

Clinical aspects of allergic disease

Evaluation of the atopy patch test and the cutaneous late-phase reaction as relevant models for the study of allergic inflammation in patients with atopic eczema

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Objective: The study was designed to evaluate the atopy patch test (APT) and the late-phase reaction (LPR) after intracutaneous allergen injection as models for the study of allergic inflammation in atopic eczema.

Methods: Immunocytochemistry was used to analyze skin biopsy specimens from sites of APTs and LPRs at 2 and 24 hours and to compare these with lesional and nonlesional skin of patients with atopic eczema.

Results: A lack of neutrophil infiltration in specimens from both the APT and lesional skin sites was observed, whereas neutrophils were abundantly present in the specimens from LPR sites. With double-staining techniques it was demonstrated that the few neutrophils present in specimens from APT sites and in lesional skin were mostly located in intravascular areas, whereas in the LPR specimens they were located predominantly in extravascular areas. Eosinophils infiltrated at an earlier time point in the LPR as compared with the APT. Furthermore, there was a decrease of intact mast cells in the LPR sites compared with the APT sites and lesional skin. No significant difference in T-cell number was observed between the two tests. Upregulation of E-selectin expression on endothelial cells occurred at an earlier time point in the LPR as compared with the APT.

Conclusion: There are important differences in cellular infiltrate between the APT and the LPR. The close macroscopic and microscopic similarities between the specimens from APT sites and lesional skin of patients with atopic eczema support the argument that the APT is a more valid *in vivo* model with which to study allergic inflammation in atopic eczema than the LPR. (*J Allergy Clin Immunol* 1996;98:1019-27.)

Key words: Atopy patch test, late-phase reaction, atopic eczema, Dermatophagoides pteronyssinus, allergic inflammation, eosinophils, neutrophil, T cell, mast cell, E-selectin

Atopic eczema (AE) is a chronic skin disease, characterized by acanthosis, spongiosis, and a dermal cellular infiltrate consisting predominantly of CD1⁺ cells and activated CD4⁺ T cells. In addition

Abbreviations used

AE:	Atopic eczema
APT:	Atopy patch test
HDM:	House dust mite
LPR:	Late-phase reaction
NHoS:	Normal horse serum
NHuS:	Normal human serum
PBS:	Phosphate-buffered saline

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to this, deposits of eosinophil-derived major basic protein and eosinophil cationic protein in the dermis suggest eosinophil involvement.^{1, 2}

TABLE I. Patients' characteristics

Patient No.	Sex	Age (yr)	Costa score	Total IgE (kU/L)	HDM-specific IgE (kU/L)
1	F	29	25	3,404	20
2	F	26	16	800	45
3	F	22	12	573	60
4	M	22	27	25,907	100
5	M	26	10	21,401	100
6	M	44	20	6,081	62
7	M	20	15	10,500	85

Eczematous skin reactions can be induced by epicutaneous application of aeroallergens (the atopy patch test [APT]). Macroscopically, these APT reactions resemble AE lesions by showing erythema, induration, papules, and/or vesicles. The maximum reaction is reached after 24 to 48 hours.³ The APT reaction is microscopically characterized by acanthosis, spongiosis, and a dermal infiltrate consisting predominantly of CD1⁺ cells, CD4⁺ T cells, and activated eosinophils.⁴ APT reactions occur in about 50% of aeroallergen-sensitized patients with AE and are specific for AE; positive APT responses do not occur in normal control subjects.³ There is a positive correlation between the APT reaction and the total and specific serum IgE levels.³ The APT has been proposed as an in vivo model for the study of allergic inflammation in AE.¹

A more generally accepted model for the study of allergic inflammation in the skin and airways is the late-phase reaction (LPR) after intracutaneous allergen administration.⁵⁻⁷ This reaction is macroscopically characterized by diffuse edema, erythema, slight induration, pruritus, and tenderness. The LPR is maximal after 6 to 10 hours, and in most cases, lasts no longer than 24 hours. Microscopically, no spongiosis or acanthosis is observed. The reaction is characterized by edema, mast cell degranulation, and a dermal infiltrate consisting of mononuclear cells, eosinophils, neutrophils, and basophils.^{8,9} Extracellular deposition of eosinophil and neutrophil granule proteins is already seen 1 hour after antigen challenge.¹⁰ Depending on the concentration of the tested allergen, all atopic individuals can have an LPR.¹¹

