

Atopy patch test reactions show a rapid influx of inflammatory dendritic epidermal cells in patients with extrinsic atopic dermatitis and patients with intrinsic atopic dermatitis

Karin Kerschenlohr, MD, Sandra Decard, Bernhard Przybilla, MD, and
Andreas Wollenberg, MD *Munich, Germany*

Background: Normal human skin harbors a single epidermal dendritic cell (DC) population, the CD1a⁺⁺⁺CD11b⁻ Langerhans cells. In many chronic inflammatory skin diseases, the epidermal DC pool bears a second population, the CD1a⁺CD11b⁺⁺⁺ inflammatory dendritic epidermal cells (IDECs). Immunophenotypic, ultrastructural, and functional aspects of IDECs have been investigated in chronic untreated skin lesions of intrinsic and extrinsic atopic dermatitis (AD), contact dermatitis (CD), and psoriasis, but little is known about freshly induced early skin lesions.

Objective: We sought to characterize enumerative and immunophenotypic changes in the epidermal DC pool during the development of eczematous skin lesions.

Methods: The atopy patch test with aeroallergens and food-protein allergens and a conventional patch test with standard-series haptens were performed as models for early skin lesions of extrinsic and intrinsic AD and CD, respectively. After 72 hours, epidermal cell suspensions were prepared, analyzed in a standardized flow cytometric technique, and compared with the results obtained from chronic lesions.

Results: The migration of IDECs into the epidermis occurs within 72 hours and is thus an early event. It continues in chronic AD, but not in chronic CD, lesions. The specific upregulation of FcεRI, especially on IDECs, occurs later during formation of extrinsic but not intrinsic AD lesions. LCs were negative for CD36 in patch test lesions, whereas in chronic skin lesions, LCs expressed CD36.

Conclusion: The DC alteration during skin lesion formation can be subdivided into early and late events, with the influx of IDECs as an early event and the alteration of the DC phenotype as a late event. (*J Allergy Clin Immunol* 2003;111:869-74.)

Key words: Atopic dermatitis, Langerhans cells, inflammatory dendritic epidermal cells, atopy patch test

Abbreviations used

AD:	Atopic dermatitis
APT:	Atopy patch test
CD:	Contact dermatitis
DC:	Dendritic cell
EDCP:	Epidermal dendritic cell phenotyping
ETFAD:	European Task Force on Atopic Dermatitis
FcεRI:	High-affinity IgE receptor
HPT:	Patch test with haptens
IDEC:	Inflammatory dendritic epidermal cell
LC:	Langerhans cell
MFI:	Mean fluorescence intensity
rFI:	Relative fluorescence index
7-AAD:	7-amino-actinomycin-D

Atopic dermatitis (AD) is a clinically defined, highly pruritic, chronic inflammatory skin disease. Frequently there is an association with increased IgE production against aeroallergens and food allergens as well as a local infiltration of mononuclear cells, mainly T cells, and antigen-presenting cells.¹⁻³ A so-called intrinsic type of AD has been delineated from the more common extrinsic AD by normal serum IgE levels, negative RAST, and negative immediate-type skin reactions toward environmental allergens.^{4,5}

In extrinsic AD, there is growing evidence to suggest that IgE-bearing Langerhans cells (LCs) initiate AD lesions and contribute to the amplification of IgE responses toward environmental allergens.⁶ This hypothesis is strengthened by the demonstration of high-affinity receptors for IgE (FcεRI) on LCs in normal skin^{7,8} and the strongly and specifically increased FcεRI expression on epidermal dendritic cells (DCs) in AD.^{9,10} Thus, extrinsic AD might involve an IgE-mediated, delayed-type hypersensitivity reaction in which FcεRI-bearing, antigen-presenting epidermal DCs act as a link between aeroallergens and lesional antigen-specific T cells.^{3,6}

AD is associated with phenotypic and functional alterations of the CD1a-expressing epidermal DCs.^{9,11-13} Two distinct CD1a⁺ epidermal DC populations have been identified in chronic AD lesions. One is LCs, which contain Birbeck granules; the other is inflammatory dendritic epidermal cells (IDECs), which do not contain Birbeck

From the Department of Dermatology and Allergy, Ludwig-Maximilians-University.

Supported by a grant from the Paul-Gerson-Unna-Stiftung, Göttingen, Germany.

Received for publication October 9, 2002; revised December 8, 2002; accepted for publication December 16, 2002.

Reprint requests: Andreas Wollenberg, MD, Department of Dermatology and Allergy, Ludwig-Maximilians-University, Frauenlobstrasse 9-11, 80337 Munich, Germany.

© 2003 Mosby, Inc. All rights reserved.

0091-6749/2003 \$30.00 + 0

doi:10.1067/mai.2003.1347

granules. Compared with LCs, the IDECs show a higher FcεRI expression, express higher amounts of costimulatory molecules,¹⁴ are capable of mannose receptor-mediated endocytosis,¹⁵ and might therefore be the key DC in AD.¹⁶ The immunophenotype of both LCs and IDECs has been analyzed simultaneously in a recently standardized flow cytometric test procedure, which is based on the quantitative data obtained from a 3-color flow cytometric test procedure.¹⁰ Whereas >800 tissue samples have been analyzed from chronic, untreated, inflamed skin lesions (Wollenberg et al, unpublished observations), including intrinsic and extrinsic AD,¹⁷ little is known about the DC infiltrate of early AD lesions.

The atopy patch test (APT) has been used as a model for early AD lesions¹⁸ and is performed like a normal patch test with haptens (HPT), only with protein allergens.^{19,20} The standardized APT technique that we used has been developed in a series of multicenter trials and is regarded as a suitable and reproducible procedure for clinical and experimental use of this test.²¹⁻²³

Using the APT and HPT as an experimental model for early AD lesions and contact dermatitis (CD) lesions, respectively, we performed the present study to compare APT lesions with the previously described data from the respective chronic skin lesions to better understand the kinetics of lesion formation in AD. Positive HPT lesions as well as samples of chronic untreated CD lesions served as controls.

METHODS

Study patients

The study was undertaken at the Department of Dermatology and Allergy, University of Munich, in 65 AD outpatients (17 male, 48 female; age, 3 to 66 years; mean age, 28.5 years). Our investigation was a substudy of a larger multicentric trial (Darsow et al, in preparation). The diagnosis of AD was made according to the criteria of Hanifin and Rajka²⁴ and was confirmed for each patient on clinical grounds by at least 2 different dermatologists.

Skin prick tests were performed with atopy patch-tested aeroallergens (100 IR, in water/glycerol) and food allergens (33 IR, in water/glycerol) in all patients with AD. Allergen-specific IgE levels were determined by CAP-FEIA (Pharmacia, Uppsala, Sweden).

The control group consisted of 12 patients with allergic CD who had a classic HPT performed with their relevant contact allergens. Shave biopsy samples were taken from 3 test lesions positive for nickel(II) sulfate and 1 positive test lesion each for cobalt, neomycin, dibromdicyanobutan, thiuram mixture, *p*-methylaminophenolsulfate, fragrance mixture, thimerosal, methyl(chloro)-isothiazolinone, and toluenediamine.

To compare our results from APT and HPT lesions with the immunobiological situation of chronic, naturally developed skin lesions, epidermal DC phenotyping (EDCP) data of 80 chronic, untreated skin lesions were analyzed from our EDCP data bank (7 intrinsic AD, 59 extrinsic AD, and 26 CD).¹⁰

This study was approved by the local ethics committee and was conducted according to the principles expressed in the Declaration of Helsinki. Written, informed consent was obtained from all patients.

APT procedure

APTs were performed according to a standardized, published procedure.²³ In brief, the patch-tested aeroallergens (house dust

mite [*Dermatophagoides pteronyssinus*], birch pollen, cat dander, and grass pollen [*Phleum pratense*], all at 200 IR in petrolatum [Stallergenes, Antony, France]), food allergens (egg white, celery, and wheat flour, all at 1/3 w/v in petrolatum [Stallergenes]), and a negative vehicle control (petrolatum [Stallergenes]) were applied on the patients' backs in large Finn chambers (11 mm in diameter) and fixed with stretch plaster. After 48 hours the allergens were removed and the tested areas were marked. The test was read after 48 and 72 hours. Positive reactions were classified according to the European Task Force on Atopic Dermatitis (ETFAD) key, which is based on the appearance of papules, erythema, and infiltration in the tested area.²⁵ Patients did not have an acute phase of eczema, nor had they received prior treatment with topical steroids or UV light on the test site.

Biopsy specimens and preparation of epidermal cell suspensions

For EDCP, shave biopsy samples were obtained under local anesthesia on day 3 from positive patch test lesions. Epidermal cell suspensions were prepared by trypsinization of skin specimens, as described.¹⁰ The resulting cell suspensions were processed for immunolabeling and analyzed by flow cytometry to determine the proportion of CD1a⁺ epidermal DCs (ie, LCs and IDECs) and the expression of selected cell surface molecules.

Immunostaining of epidermal cell suspensions

Flow cytometric analysis was performed in a triple-staining method, as described previously.¹⁰ In short, approximately 200,000 crude epidermal cells were incubated with 1 of the following antibodies at a concentration of 2.5 μg/mL for 30 minutes. The IgE-binding α-chain of FcεRI was detected by mAb 22E7 (IgG1; a generous gift of Dr J. Kochan, Hoffmann-La Roche Co, Nutley, NJ); this antibody does not interfere with the binding site for IgE on the receptor.²⁶ The mAb IV.3 (IgG2b, Medarex, West Lebanon, NH) is directed against the human low-affinity IgG receptor FcγRII/CD32. The FcεRI/FcγRII expression ratio is a highly specific marker for AD.²⁷ The mAb IOP36 (IgG2b, Immunotech, Marseille, France) is specific for CD36, the thrombospondin receptor, which represents a putative collagen-binding structure. In situ expression of CD36 is indicative of an inflammatory microenvironment.¹⁰ The mAb BEAR1 (IgG1, Immunotech) reacts with the integrin chain CD11b, which is highly expressed on IDECs but not on LCs.¹⁰ MOPC (IgG1, Sigma, Deisenhofen, Germany) and UPC10 (IgG2b, Sigma) were used at the same concentration as the isotype control mAb. After primary labeling, the cells were washed in PBS containing 1% FCS and 0.1% sodium azide and then incubated for 30 minutes with FITC-conjugated goat antimouse antibody (Jackson Laboratories, West Grove, Pa) at a dilution of 1:100. Blocking was carried out for 15 minutes with normal mouse serum (Sigma, 1:10). The cells were again washed and then counterstained for 30 minutes with a mixture of the phycoerythrin-labeled T6/RD1 antibody (Coulter, Krefeld, Germany) and 7-amino-actinomycin D (7-AAD, Sigma), both at a concentration of 1 μg/mL. Phycoerythrin-labeled T6/RD1 antibody labels CD1a⁺ epidermal DCs—ie, LCs and IDECs. The 7-AAD, which emits in the far red end of the spectrum, labels dead keratinocytes and debris, which can be strongly nonspecifically labeled with the anti-CD1a antibody.¹⁰ After a final wash, the cells were analyzed by flow cytometry. All incubations and washes were performed at 4°C.

Flow cytometric analysis of epidermal DCs

Unfixed cells were analyzed on a fluorescence-activated cell-sorting system (FACScan, Becton Dickinson, Mountain View, Calif). The vital CD1a⁺ DC populations were gated out by a com-

bination of forward and side scatters and a CD1a/7-AAD gate set, as described previously.¹⁰ Fluorescence parameters were collected through use of a built-in logarithmic amplifier, and the data of approximately 10,000 cells were collected and analyzed with the CellQuest program (Becton Dickinson). For quantitative evaluation, the CD1a⁺ populations (ie, LCs and IDECs) were gated out manually. Their proportions in the CD1a⁺ cell pool as well as the mean fluorescence intensity (MFI) for each surface molecule were determined for each population of interest through use of CellQuest software.¹⁰ Relative fluorescence indices (rFIs) of all surface receptors were determined as follows:

$$\text{rFI} = (\text{MFI}_{\text{receptor}} - \text{MFI}_{\text{control}}) / \text{MFI}_{\text{control}}$$

RESULTS

Clinical aspects of APT reactivity

The APT was performed on 65 patients with AD, of whom intrinsic AD was diagnosed in 6 (9%).¹⁷ Positive APT reactions after 72 hours were seen in 26 patients (40%), and 15 of them volunteered to have their positive APT skin reaction shave-biopsied. The causative allergens of the 15 biopsied lesions were house dust mite (*Dermatophagoides pteronyssinus*; n = 11), birch pollen (n = 2), grass pollen (*Phleum pratense*; n = 1), and wheat flour (n = 1). Whereas 12 of the biopsied patients were classified as having extrinsic AD, 3 patients fulfilled the criteria of having intrinsic AD. The range of positive APT reactions lay between + and +++++, and biopsy samples were taken from + to +++ reactions. Most of the reactions were +++. There was no relation between the tested allergen and the intensity of the test reaction.

Of the 6 patients with intrinsic AD, 3 showed positive APT reactions to house dust mite (n = 2) or birch pollen (n = 1). All 3 had negative prick test results, negative RAST results, low total serum IgE levels (23 kU/L, 55 kU/L, and 10 kU/L), and a negative family history for atopic diseases.²⁸ Nevertheless, the patients' reported histories had already suggested a clinically relevant sensitization against the house dust mite (worsening of skin condition after dust exposure) or birch pollen (worsening of skin condition during birch pollen flight, especially in exposed skin areas). EDCP of these patients' APT reactions showed a rather high number of IDECs compared with the extrinsic AD test lesions but a comparably low FcεRI expression, a pattern previously seen in naturally developed intrinsic AD lesions.¹⁷ Hence, a clinically suggestive sensitization to aeroallergens is detectable in a subset of patients with intrinsic AD, indicating an allergic background even in the absence of allergen-specific IgE.

IDEC migration into the epidermis starts early during formation of AD lesions

The presence of IDECs is a hallmark of the skin lesions in AD and CD, whereas nonlesional skin of patients with AD and patients with CD does not contain relevant numbers of IDECs.¹⁰ In the APT-induced lesions, the amount of CD1a⁺ cells was already increased in intrinsic and patients with extrinsic AD in comparison with normal skin. Moreover, a distinct IDEC population could already be detected at day 3 in both APT and HPT (Fig 1). Chron-

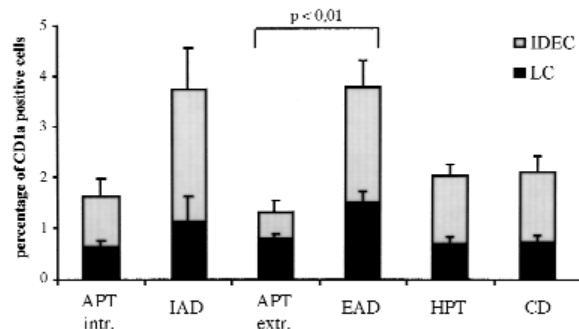


FIG 1. Mean proportions of CD1a⁺ epidermal DCs and their subclassification into inflammatory dendritic epidermal cells (IDEC) and Langerhans cells (LC). Mean proportions of IDECs and LCs were determined by flow cytometric analysis of APT and HPT lesions and chronic naturally grown lesions (APT intrinsic, n = 3; IAD, n = 5; APT extrinsic, n = 12; EAD, n = 30; HPT, n = 12; CD, n = 21). Each column shows the proportion of CD1a⁺ cells as a percentage of the total epidermal cells. Highest percentages are present in chronic lesions of both intrinsic and extrinsic AD. The amount of CD1a⁺ cells in patch test lesions is already increased compared with normal skin. APT lesions of patients with IAD showed a higher number of IDECs than did the APT lesions of EAD. Naturally developed lesions of both AD subtypes showed an increased number of IDECs compared with test lesions, which became significant for extrinsic AD.

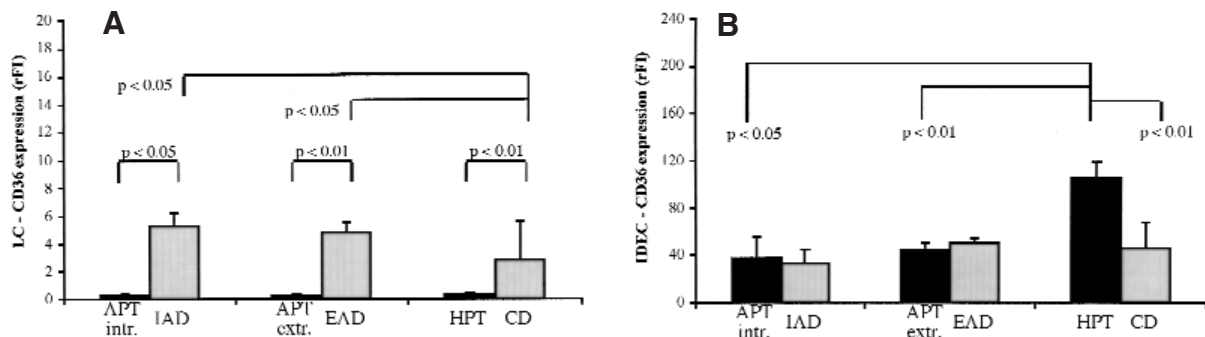
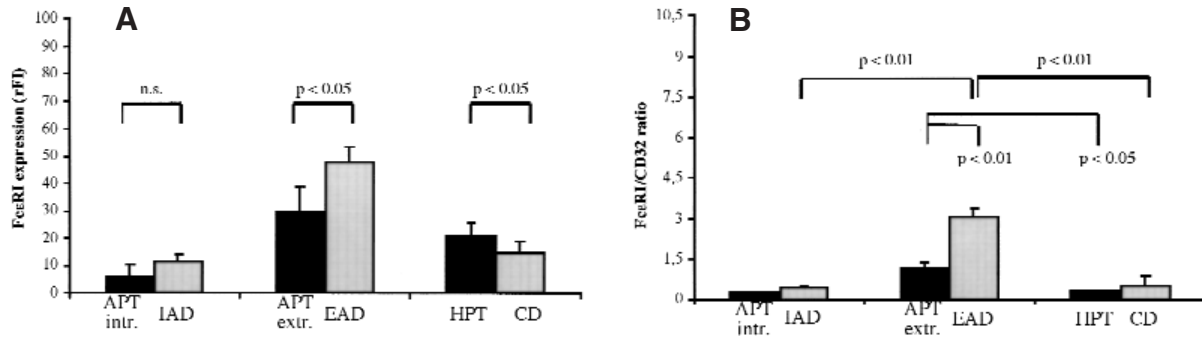
ic lesions of intrinsic and extrinsic AD are characterized by further increased numbers of IDECs, whereas in CD, the IDEC population does not increase over time (Fig 1). Taken together, there is an early immigration of IDECs in AD and CD, but an ongoing influx of IDECs is present only in both intrinsic and extrinsic AD.

Evidence for a time course of disease-specific FcεRI expression

In general, chronic extrinsic AD lesions are characterized by high expression of FcεRI on the DC surface.¹⁷ The APT lesions of patients with intrinsic AD showed a lower FcεRI expression on DCs than did HPT lesions, although chronic intrinsic AD lesions showed higher FcεRI expression than did chronic CD lesions (Fig 2, A). In addition, the FcεRI expression on DCs increases over time in intrinsic and extrinsic AD, whereas it decreases in CD (Fig 2, A). These results indicate distinctive, dynamic changes of the DC phenotype during formation of the lesion in AD and CD and identify the disease-specific upregulation of FcεRI as a late event during the formation of an extrinsic AD lesion.

The established FcεRI/FcγRII ratio is not diagnostic for APT lesions

A high FcεRI/FcγRII expression ratio (>1.5) on epidermal DCs is an established diagnostic marker for chronic extrinsic AD.^{17,27} All chronic and patch test lesions of both CD and intrinsic AD showed an FcεRI/FcγRII expression ratio on epidermal DCs below 1.5, whereas the majority of chronic AD lesions exceeded the 1.5 threshold (Fig 2, B). In APT lesions this threshold value was reached in only 4 of 15 samples, which were all taken



from patients with extrinsic AD. A low FcεRI/FcγRII expression ratio (<1.5) was seen in 11 of 15 APT lesions, including the 3 intrinsic AD cases with ratios of 0.26 (37% IDECs), 0.23 (61% IDECs), and 0.23 (70% IDECs). With the frequently low FcεRI/FcγRII ratio on DCs in APT lesions, the diagnostic use of EDCP is restricted to chronic skin lesions.

CD36 expression on LCs is a marker for chronic lesions

For many years it has been known that IDECs are characterized by a high CD36 expression, whereas LCs express little or no CD36 on their cell surfaces.⁹ We were able to show for the first time that just as in normal human skin, LCs do not express CD36 in patch test lesions, whereas a low but significant CD36 expression was seen on LCs in chronic lesions of AD and CD (Fig 3, A). This extends the diagnostic possibilities of EDCP

to assign an early or chronic stage to each biopsy sample. Second, CD36 expression on IDECs was significantly higher in HPT lesions than in APT lesions and decreased during formation of chronic CD lesions (Fig 3, B).

DISCUSSION

By using the APT and the HPT as a model for early AD and CD lesions, respectively, we were able to demonstrate that the migration of IDECs into the epidermis occurs within 72 hours and is thus an early event. This immigration of IDECs continues in chronic AD, but not in chronic CD, lesions. The specific upregulation of FcεRI, especially on IDECs, occurs later during formation of extrinsic, but not intrinsic, AD lesions. Consequently, it is impossible to reliably differentiate early inflammatory skin lesions by using the established high FcεRI/FcγRII expression ratio. There is no CD36 expres-

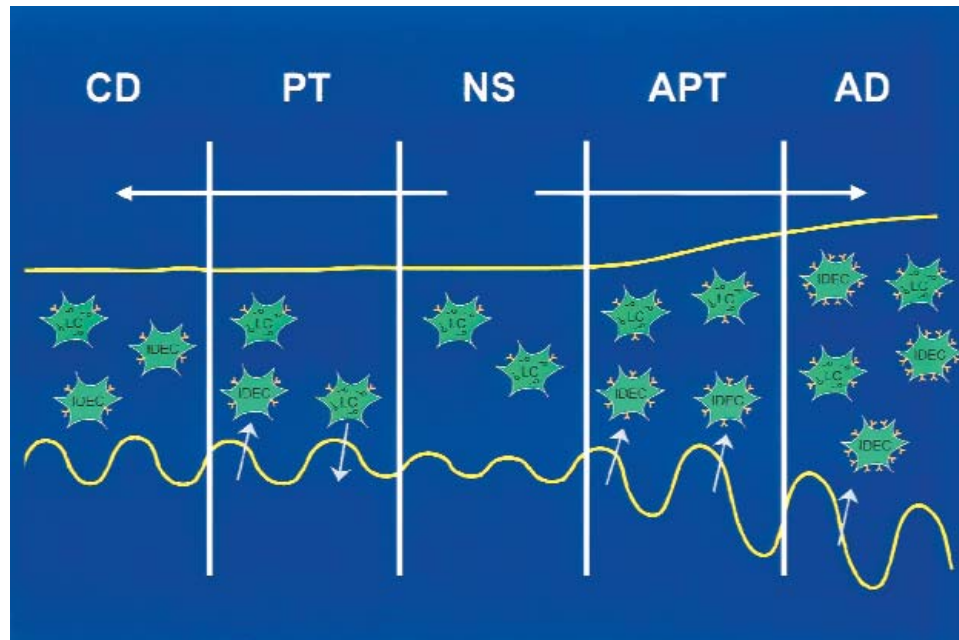


FIG 4. Kinetics of epidermal DC trafficking. The influx of IDECs into the epidermal compartment of normal human skin (*NS*) is a relatively early event during the formation of an eczematous skin lesion, inasmuch as it can be witnessed in hapten patch test (*PT*) lesions and atopy patch test (*APT*) lesions. Later, atopic dermatitis (*AD*) is characterized by a prolonged influx of IDECs, whereas in contact dermatitis (*CD*), FcεRI is downregulated and LCs are depleted from the epidermis, as witnessed by a constant number of epidermal DCs with an increased proportion of IDECs.

sion on LCs in patch test lesions, but CD36 is regularly present on LCs in chronic skin lesions.

The characterization of early skin lesions by analysis of biopsy samples from naturally grown lesions of AD and other chronic inflammatory skin diseases is hampered by (1) the rareness of early lesions in our outpatient setting and (2) the inaccurate history most patients are able to provide for a single lesion. Although APT lesions have not formally been shown to develop into chronic eczematoid skin lesions, freshly induced APT and HPT lesions have been used as a model for early AD and CD lesions before.^{18,29,30} We have followed this approach because it seems reasonable and guarantees uniform conditions for the type and quality of the allergens, the exposure time, and the clinical setting as such.

At present, little is known about the cascade of events during the formation of an eczematous skin lesion. From histology and immunohistology we know that lymphocytes, histiocytes (monocytes, macrophages, and DCs), eosinophils, and other cell types are accumulating in the inflamed skin.³¹ In HPT lesions, the pro-inflammatory cytokine IL-1b is the first detectable cytokine, followed by TNFα, which induces the migration of LCs from the epidermal compartment.³²⁻³⁴ The switch from a T_H2-dominated to a T_H1-dominated microenvironment in the AD lesion might be induced by IL-12, and eosinophils as well as IDECs might be the relevant source of this cytokine.^{35,36} The chemokine MIP-3a, which is produced by keratinocytes, induces the migration of CCR6-expressing LCs into the epidermis, whereas other “inflammatory” DCs, such as IDECs, might migrate in

response to a wide array of different chemokines.³⁷

On the basis of our assumption that the immunophenotype of the epidermal DCs might reflect the disease-specific inflammatory microenvironment of the underlying skin disease, we proposed the technique of EDCP as a diagnostic tool in inflammatory skin diseases.^{10,27} Diagnostic EDCP criteria for AD and psoriasis have been identified.^{10,38} The high FcεRI/FcγRII expression (>1.5), which is characteristic for naturally grown extrinsic AD lesions, is not diagnostic for early AD as induced with the APT, neither for extrinsic nor intrinsic AD. Hence, the diagnostic value of EDCP will continue to lie within the limits of chronic untreated skin lesions.

Early lesions of CD and AD, as induced by the HPT and APT, do not differ much in terms of the epidermal DC immunophenotype. The early differences are an accumulation of epidermal DCs in AD skin, as opposed to the emigration of resident LCs from the epidermal compartment toward the lymph nodes.³⁹ Hence, the generation of eczematous skin lesions might involve an early phase with DC immigration, followed by an alteration of the DC immunophenotype. Because differences have been identified on the RNA level between clinically distinct eczematous skin lesions,⁴⁰ the modulation of the DC immunophenotype might follow an earlier differentiation on the RNA level.

The expression of CD36 has repeatedly been attributed to be a specific delineation marker between LCs and IDECs.⁴¹ Here we confirmed the previously known strong expression of CD36 on IDECs and could confirm the previously suspected relation between the duration of a single

lesion and the expression of CD36 on the lesional LC.¹⁰ Because LCs might express CD36 on inflammation, CD36 is not a useful delineation marker between LCs and IDECs.

In conclusion, the role of DCs during the formation of inflamed skin lesions can be subdivided into early and late events, with the influx of IDECs as an early event and the alteration of the DC phenotype as a late event (Fig 4).

We are grateful to Dr J. Kochan, Nutley, NJ, for providing anti-FcεRIα mAb; to Mr A. Didierlaurent, Stallergenes, Antony, France, for the generous gift of the APT substances; and to Ulf Darsow, Munich, Germany, for organizing the clinical multicenter trial. We thank Prof Dr H. C. Gerd Plewig, Munich, Germany, and Prof Walter Burgdorf, MD, Tübingen, Germany, for critical reading of the manuscript.

REFERENCES

- Cooper KD. Atopic dermatitis: recent trends in pathogenesis and therapy. *J Invest Dermatol* 1994;102:128-37.
- Leung DY. Atopic dermatitis: new insights and opportunities for therapeutic intervention. *J Allergy Clin Immunol* 2000;105:860-76.
- Wollenberg A, Bieber T. Atopic dermatitis: from the genes to skin lesions. *Allergy* 2000;55:205-13.
- Wüthrich B. Neurodermitis atopica sive constitutionalis: ein pathogenetisches Modell aus der Sicht des Allergologen. *Akt Dermatol* 1983;9:1-7.
- Schmid-Grendelmeier P, Simon D, Simon HU, Akdis CA, Wüthrich B. Epidemiology, clinical features, and immunology of the 'intrinsic' (non-IgE-mediated) type of atopic dermatitis (constitutional dermatitis). *Allergy* 2001;56:841-9.
- Novak N, Kraft S, Bieber T. Unraveling the mission of FcεRI on antigen presenting cells. *J Allergy Clin Immunol* 2003;111:38-44.
- Bieber T, de la Salle H, Wollenberg A, Hakimi J, Chizzonite R, Ring J, et al. Human epidermal Langerhans cells express the high affinity receptor for immunoglobulin E (FcεRI). *J Exp Med* 1992;175:1285-90.
- Wang B, Rieger A, Kilgus O, Ochiai K, Maurer D, Föding D, et al. Epidermal Langerhans cells from normal human skin bind monomeric IgE via FcεRI. *J Exp Med* 1992;175:1353-65.
- Wollenberg A, Kraft S, Hanau D, Bieber T. Immunomorphological and ultrastructural characterization of Langerhans cells and a novel, inflammatory dendritic epidermal cell (IDEC) population in lesional skin of atopic eczema. *J Invest Dermatol* 1996;106:446-53.
- Wollenberg A, Wen S, Bieber T. Phenotyping of epidermal dendritic cells: clinical applications of a flow cytometric micromethod. *Cytometry* 1999;37:147-55.
- Leung DY, Schneeberger EE, Siraganian RP, Geha RS, Bhan AK. The presence of IgE on macrophages and dendritic cells infiltrating into the skin lesion of atopic dermatitis. *Clin Immunol Immunopathol* 1987;42:328-37.
- Cooper KD. Pathogenesis and pharmacology of atopic dermatitis. In: Czernielewski JM, editor. *Immunological and pharmacological aspects of atopic and contact eczema*. Basel: Karger, 1991, vol 4, p. 119-27.
- Taylor RS, Baadsgaard O, Hammerberg C, Cooper KD. Hyperstimulatory CD1a+CD1b+CD36+ Langerhans cells are responsible for increased autologous T lymphocyte reactivity to lesional epidermal cells of patients with atopic dermatitis. *J Immunol* 1991;147:3794-802.
- Schuller E, Teichmann B, Haberkost J, Moderer M, Bieber T, Wollenberg A. In situ-expression of the costimulatory molecules CD80 and CD86 on Langerhans cells and inflammatory dendritic epidermal cells (IDEC) in atopic dermatitis. *Arch Dermatol Res* 2001;293:448-54.
- Wollenberg A, Mommaas M, Oppel T, Schottdorf EM, Günther S, Moderer M. Expression and function of the mannose receptor CD206 on epidermal dendritic cells in inflammatory skin diseases. *J Invest Dermatol* 2002;118:327-34.
- Wollenberg A, Bieber T. Antigen presenting cells. In: Bieber T, Leung DYM, editors. *Atopic dermatitis*. 1st ed. New York: Marcel Dekker, 2002, p. 267-83.
- Oppel T, Schuller E, Günther S, Moderer M, Haberkost J, Bieber T, et al. Phenotyping of epidermal dendritic cells allows the differentiation between extrinsic and intrinsic form of atopic dermatitis. *Br J Dermatol* 2000;143:1193-8.
- Billmann-Eberwein C, Rippke F, Ruzicka T, Krutmann J. Modulation of atopy patch test reactions by topical treatment of human skin with a fatty acid-rich emollient. *Skin Pharmacol Appl Skin Physiol* 2002;15:100-4.
- Ring J, Darsow U, Gfesser M, Vieluf D. The 'atopy patch test' in evaluating the role of aeroallergens in atopic eczema. *Int Arch Allergy Immunol* 1997;113:379-83.
- de Bruin-Weller MS, Knol EF, Bruijnzeel-Koomen CA. Atopy patch testing—a diagnostic tool? *Allergy* 1999;54:784-91.
- Darsow U, Vieluf D, Ring J. Atopy patch test with different vehicles and allergen concentrations: an approach to standardization. *J Allergy Clin Immunol* 1995;95:677-84.
- Darsow U, Vieluf D, Ring J. The atopy patch test: an increased rate of reactivity in patients who have an air-exposed pattern of atopic eczema. *Br J Dermatol* 1996;135:182-6.
- Darsow U, Vieluf D, Ring J. Evaluating the relevance of aeroallergen sensitization in atopic eczema with the atopy patch test: a randomized, double-blind multicenter study. *J Am Acad Dermatol* 1999;40:187-93.
- Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol (Stockh) Suppl* 1980;92:44-7.
- Darsow U, Ring J. Airborne and dietary allergens in atopic eczema: a comprehensive review of diagnostic tests. *Clin Exp Dermatol* 2000;25:544-51.
- Riske F, Hakimi J, Mallamaci M, Griffin M, Pilsen B, Tobkes N, et al. High affinity human IgE-Receptor (FcεRI): analysis of functional domains of the α-subunit with monoclonal antibodies. *J Biol Chem* 1991;266:11245-51.
- Wollenberg A, Wen S, Bieber T. Langerhans cell phenotyping: a new tool for differential diagnosis of inflammatory skin diseases. *Lancet* 1995;346:1626-7.
- Kerschenlohr K, Decard S, Darsow U, Ollert M, Wollenberg A. Clinical and immunologic reactivity to aeroallergens in 'intrinsic' atopic dermatitis patients. *J Allergy Clin Immunol* 2003;111:195-7.
- Grewe M, Walther S, Gyufko K, Czech W, Schopf E, Krutmann J. Analysis of the cytokine pattern expressed in situ in inhalant allergen patch test reactions of atopic dermatitis patients. *J Invest Dermatol* 1995;105:407-10.
- Thepen T, Langeveld Wildschut EG, Bihari IC, van Wichen DF, van Riejen FC, Mudde GJ, et al. Biphasic response against aeroallergen in atopic dermatitis showing a switch from an initial TH2 response to a TH1 response in situ: an immunocytochemical study. *J Allergy Clin Immunol* 1996;97:828-37.
- Eckert F. Histopathological and immunohistological aspects of atopic dermatitis. In: Ruzicka T, Ring J, Przybilla B, editors. *Handbook of atopic dermatitis*. Berlin: Springer, 1991, p. 127-31.
- Kimber I, Cumberbatch M. Stimulation of Langerhans cell migration by tumor necrosis factor alpha (TNF-alpha). *J Invest Dermatol* 1992;99:48S-50S.
- Enk AH, Angeloni VL, Udey MC, Katz SI. An essential role for Langerhans cell-derived IL-1 beta in the initiation of primary immune responses in skin. *J Immunol* 1993;150:3698-704.
- Cumberbatch M, Dearman RJ, Kimber I. Interleukin 1 beta and the stimulation of Langerhans cell migration: comparisons with tumor necrosis factor alpha. *Arch Dermatol Res* 1997;289:277-84.
- Grewe M, Czech W, Morita A, Werfel T, Klammer M, Kapp A, et al. Human eosinophils produce biologically active IL-12: implications for control of T cell responses. *J Immunol* 1998;161:415-20.
- Wittmann M, Larsson VA, Schmidt P, Begemann G, Kapp A, Werfel T. Suppression of interleukin-12 production by human monocytes after preincubation with lipopolysaccharide. *Blood* 1999;94:1717-26.
- