of the T cells, both in APT reaction sites[6-8] and in the clinically noninvolved skin[9] of patients with AE, is allergen-specific. In addition, epidermal Langerhans' cells (LCs) in the clinically noninvolved skin of a subgroup of patients with AE were demonstrated to bear IgE.[10,11] Membrane-bound IgE improves the allergen-presenting capacity of dendritic cells; epidermal IgE$^+$ CD1a$^+$ cells efficiently presented allergen to autologous T cells from peripheral blood, whereas epidermal IgE$^-$ CD1a$^+$ cells were only weak stimulators of T-cell proliferation.[12] It is now established that IgE is bound by the high-affinity receptor for IgE (FcεRI).[13-15] We hypothesize that the APT reaction is initiated by binding of allergens to epidermal IgE$^+$ CD1a$^+$ cells, which present allergens to allergen-specific T_{H2} cells in the dermis. Subsequent release of T_{H2} cell–derived cytokines will lead to an inflammatory reaction in which among others skin-infiltrating eosinophils are involved.[4,16]

The objective of the present study was to elucidate why not all patients with AE who have elevated serum levels of house dust mite–specific IgE show a positive APT response. First, we examined whether differences exist in clinical features of 10 patients with AE who had positive

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Abbreviations used
AE: Atopic eczema
APC: Antigen-presenting cell
APT: Atopy patch test
CM: Culture media
FcεRI: High-affinity receptor for IgE
HDM: House dust mite
LC: Langerhans' cell
LST: Lymphocyte stimulation test
SLS: Sodium lauryl sulfate
TCL: T-cell line

APT responses and 10 who had negative APT responses. For this purpose, Costa scores and distribution of eczema were recorded. Second, to appreciate a study of immunologic parameters in clinically noninvolved skin, we investigated whether macroscopically negative APT sites differed histologically from macroscopically positive APT reactions. Therefore the numbers of (activated) T cells and eosinophils were assessed in biopsy specimens from both macroscopically positive and negative APT sites by means of immunohistochemistry. Third, we investigated whether immunologic differences exist in clinically noninvolved skin of patients with AE who have positive APT responses versus those who have negative APT responses. Here, we also included 10 atopic patients without AE and 10 nonatopic volunteers. The immunologic parameters, being (1) composition of the cellular infiltrate, (2) presence of allergen-specific T cells, and (3) presence of IgE^+ cells, $FcεRI^+$ cells, or both, were determined by means of immunohistochemistry (for 1 and 3) and by means of in vitro examination of allergen specificity in polyclonal T-cell cultures (for 2).

METHODS

Patients and control subjects

Ten patients with AE and a positive APT response (time = 24 hours) for house dust mite (HDM) and 10 patients with AE and a negative APT for HDM were randomly selected from the outpatient clinic. Importantly, none of the patients with a negative APT response showed a positive APT reaction to other aeroallergens for which sensitization was diagnosed. As control subjects, 10 atopic patients with allergic rhinitis, allergic asthma, or both, but without AE and 10 healthy nonatopic volunteers were randomly selected. None of the atopic or nonatopic control subjects had a positive APT reaction.

All atopic subjects, either with or without AE, were sensitized to HDM, as determined by measuring serum IgE specific for HDM (CAP method; Pharmacia, Uppsala, Sweden). Total serum IgE levels of all patients and control subjects were determined by using the paper radioimmunosorbent test (Pharmacia).

All patients with AE fulfilled the criteria of AE according to Hanifin and Raijka.[17] The disease severity of AE was measured by using the Costa score.[18] Furthermore, the distribution of eczema (predominantly on air-exposed areas, predominantly in flexural areas, or predominantly wide spread) was carefully investigated and registered. At least 7 days before testing, intake of oral antihistamines, topical treatment with corticosteroids, and tar preparations on the back were discontinued. None of the patients received UV therapy or systemic steroids before testing. The characteristics of the patients and control subjects are shown in Table I. All participating persons gave their informed consent.

Allergens

A commercially available aqueous preparation of *Dermatophagoides pteronyssinus* (HDM) allergen (Haarlem's Allergenen Laboratorium, Haarlem, The Netherlands) was used for atopy patch testing (concentration, 10,000 US AU/mL). In patients with AE and negative APT reactions for HDM, additional APTs were performed with commercially available aqueous preparations of other allergens (grass pollen, birch pollen, cat dander, and dog dander at 10,000 US AU/mL; Haarlem's Allergenen Laboratorium).

APTs and punch biopsies

APTs were performed as described previously.[3] In short, clinically noninvolved skin of the back was stripped 10 times with hypoallergenic adhesive tape (Transpor; 3M Health Care, St Paul, Minn), after which the above-mentioned allergenic solution (0.08 mL) was applied by using a Leucotest patch (Beiersdorf AG, Hamburg, Germany). APT results were read after 24, 48, and 72 hours. The APTs were recorded as either 1+ (for erythema with slight induration), 2+ (for erythema with papules), or 3+ (for erythema, papules, and vesicles). APTs were scored as negative when repeated testing (two times) gave a negative result at all time points. In all patients the diluent alone served as a negative control.

Punch biopsy specimens (3 mm) were taken after achievement of local anesthesia (1% lidocaine) from clinically noninvolved skin of the back. Two biopsy specimens were taken from each subject: one was snap-frozen in liquid nitrogen for immunohistochemistry, and another was enzymatically digested for culturing of T cells. In 6 patients with AE and positive APT reactions, additional biopsy specimens were taken from a 24-hour APT site and from a site patch tested with diluent for 24 hours. The latter biopsy specimen was taken to control the effect of tape stripping and occlusion. To verify whether histologic changes occur in a macroscopically negative APT response, additional biopsy specimens were taken from a 24-hour APT site in 6 patients with AE and negative APT reactions.

Irritant reactions

Irritant reactions were induced on clinically noninvolved skin without tape stripping with sodium lauryl sulfate (SLS) in distilled water by using Leucoplasts patches (Beiersdorf AG). SLS was applied in increasing concentrations, ranging from 0.01% to 1%. Patches were removed after 24 hours and read 24 hours later.

Immunohistochemistry

Both single- and double-staining procedures were performed, as described earlier.[19] The monoclonal mouse antibodies used in single-staining experiments were CD3 (Leu-4; Becton Dickinson, San Jose, Calif), CD25 (Dako M-0721; Dako Corp, Carpenteria, Calif), EG2 (Pharmacia), anti-IgE (MH25-1 MS-1; CLB, Amsterdam, The Netherlands), and 15.1 (recognizing the unoccupied FcεRI; kindly provided by Dr J.-P. Kinet, National Institutes of Health, Rockville, Md, and purified by Dr F. Effenberger, Novartis Research Institute, Vienna, Austria). Double staining was performed with anti-IgE and CD1a (Dako M-0731) as primary antibodies.

Quantification

Of each subject, two complete 7-μm sections were counted per staining; positively stained cells in the epidermis were counted per 200 basal cells, whereas positively stained cells in the dermis were counted per square millimeter in an area ranging from the basal membrane to 0.7 mm below the epidermis. In fields containing sweat ducts and hair shafts, only intervening dermal regions were counted. All cells were counted at 400× magnification.

TABLE I. Patient characteristics

Group	Patient No.	Costa score	Distribution	Total IgE	HDM-specific IgE	IgE epidermis	LST data
AE^+APT^+	1	27	d	1340	38	14	Positive
	2	21	d	6081	62	15	Positive
	3	18	d	7762	100	9	Positive
	4	38	d	7620	100	15	Positive
	5	29	hn	3070	63	25	Positive
	6	43	d	18,520	4	48	Negative
	7	32	d	5270	91	23	Negative
	8	22	d	4598	63	14	Positive
	9	22	hn	2300	1.9	17	Negative
	10	15	fl	5190	100	50	Negative
Average		26.7		6175	62	23	
AE^+APT^-	1	21	d	2030	25	9	Negative
	2	25	hn	882	33	0	Negative
	3	15	fl	469	4	0	Negative
	4	20	d	1992	100	0	Positive
	5	21	hn	146	26	0	Positive
	6	30	d	3560	21	19	Positive
	7	31	d	9586	100	5	Negative
	8	10	hn	215	7	0	Negative
	9	25	d	896	60	0	Negative
	10	19	fl	2562	19	0	Negative
Average		21.7		2234	40.2	3.3	
AC	1	0	—	226	27	0	Positive
	2	0	—	23	5	0	Negative
	3	0	—	192	0.9	0	Negative
	4	0	—	164	14	0	Negative
	5	0	—	200	4	0	Negative
	6	0	—	358	63	0	Negative
	7	0	—	117	2	0	Negative
	8	0	—	39	9	0	Negative
	9	0	—	427	1.6	0	Negative
	10	0	—	239	10	0	Negative
Average		0		199	13.7	0	
NC	1	0	—	20	0	0	Negative
	2	0	—	26	0	0	Negative
	3	0	—	26	0	0	Negative
	4	0	—	219	0	0	Negative
	5	0	—	11	0	0	Negative
	6	0	—	61	0	0	Negative
	7	0	—	5	0	0	Negative
	8	0	—	22	0	0	Negative
	9	0	—	40	1.4	0	Negative
	10	0	—	22	0.7	0	Negative
Average		0		45.2	0.21	0	

Distribution, Distribution type of eczema; *IgE epidermis,* number of IgE^+ cells in the epidermis per 200 basal cells; *AE^+APT^+,* patients with AE and positive APT reactions; *AE^+APT^-,* patients with AE and negative APT reactions; *AC,* atopic control subjects; *NC,* normal control subjects; *d,* diffuse; *hn,* head and neck; *fl,* flexural.

Isolation and culturing of T-cell lines derived from clinically noninvolved skin

Culture media (CM) were based on RPMI-1640 supplemented with $NaHCO_3$ (2 mg/mL), penicillin (100 IU/mL), streptomycin (100 μg/mL), and glutamine (2 mmol/L; Gibco, Grand Island, NY). CM-ABS contained 10% pooled human blood group AB^+ serum (Red Cross Blood Bank, Utrecht, The Netherlands). CM-FCS contained 10% FCS (Gibco). Human recombinant IL-2 and IL-4 were kind gifts from Dr E. Liehl (Novartis Research Institute, Vienna, Austria) and were both used at a concentration of 50 U/mL.

From each punch biopsy specimen obtained from clinically noninvolved skin, a cell suspension was prepared according to the method described by Sager et al.[8] Briefly, the biopsy specimen was incubated for 5 hours at 37°C in HBSS (Gibco), which was supplemented with FCS (10%), collagenase H (0.1%; Sigma, St Louis, Mo), DNase (20 μg/mL), dispase (1.2 U/mL), hyaluronidase (0.1%; Boehringer Mannheim GmbH, Mannheim, Germany), and versene (0.003%; Gibco). Subsequently, the biopsy specimen was filtered through a nylon gauze (Gibco) that was thereafter rinsed with PBS containing 1% BSA (Sigma). The cell suspension was washed twice (250*g* for 10 minutes at room temperature) and cocultured with 2×10^6 irradiated (20 Gy) PBMCs in the presence of 50 μg/mL HDM to select for HDM-specific T cells. Before coculturing, PBMCs were washed once (250*g* for 10 minutes at room temperature) to remove radicals. Coculturing was performed in a 24-well plate in CM-ABS supplemented with IL-2 and IL-4.

T-cell cultures were restimulated twice (3 and 5 weeks after culture onset) with HDM-preincubated autologous EBV-transformed B cells (EBV-B cells) as antigen-presenting cells (APCs). Preincubation was performed in CM-FCS (overnight at 37°C) with 50 μg/mL HDM, after which cells were irradiated (40 Gy) and washed twice. For restimulation, 1×10^6 T cells were cocultured with 1×10^6 HDM-preincubated EBV-B cells in CM-ABS with IL-2 and IL-4. HDM specificity was tested 3 times (3, 5, and 7 weeks after the start of culturing) in lymphocyte stimulation tests (LSTs).

Lymphocyte stimulation tests

LSTs were performed in triplicate in 96-well U-bottom plates in CM-ABS. Each well contained 4×10^4 resting T cells, 4×10^4 APCs (EBV-B cells), and IL-2. Before coculturing with T cells, EBV-B cells were incubated overnight at 37°C with 50 μg/mL HDM in CM-FCS. Nonpreincubated EBV-B cells were prepared as blank stimulation of T cells. APCs were irradiated (40 Gy), and excess allergen was removed by washing twice. After 4 days, 1 μCi of tritiated thymidine was added per well, and incorporation was measured 16 hours later.[6,7]

The best stimulation of three is presented for each subject. Polyclonal T-cell lines (TCLs) were considered HDM specific when the stimulation with HDM was at least 5000 cpm and twice as high as the blank stimulation.

Statistical analysis

Statistical analysis was performed by using the program SPSS for Windows (version 6.1.3, 1995). The differences between groups were analyzed by using the nonparametric Kruskal-Wallis test, and if results were significant, the Mann-Whitney test was applied to compare any two groups. The Wilcoxon signed-rank test was used for paired samples. Relationships between two parameters were studied by using the Spearman rank correlation coefficient. The Fischer exact test was used to determine differences among the 4 groups concerning the presence of allergen-specific T cells. A P value of less than .05 was considered significant. The results are expressed as mean values ± SEM.

RESULTS

Evaluation of clinical features

No significant differences were found between the patient group with positive APT reactions and the patient group with negative APT reactions regarding severity or distribution type of eczema. The mean Costa score in patients with AE and a positive APT reaction (n = 10) was 26.7, and that in patients with AE and a negative APT reaction (n = 10) was 21.7 (Table I). Therefore the outcome of the APT does not seem to be determined by severity or distribution of the eczema.

Evaluation of irritant reactions versus APT reactions

For the comparison of the outcome of the APT and an irritant reaction, the skin reactivity toward the irritant SLS was determined in an additional group of 43 patients with AE. The response toward a concentration range of SLS was compared in patients with AE and positive APT reactions and those with negative APT reactions. As is clear from Table II, no differences were observed in the response toward SLS in the patients with AE and negative APT reactions compared with the patients with AE and positive APT reactions.

Evaluation of the cellular infiltrate of positive and negative APT sites

Biopsy specimens from APT reaction sites (time = 24 hours) of patients with AE and positive APT reactions (n = 6) showed an increase in the numbers of $CD3^+$ T cells, activated $CD25^+$ T cells, and $EG2^+$ eosinophils when compared with the clinically noninvolved skin of the same patients ($P < .05$, Fig 1). In contrast, no significant differences in the numbers of T cells, $CD25^+$ T cells, or $EG2^+$ eosinophils were observed in biopsy specimens from APT sites (time = 24 hours) of patients with AE and negative APT reactions (n = 6) when compared with the noninvolved skin of the same patients ($P < .05$, Fig 1). Together, these data indicate that a negative APT reaction is immunohistochemically different from a positive APT reaction. When patients with AE and positive APT reactions were patch tested with the diluent solution, no increased cell numbers were observed in biopsy specimens taken after 24 hours (results not shown), demonstrating that the cellular infiltration in APT reaction sites was not caused by tape stripping or occlusion.

Composition of the cellular infiltrate in clinically noninvolved skin

To assess whether the outcome of the APT is determined by differences in the composition of the cellular infiltrate in clinically noninvolved skin, macroscopically normal skin from all 4 subject groups was investigated with regard to the number of T cells, $CD25^+$ T cells, and $EG2^+$ eosinophils by means of immunohistochemistry. No significant differences were seen between the patients with AE and positive APT reactions (n = 10) and those with negative APT reactions (n = 10, Fig 1). In contrast, both AE patient groups showed higher numbers of all the above-mentioned cells when compared with skin of both atopic subjects without AE (n = 10) and healthy nonatopic control subjects (n = 10, $P < .05$, Fig 1).

Presence of allergen-specific T cells in clinically noninvolved skin

Polyclonal TCLs were initiated from the clinically noninvolved skin of all groups. The polyclonal TCLs were tested for HDM specificity in LSTs. HDM-specific T cells were detected in 6 of 10 patients with AE and positive APT reactions. Allergen-specific T cells were, however, also demonstrated in polyclonal TCLs from 3 of 10 patients with AE and negative APT reactions and even in a polyclonal TCL from 1 of 10 atopic control subjects (Table I). Thus no discrimination can be made between patients with AE and positive APT reactions and those with negative APT reactions on the basis of the presence of allergen-specific T cells.

Numbers of IgE^+ cells and $Fc\varepsilon RI^+$ cells in clinically noninvolved skin

IgE^+ cells in the epidermis of noninvolved skin were seen in all patients with AE and positive APT reactions (n = 10), whereas these were observed in only 3 of 10

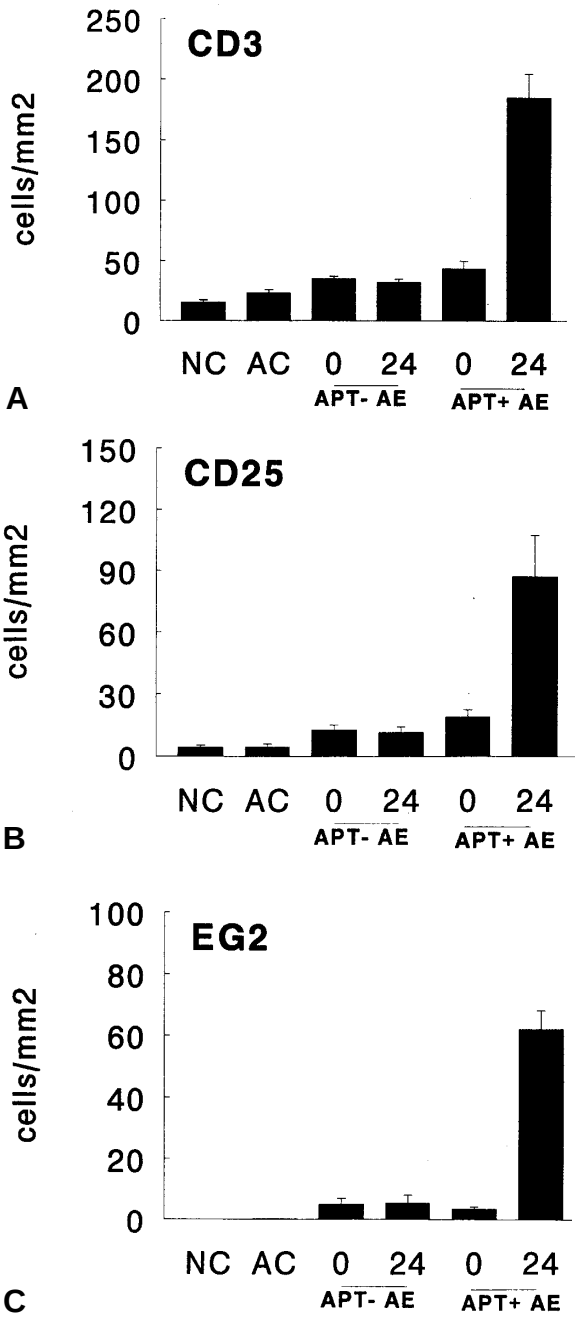

FIG 1. Number of CD3+, CD25+, and EG2+ cells per square millimeter in the dermis of normal control subjects *(NC)*, atopic control subjects *(AC)*, patients with AE and negative APT reactions 0 and 24 hours after patch testing *(APT- AE)*, and patients with AE and positive APT reactions 0 and 24 hours after patch testing *(APT+ AE)*. Results are shown as mean values ± SEM of 6 different individuals per group.

patients with AE and negative APT reactions. The mean number of IgE^+ epidermal cells was higher in the patient group with positive APT reactions compared with the patient group with negative APT reactions ($P < .05$). No epidermal cells with surface IgE were observed in atopic control patients without AE (n = 10) or in healthy

TABLE II. Irritant reactions in patients with either positive or negative APT reactions

	AE^+APT^-	AE^+APT^+
SLS 0.01%	1 (6%)	5 (19%)
SLS 0.05%	2 (12%)	4 (15%)
SLS 0.1%	3 (18%)	1 (4%)
SLS 0.5%	4 (24%)	5 (19%)
SLS 1%	4 (24%)	6 (23%)
No reaction	3 (18%)	5 (19%)
Total	17	26

A total of 43 patients with AE were simultaneously tested with APTs and irritant reactions with SLS (concentration range, 0.01%-1% in distilled water). Patches were removed after 24 hours, and reactions after APT and SLS applications were recorded. The lowest concentration of SLS that induced a positive reaction per patient is depicted.
AE^+APT^+, Patients with AE and positive APT reactions; *AE^+APT^-*, patients with AE and negative APT reactions.

nonatopic control subjects (n = 10, Fig 2). By using double-staining techniques, it appeared that all IgE^+ cells in the epidermis were $CD1a^+$. The total number of $CD1a^+$ cells in the epidermis did not differ between patients with AE and positive APT reactions and those with negative APT reactions (results not shown). The clinically noninvolved skin of patients with AE and positive APT reactions also contained higher numbers of IgE^+ dermal cells than did that of patients with AE and negative APT reactions ($P < .05$). The noninvolved skin of both patients with AE and positive APT reactions and those with negative APT reactions showed a higher number of IgE^+ dermal cells than the normal skin of the control groups ($P < .05$). The number of IgE^+ cells in the dermis of atopic control patients without AE was significantly higher than that found in healthy nonatopic control persons ($P < .05$, Fig 2). Representative photomicrographs of the binding of anti-IgE in the patients with AE and negative APT reactions versus those with positive APT reactions are given in Fig 3. As is clear, the most profound difference is the presence of IgE in the epidermis only in the patients with positive APT reactions, whereas IgE^+ dermal cells are present in both types of patients with AE.

The number of $Fc\varepsilon RI^+$ cells in both the epidermis and dermis of noninvolved skin did not differ significantly between the two groups of patients with AE (Fig 2, *C* and *D*).

Within the two groups of patients with AE, there was a positive correlation between the total serum IgE level and both the number of epidermal IgE^+ cells and the number of dermal IgE^+ cells ($P < .05$).

DISCUSSION

This study was designed to elucidate why 50% of patients with AE who are sensitized to HDM have a positive APT reaction after epicutaneous application of HDM, whereas the other 50% do not. In our attempt to evaluate the factors that determine the outcome of the APT, we noticed that clinical features do not define whether a patient with AE will have a positive or a negative APT reaction. Between patients with AE and positive

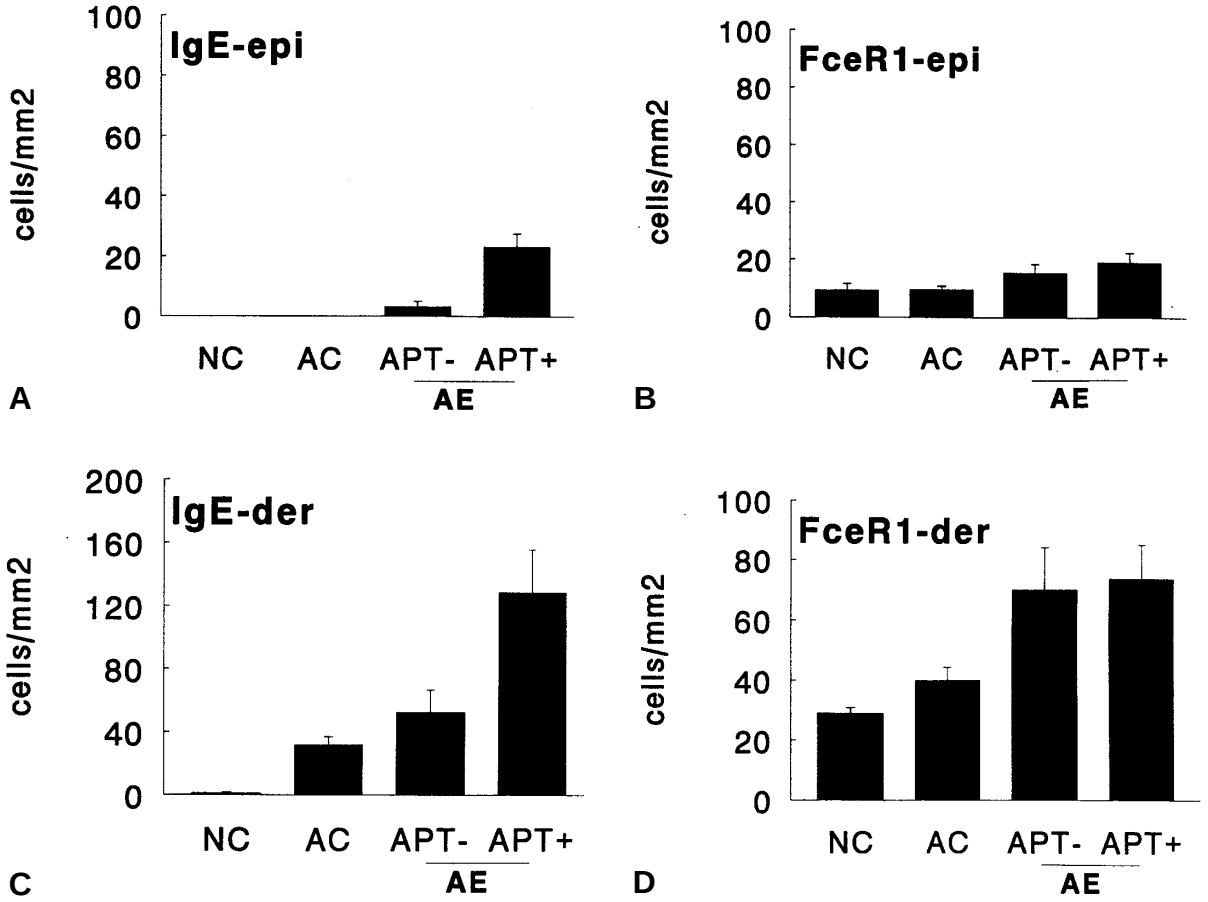

FIG 2. Number of IgE+ and unoccupied FcεRI+ cells in epidermis (*epi*, per 200 basal membrane cells) and dermis (*der*, per square millimeter) in normal control subjects *(NC)*, atopic control subjects *(AC)*, patients with AE and negative APT reactions *(APT- AE)*, and patients with AE and positive APT reactions *(APT+ AE)*. Biopsy specimens were obtained from untreated skin. Results are shown as mean values ± SEM of 10 different individuals per group.

APT reactions and those with negative APT reactions, no differences were observed in either severity or distribution type of the eczema. This observation contrasts with data from Darsow et al,[20] who showed an increased frequency of positive APT reactions in patients with AE in whom air-exposed areas of the skin were affected preferentially. The discrepancy may be explained by the fact that they performed the APT with 3 different allergens (HDM, cat, and grass pollen), whereas we only used HDM. HDM, unlike grass pollen, can be found in clothing and in beds, thereby challenging the entire body surface continuously.[21] Recently, Tabata et al[22] analyzed the histologic pattern after a 24-hour occlusive exposure to 1% SLS. A resemblance was noted with the initial cellular infiltrate after SLS in patients with atopic dermatitis, as described previously after APT. An increase in eosinophils was noted as in APT,[23] although the eosinophil infiltrate persisted in SLS reactions and normally decreased after 48 hours in APT reactions. Previously, it was described that the percentages of infiltrating $CD3^+/CLA^+$ T lymphocytes are comparable after APT and SLS.[24] Whereas HDM can contain proteolytic irritant activity,[25] our APT reactions induced by HDM could be due to irritant responses instead of an IgE-mediated mechanism. Therefore we have analyzed the response in the APT versus the response to an irritant in a large group of patients with AE. As is clear from Table II, patients' patterns of responses are different between the APT and irritant reactions; that is, there is no tendency for a positive response after SLS at lower concentrations in the patients with positive APT reactions. We conclude therefore that in our hands the APT induced by HDM is, to a large extent, determined by its allergenic properties. In addition, as was demonstrated before, serum IgE is correlated to the outcome of the APT.[3,26,27] Moreover, as is shown in Fig 2, a correlation between IgE in the epidermis and dermis and the outcome of the APT also indicates the importance of IgE-mediated mechanisms in the APT reactions. Another implication from the sensitivity toward patients with positive APT reactions versus those with negative APT reactions is that the sensitivity of the skin as such between patients with positive and negative

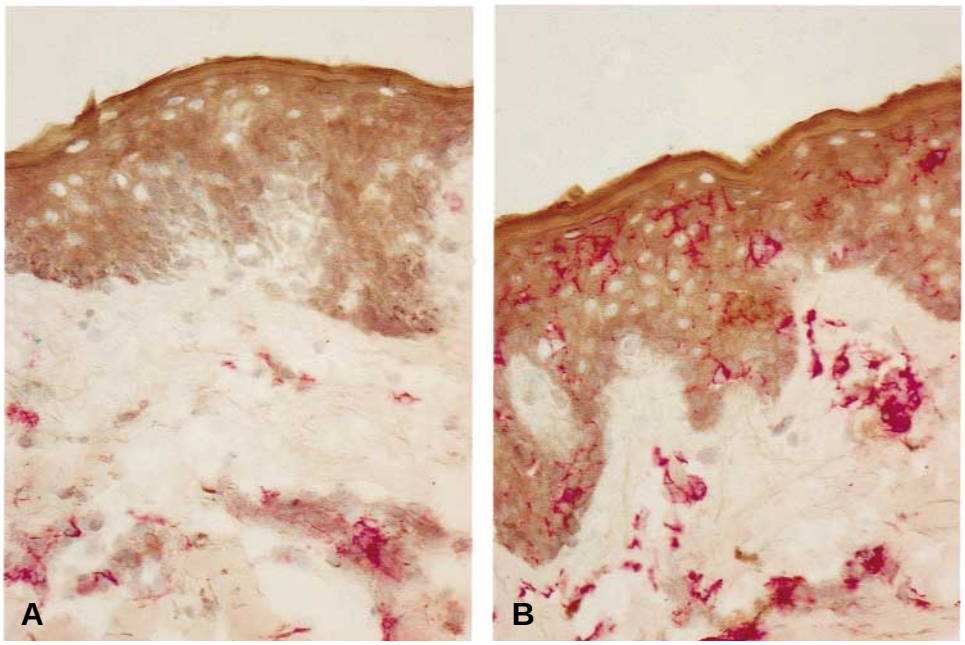

FIG 3. Binding of anti-IgE in skin of patients with AE and negative APT reactions (**A**) and patients with AE and positive APT reactions (**B**). Biopsy specimens were obtained from untreated skin. Two representative photomicrographs are shown (objective magnification 20×).

APT reactions is similar. These data suggest that although APT and irritant responses share several characteristics in their outcome, the induction of these two reactions is different.

Buckley et al[28] reported the infiltration of mononuclear cells in macroscopically negative APT sites that had not been tape stripped before patch testing. These APT sites, however, became positive when performed on tape-stripped skin. Therefore the authors emphasized the necessity of tape stripping before patch testing to prevent the occurrence of false-negative APT reactions. We also addressed the question of whether immunohistochemical changes occur in clinically negative APT sites, as opposed to clinically noninvolved AE skin. All APTs in our study were performed on tape-stripped skin. In the group of patients with AE and negative APT reactions, no significant differences were observed between cellular infiltrates of clinically noninvolved skin and negative APT sites. For this reason, it may be excluded that the negative APT reactions in the present study were false-negative reactions.

The degree of cellular activation in clinically noninvolved skin was quantified by counting $CD3^+$ T cells, activated $CD25^+$ T cells, and $EG2^+$ eosinophils in situ. Higher numbers of all 3 markers were observed in clinically noninvolved skin from patients with AE when compared with atopic control subjects without AE and healthy nonatopic volunteers. This finding is in accordance with data from Uehara and Miyauchi,[29] which indicate that the clinically noninvolved skin of patients with AE is subclinically inflamed. No differences, however, were observed between patients with AE and positive APT reactions and those with negative APT reactions. Therefore the outcome of the APT cannot be predicted by examination of the above-mentioned activation parameters in clinically noninvolved skin.

By using a T-cell clone tracing procedure, we recently detected APT-derived HDM-specific T-cell clone in the clinically noninvolved skin of a patient with AE and a positive APT reaction.[9] This finding suggests a role for skin-residing allergen-specific T cells in the initiation of APT reactions. However, in the present study HDM-specific T cells could not be isolated from the clinically noninvolved skin of 4 of 10 patients with AE and positive APT reactions. Probably a sampling error or in vitro loss of allergen-specific T cells has caused the lack of detectable specificity in these patients. Alternatively, retention in the skin may not be necessary for T cells to play a role in the development of a positive APT reaction. In this case early recruitment of allergen-specific T cells from the circulation may occur. HDM-specific T cells were also isolated from the clinically noninvolved skin of 3 patients with AE and negative APT reactions and even from normal skin of one atopic control subject without AE. Moreover, Virtanen et al[30] isolated allergen-specific T cells from nonatopic skin. Taken together, the presence of allergen-specific T cells in clinically noninvolved skin is not a discriminating factor between patients with AE and positive APT reactions and those with negative APT reactions.

Differences between patients with AE and positive APT reactions and those with negative APT reactions emerged when IgE-bearing (IgE^+) cells were enumerated in the epidermis and dermis of clinically noninvolved skin. IgE^+ $CD1a^+$ cells were observed in the epidermis of

all patients with AE and positive APT reactions, whereas the epidermis of only 3 of 10 patients with AE and negative APT reactions contained these cells. Furthermore, the number of epidermal IgE^+ $CD1a^+$ cells observed in patients with AE and positive APT reactions was considerably higher than that found in patients with negative APT reactions. In addition, the number of dermal IgE^+ cells was higher in patients with AE and positive APT reactions than in those with negative APT reactions. In the dermis IgE^+ cells were mainly mast cells and dendritic cells (not shown). Our current results are in accordance with an earlier report, in which a positive correlation was observed between the outcome of the APT and in vitro allergen-presenting capacity of freshly isolated epidermal LCs.[12] In that study epidermal IgE^+ $CD1a^+$ cells were isolated from 9 of 11 patients with AE and positive APT reactions and from only 1 of 5 patients with AE and negative APT reactions.

IgE^+ LCs were also present in the epidermis of 3 of 10 patients with AE and negative APT reactions. Therefore despite the presence of increased numbers of IgE^+ $CD1a^+$ cells in the clinically noninvolved skin of patients with AE and positive APT reactions, IgE-binding capacity of epidermal $CD1a^+$ cells does not discriminate between a positive and a negative APT reaction.

Thus although the importance of IgE^+ $CD1a^+$ cells in the epidermis has been indicated, additional properties seem crucial for the development of a positive APT reaction; for example, HDM-processing by IgE^+ $CD1a^+$ cells in patients with AE and positive APT reactions may be executed more efficiently as a consequence of higher levels of cell-bound HDM-specific IgE. However, immunohistochemical double staining with CD1a and biotinylated HDM did not reveal specific binding of HDM in the epidermis of patients with AE and positive APT reactions.[31] Nevertheless, HDM binding was evident in the dermis of patients with AE. IgE receptors were functional even in the epidermis because passive sensibilization with human serum containing high concentrations of HDM-specific or grass pollen–specific IgE resulted in allergen binding in the epidermis (data not shown). An explanation for the failure of allergen binding in the original epidermis of patients with AE and positive APT reactions could be the relative low ratio of allergen-specific IgE/total IgE in patients with AE.

We previously reported that positive APT reactions are associated with elevated levels of allergen-specific serum IgE.[3] Accordingly, the patients with AE and positive APT reactions in the present study had a significantly higher total and allergen-specific serum IgE than the patients with AE and negative APT reactions ($P < .05$). In addition, we found a positive correlation between the levels of both total and specific IgE and elevated numbers of IgE^+ cells in the epidermis and dermis of the clinically noninvolved skin of patients with AW. This is in accordance with data from Bieber et al,[32] who reported that serum IgE levels higher than 300 kU/L go with the presence of IgE^+ epidermal LCs in the lesional skin of patients with AE. In the present study, which was performed with the clinically noninvolved skin, this was not always the case. Several patients with AE and negative APT reactions who had high serum IgE levels did not have detectable IgE^+ epidermal LCs in noninvolved skin. Furthermore, numerous unoccupied FcεRI molecules were detected even in the dermis and epidermis of patients with atopic dermatitis and positive APT reactions who had extremely high serum IgE levels; positive staining with mAb 15.1 indicates the presence of unoccupied FcεRI molecules because IgE incubation in vitro inhibits binding of mAb 15.1 to FcεRI.[33] Therefore binding of IgE to FcεRI seems not to be determined by the availability of IgE only. Recent data indicate that FcεRI-associated molecules control the capacity of FcεRI to bind monomeric IgE.[34]

Eczema in patients with AE and negative APT reactions is most likely not caused by skin contact with aeroallergens. Aeroallergens probably enter the skin of these patients through the circulation after respiratory challenge. Support for this option was recently provided by two independent studies. By bronchial challenge with HDM, Tupker et al[35] induced pruritic, erythematous skin lesions in 9 of 20 patients with AE, whereas Brinkman et al[36] induced exacerbation of skin lesions in 13 of 16 patients with AE.

In conclusion, the clinically noninvolved skin of patients with AE and positive APT reactions differs from that of patients with AE and negative APT reactions in having higher numbers of IgE^+ cells in both the dermis and epidermis. IgE^+ $CD1a^+$ cells in the epidermis of clinically noninvolved skin do not, however, provide a marker to fully discriminate between patients with AE and positive or negative APT reactions.

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