The purpose of this study was to compare the cellular changes in the cutaneous inflammation of the APT reaction and the LPR and to relate these to lesional skin in the same patients with AE. Because histologic changes in APT reactions can be observed between 2 and 24 hours after antigen

challenge⁴ and in LPRs already 1 hour after antigen challenge,¹⁰ we decided to study the cellular infiltrate of the APT reaction and the LPR at two time points, namely 2 hours and 24 hours after allergen challenge.

METHODS

Patients

Seven patients with AE, diagnosed according to the criteria of Hanifin and Rajka,¹² were selected on the basis of demonstration of a positive APT reaction at 24 hours and a positive LPR to house dust mite (HDM) (defined as ≥ 5 mm induration 2 hours after intradermal allergen challenge). The patients ranged in age from 22 to 44 years (mean age, 27 years). Clinical activity of the skin was established according to the Costa score.¹³ The Costa score varied between 10 and 27 (mean, 18). Total serum IgE level of all patients was determined by using the paper radioimmunosorbent test (PRIST; Pharmacia, Uppsala, Sweden) according to the manufacturer's instructions (mean, 9809 kU/L). Specific serum IgE for HDM of all patients (mean, 67 kU/L) was measured by the CAP method (Pharmacia) according to the manufacturer's instructions. The characteristics of the patients are shown in Table I. Patients did not receive oral antihistamines and/or topical treatment with corticosteroids or tar preparations on the back for at least 7 days before testing. None of the patients received UV therapy or systemic steroids before testing. All participating patients gave their informed consent.

Allergens

The same batch of *Dermatophagoides pteronyssinus* (Haarlem's Allergen Laboratorium, Haarlem [HAL], The Netherlands) was used for both the APT (concentration, 10,000 AU/ml) and the intracutaneous tests (concentration, 100 AU/ml). The allergens were dissolved in HAL buffer solution consisting of a buffered salt solution (pH 6.8 to 6.9) containing 6-aminohexanoic acid (1.31% wt/vol), dipotassium hydrogen phosphate (0.91% wt/vol), potassium dihydrogen phosphate (0.12% wt/vol), with human serum albumin (0.03% wt/vol) as stabilizer and phenol (0.5% wt/vol) as preservative. The allergenic extracts were standardized by the manufacturer in vivo by an "in house reference" determined by Turkeltaub's method.¹⁴ The manufacturer determined the allergen activity of separate charges of allergen extracts in vitro by an IgE inhibition test and expressed this in allergen units per milliliter.¹⁰ The solutions were stored between 4° and 8° C for a maximum of 3 months.

APT reaction

The allergen preparation described above (0.08 ml per test), at a concentration of 10,000 AU/ml, was used for performing APTs on each patient's back (A detailed description of the APT technique can be found in a previously published study.³) The APT reactions were

TABLE II. List of primary antibodies used and dilutions

Antibody	Dilution	Source	Specificity
CD-3	1:50	Becton Dickinson	T lymphocytes
CD-4	1:20	Dako	T-helper lymphocytes
CD-8	1:50	Becton Dickinson	T-cytotoxic lymphocytes
EG-2	1:400	Pharmacia	Eosinophils
AA-1	1:100	Dako	Mast cell tryptase
Elastase	1:50	Dako	Neutrophils
<i>Ulex</i> -biotin	1:50	Sigma	Vascular endothelium
CD-62E	1:10	Genzyme Diagnostics	E-selectin

recorded as: +, erythema with slight induration; 2+, erythema with papules; and 3+, erythema, papules, and vesicles. In all patients the diluent alone, as described above, was tested as a negative control.

LPR

The allergen preparation described above (0.02 ml) at a concentration of 100 AU/ml was injected intradermally on the flexor aspect of the forearm by using a 25-gauge needle and 1 ml plastic syringe. Histamine dihydrochloride (0.1 mg/ml) was used as a positive control, and the HAL buffer solution (for description see above) was used as a negative control. The immediate reactions were recorded according to the method described by Dreborg et al.¹⁵ The LPRs were characterized by swelling, erythema, and induration. The diameter (in millimeters) of edema and induration was measured after 2 and 24 hours.

Tissue processing and staining

Punch biopsy specimens (3 mm) were taken after local anesthesia had been induced (1% lidocaine) from the center of the APT and LPR sites and from nonlesional skin of the back and chronic lichenified lesional skin from the antecubital site of the elbow or knee in the same patients with AE. In seven patients with AE, biopsy specimens were taken from APT sites at 2 and 24 hours, from LPR sites at 2 and 24 hours, and from lesional and nonlesional skin. The biopsy specimens were snap-frozen in liquid nitrogen and stored at -70°C before use. From these biopsy specimens, 7 μm sections were prepared on a freezing microtome and mounted on slides coated with 3-aminopropyltriethoxysilane (Sigma Chemical Co., St. Louis, Mo.). Sections were fixed with dry acetone (Sigma) for 7 minutes, air-dried, and preincubated for 20 minutes in 10% normal horse serum (NHoS) and 10% normal human serum (NHuS) in phosphate-buffered saline (PBS). Primary antibodies (see Table II) were diluted in PBS with 1% NHoS/NHuS and incubated with the sections for 60 minutes. Slides were washed (3 times for 5 minutes in PBS + 0.05% Tween-20 [Sigma]), after which a second layer of biotinylated horse-anti-mouse (IgG [H+L] Vector BA-2000, 1:800 in PBS 1% NHoS/NHuS) was applied for 30 minutes. After washing with PBS-Tween (3 times for 5

minutes), the sections were incubated with streptavidin-conjugated alkaline phosphatase (1:300 in PBS-1% NHuS; Dako Corp., Carpinteria, Calif.) for 30 minutes and washed in Tris-HCL (3 times for 5 minutes, 0.1 mol/L, pH 8.5). Alkaline phosphatase reactivity was demonstrated by using naphthol AS-BI phosphate (sodium salt, 50 mg/100 ml; Sigma) as substrate and new fuchsin (10 mg/100 ml; Merck & Co., Inc., Whitehouse Station, N.J.) as chromogen dissolved in 0.1 mol/L Tris-HCL, pH 8.5, resulting in a pink-red staining. Endogenous alkaline phosphatase activity was inhibited by addition of levamisole (35 mg/100 ml, Sigma) to the reaction mixture. Slides were thereafter slightly counterstained with hematoxylin and embedded in gelatin. As a control, the primary antibody was replaced by an IgG₁-isotype control (mouse IgG₁ standard 102-01; Southern Biotechnology Association, Birmingham, Ala.) in the appropriate dilution, after which the sections were all negative.

Double staining

To determine E-selectin expression on endothelial cells or localization of neutrophils, sections were double-stained with anti-E-selectin monoclonal antibody and elastase, respectively (Table II); and biotinylated *Ulex europaeus* agglutinin 1 (UEA-1) was used as a panendothelial cell marker.¹⁶ After fixation with dry acetone with H₂O₂ (30%, 100 μl /100 ml, 7 minutes), sections were preincubated for 20 minutes in 10% NHuS-10% normal rabbit serum (Dako), after which incubation with anti-E-selectin (1:10) or elastase (1:50) in combination with *Ulex*-biotin (1:50) in 1% NHuS-1% normal rabbit serum followed. The sections were incubated with a mixture of streptavidin-conjugated peroxidase (Boehringer Mannheim GmbH, Mannheim, Germany) and alkaline phosphatase-conjugated rabbit-anti-mouse (1:50, Dako) in 1% NHuS-1% normal rabbit serum. In between incubations, sections were washed with PBS-Tween (3 times for 5 minutes). First, alkaline phosphatase activity was demonstrated by using naphthol AS MX phosphatase (Sigma) as substrate and fast blue BB base (Sigma) as chromogen, which resulted in blue staining. After rinsing twice with PBS and once with NaAc (0.1 mol/l, pH 5.0), peroxidase activity was revealed by using 3-amino-9-ethylcarbazole (Sigma) as substrate, resulting in a red

TABLE III. Macroscopic reactions of APTs and LPRs

Patient No.	APT		LPR (mm)*	
	2 hr	24 hr	2 hr	24 hr
1	Neg	1+	18	20
2	Neg	1+	15	18
3	Neg	2+	20	25
4	Neg	2+	30	38
5	Neg	2+	20	27
6	Neg	1+	18	28
7	Neg	1+	20	22

1+, Erythema with induration; 2+, erythema, induration, and papules.

*The diameter of edema and/or induration of LPRs.

color. With this method, *Ulex*-positive cells stained red, whereas E-selectin- and elastase-expressing cells stained blue. E-selectin-expressing endothelial cells appear as purple cells.

The specificity of the double staining was established by using the following controls: anti-E-selectin or elastase was replaced by IgG₁-isotype control, which resulted in red-staining cells only; biotinylated *Ulex* was omitted, resulting in blue-staining cells only.

Quantification of staining and statistical analysis

Biopsy specimens were coded, and the number of infiltrating cells in each section was assessed in blinded fashion by two observers. The mean interobserver coefficient of variation was less than 10%. All cells between the dermal-epidermal junction to the base of the hair follicles were counted at 400 $\times$ magnification, and the number of positive cells per square millimeter was calculated. In fields containing sweat ducts and hair shafts, only intervening dermal regions were counted. Statistical differences between densities of the observed cellular infiltrates were evaluated by Wilcoxon's signed-rank sum test for paired data. A *p* value of 0.05 or less was considered significant.

The percentage of dermal vessels expressing E-selectin was assessed by calculating the ratio of vessels staining positive for both *Ulex* and E-selectin and cells staining positive for *Ulex* only.

RESULTS

Comparison of macroscopic reactions of the APT and the LPR

All patients with AE showed positive APT reactions and positive LPRs after HDM challenge, which was an inclusion criterion for the study. In the APT site after 2 hours, no macroscopic reaction was seen. After 24 hours, however, a reaction resembling lesional AE skin was observed, charac-

terized by erythema, induration, and papules. The macroscopic reaction of the LPR consisted of swelling with induration and erythema, which did not resemble lesional AE skin. The LPR had already started at 2 hours and was still present after 24 hours. The scores of the APTs and the diameters of the LPRs at different time points are shown in Table III.

Comparison of cellular composition in the APT reaction with that in nonlesional and lesional skin

At 2 hours (Fig. 1), the neutrophil number was very small and did not differ significantly from nonlesional or lesional skin. In one patient with AE, an influx of activated eosinophils (EG2⁺ cells) had occurred, in spite of a negative macroscopic reaction. Eosinophils were not observed in any of the other patients with AE at this time point. The T-cell number did not differ significantly from that of nonlesional skin, but it was lower compared with that of lesional skin (*p* < 0.02). The mast cell number did not differ significantly from that of nonlesional skin or lesional skin.

At 24 hours (Fig. 1), the number of neutrophils remained small and not significantly different from that of lesional skin. However, there was a slight increase of neutrophils compared with nonlesional skin (*p* < 0.05). There was an increase of activated eosinophils compared with nonlesional and lesional skin (*p* < 0.02). The number of T cells was increased compared with nonlesional skin (*p* < 0.02) but lower compared with lesional skin (*p* < 0.02). The mast cell number did not differ significantly from that of nonlesional or lesional AE skin.

Comparison of cellular composition in the LPR with that in nonlesional and lesional skin

At 2 hours (Fig. 1), an extensive infiltration of neutrophils was present; their number was increased compared with both nonlesional and lesional skin (*p* < 0.02). The LPR also contained more eosinophils than nonlesional and lesional skin (*p* < 0.02). The T-cell number was higher compared with that of nonlesional skin (*p* < 0.02) but lower compared with lesional skin (*p* < 0.02). There was a lower number of mast cells compared with both nonlesional skin and lesional skin (*p* < 0.05).

At 24 hours (Fig. 1), the number of neutrophils and eosinophils was higher compared with nonlesional and lesional skin (*p* < 0.02). The T-cell number was higher compared with nonlesional

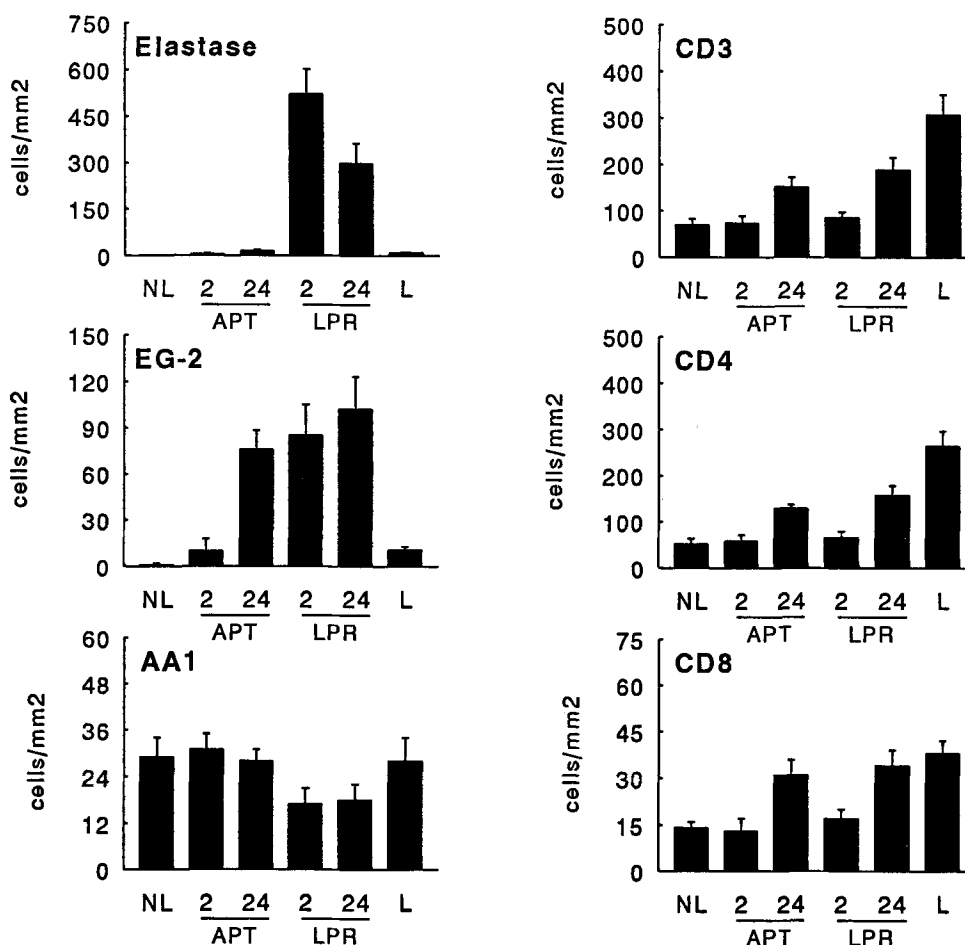


FIG. 1. Elastase-, Eg-2-, AA1-, CD3-, CD4-, and CD8-staining cells (+ SEM) per square millimeter in nonlesional (NL) skin, APT reactions at 2 and 24 hours, LPRs at 2 and 24 hours, and lesional (L) skin.

skin ($p < 0.02$) but lower compared with lesional skin ($p < 0.02$). There were fewer mast cells present than in nonlesional and lesional skin ($p < 0.05$).

Comparison of cellular composition between APT reactions and LPRs

At 2 hours (Fig. 1), the neutrophil number was much higher in LPRs compared with that in APT reactions ($p < 0.02$). Also, the eosinophil number in LPRs was much higher compared with that in APT reactions ($p < 0.02$). There was no significant difference in T-cell numbers between APT reactions and LPRs. The number of mast cells was lower in LPRs compared with APT reactions at 2 hours ($p < 0.05$).

At 24 hours (Fig. 1), the neutrophil number was much higher in LPRs compared with APT reactions ($p < 0.02$). There was no significant differ-

ence in eosinophil number or in T-cell number. The mast cell number was lower in LPRs compared with APT reactions ($p < 0.05$).

E-selectin expression by endothelial cells

In nonlesional skin a minimal staining for E-selectin was noted on endothelial cells (Fig. 2). At 2 hours, an increase of the percentage of dermal vessels expressing E-selectin was seen in the LPRs, but not in the APT reactions, compared with nonlesional skin ($p < 0.02$). At 24 hours, however, in both the APT reaction and the LPR, an increase of the percentage of dermal vessels expressing E-selectin was observed compared with nonlesional skin ($p < 0.02$). At 24 hours, the percentage of dermal vessels expressing E-selectin was significantly higher in the LPR than in the APT reaction ($p < 0.02$).

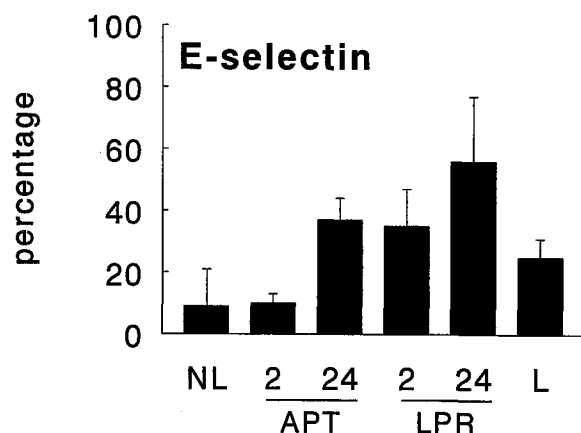


FIG. 2. Percentage of vessels (+ SEM) double-staining for both *Ulex* and E-selectin in nonlesional (NL) skin, APT reactions at 2 and 24 hours, LPRs at 2 and 24 hours, and lesional (L) skin.

Localization of neutrophils in the APT reaction and LPR

In the APT reaction (at both 2 and 24 hours) and in lesional skin, most of the few neutrophils present in the dermis were located in the intravascular area (Fig. 3, *A*), whereas in the LPR (at both 2 and 24 hours), most of the abundantly present neutrophils were located in the dermis, outside the blood vessels (Fig. 3, *B*), which was shown by double staining with *Ulex* (staining endothelial cells) and elastase (staining neutrophils).

DISCUSSION

Our study was aimed at evaluating the APT and the LPR as in vivo models for the study of allergic inflammation in patients with AE. For this reason we compared (both macroscopically and microscopically) the APT reaction, the LPR (at both 2 and 24 hours after antigen challenge), and lesional skin in the same patients with AE. Macroscopically, the APT reaction showed great similarity with lesional AE skin; both were characterized by erythema, induration, and papules. The LPR, however, was clinically characterized by diffuse edema, induration, erythema, and tenderness and did not resemble lesional AE skin.

Microscopically, the APT reaction at 24 hours resembled lesional AE skin; in both, a dermal infiltrate consisting of CD4⁺ T-helper cells and activated eosinophils were observed. At 2 hours, however, no microscopic reaction was observed in the APT site, except in one patient, who showed an increase of activated eosinophils at that time point, in the absence of clinical symptoms. The eosinophil number in the APT reaction at 24 hours was

higher than in lesional AE skin, which could be explained by the acute nature of the inflammation in the APT reaction compared with the chronic character of the inflammation in lesional AE skin. This is in accordance with a study by Wakita et al.,¹⁷ who observed an infiltrate rich in eosinophils in acute AE lesions and a much lower number in chronic AE lesions. We found a very low number of neutrophils in APT reactions, which was in the same range as the number of neutrophils in lesional AE skin.

In the LPR we observed infiltration of neutrophils, eosinophils, and CD4⁺ T cells and a decreased number of mast cells; in fact, this confirms the results of Leiferman et al.¹⁰ When we compared the infiltrate of the LPR with lesional AE skin, we observed remarkable differences; a huge infiltrate of neutrophils was seen in the LPR, which was not present in lesional AE skin, and a decreased number of intact mast cells was observed in the LPR. These data confirm the results of Ott et al.,¹⁸ who recently showed that neutrophils are prominent in the LPR but not in lesional skin of patients with AE.

Comparing the kinetics of the APT reaction and the LPR, we found that in the LPR the eosinophils accumulate at an earlier time point (already at 2 hours) compared with the APT reaction, except in one patient, who also showed an early eosinophil infiltration in the APT reaction. The most striking difference between the two tests was the number of neutrophils, which was much higher in the LPR than in the APT reaction. The neutrophils in the LPR were predominantly located in the extravascular area, in contrast to the few neutrophils present in the APT reaction, which were predominantly located in the intravascular area (Fig. 3, *A* and *B*). Another difference was the number of mast cells, which was slightly lower in the LPR than in the APT reaction, probably caused by degranulation of mast cells in the LPR.

We hypothesize that observed differences between the APT and the LPR are caused by different mechanisms of allergen presentation as a result of different routes of allergen administration. In the APT reaction, aeroallergens penetrate the epidermis and may attach to FcεR1-bound allergen-specific IgE molecules on epidermal Langerhans cells, which subsequently present allergens to allergen-specific T_{H2} cells in the dermis.¹⁹ Allergen-specific T_{H2} cells could be cultured from APT reactions in HDM-sensitized patients with AE.^{20, 21} T_{H2} cells release, among other things, IL-4 and IL-5. IL-4 controls, among other things, selec-

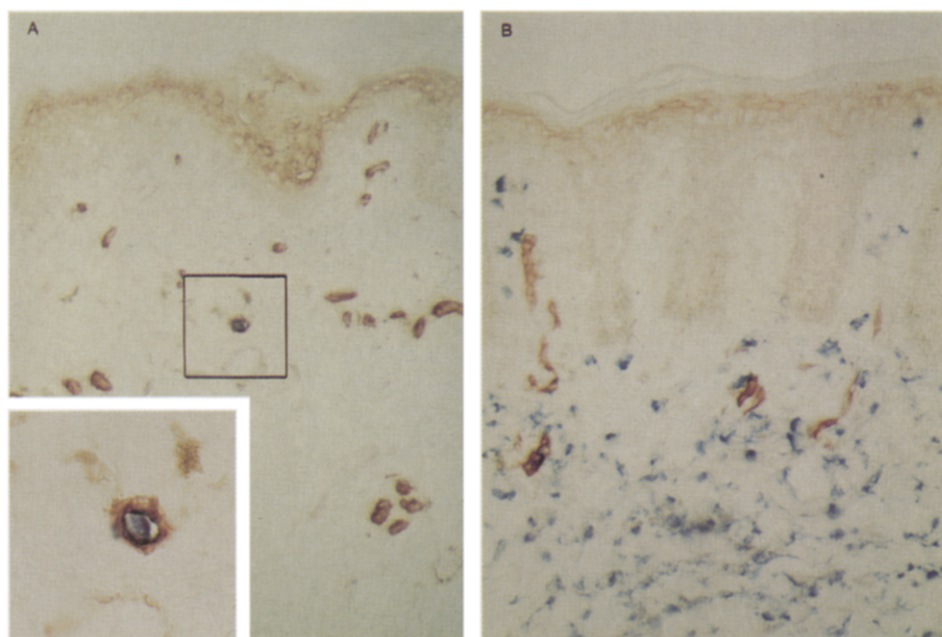


FIG. 3. **A**, APT reaction at 2 hours: double-staining with elastase (blue) and *Ulex* (red); neutrophils are only scarcely present and located in intravascular areas. (Original magnification $\times 20$.) *Left corner*, Detail showing one elastase-positive cell inside a blood vessel (original magnification $\times 60$.) **B**, LPR at 2 hours: double-staining with elastase (blue) and *Ulex* (red); neutrophils are abundantly present in the dermis and located predominantly in extravascular areas (original magnification $\times 20$.)

tive endothelium-driven transmigration of eosinophils from atopic individuals.²² Furthermore, IL-4 is chemotactic for eosinophils of patients with AE.²³ IL-5 is an important cytokine for eosinophil differentiation and proliferation. In the initiation phase of the APT reaction (24 hours), IL-4 production by T_{H2} and T_{H0} cells predominates over interferon- γ production by T_{H1} and T_{H0} cells. In the late phase (48 hours) and the chronic phase (72 hours), the situation is reversed, and interferon- γ production by T_{H1} and T_{H0} cells predominates over IL-4 production by T_{H2} and T_{H0} cells.²⁴

In the LPR, aeroallergens are directly injected into the dermis, bypassing the Langerhans cells in the epidermis. The aeroallergens can bind to Fc ϵ R1-bound allergen-specific IgE on mast cells in the dermis, which results in degranulation and release of many potent inflammatory mediators, including histamine, proteoglycans, tryptase, chymase, carboxypeptidase, acid hydrolases, prostaglandins, leukotrienes, and platelet activating factor.^{25, 26} Furthermore, mast cells may produce a variety of cytokines such as IL-4, IL-5, IL-6, interferon- γ , and tumor necrosis factor- α , which are known to be involved in inflammatory responses in the skin.^{27, 28} In the LPR a T_{H2} -type cytokine

pattern could be demonstrated by in situ hybridization techniques.²⁹

How can we explain the differences in eosinophil and neutrophil infiltration between the APT and the LPR, taking into account the different reaction mechanisms of the two tests?

One explanation may be that mast cell mediators released in the LPR alter the expression of adhesion molecules on endothelium cells, which regulate the number and type of leukocytes emigrating from the blood into inflammatory tissues. Tumor necrosis factor- α , for example, which can be released by mast cells, can induce expression of E-selectin on dermal microvascular endothelium,^{28, 30} which is important in the adhesion of neutrophils. A selective E-selectin expression in the LPR could thus be an explanation for the neutrophil infiltrate. For this reason we compared the E-selectin expression on vascular endothelial cells in APT reactions and LPRs. Already at 2 hours, an upregulation of E-selectin was observed in the LPR, whereas there was no upregulation of E-selectin in the APT reaction at that time point. At 24 hours, however, in both the APT reaction and the LPR an upregulation of E-selectin on vascular endothelial cells was seen, which could

not explain the selective neutrophil involvement in the LPR at that time point. Another explanation could be the difference in release of chemotactic mediators. In the APT reaction the influx of eosinophils must be caused by a mediator, which selectively attracts eosinophils and not neutrophils. This eosinophil-selective mediator is probably IL-4,²³ released from T_{H2} cells, because mast cells do not seem to play an important role in the APT reaction, as observed in the lack of change in mast cell number in the APT sites in our experiments. In the LPR, however, binding of allergens to mast cells results in degranulation, as suggested in our experiments by a decrease in mast cell number. The early infiltration of neutrophils and eosinophils in the LPR might therefore be caused by the local release of chemotactic mast cell mediators, such as leukotriene B₄ and platelet activating factor.⁶

The striking differences between the LPR and lesional skin of patients with AE, macroscopically as well as microscopically, do not support the argument that the LPR is a suitable in vivo model for studying the allergic inflammation in AE. The macroscopic and microscopic similarities between the APT sites and lesional AE skin support the idea that the APT is a more valid model with which to study allergic inflammation in AE than the LPR.

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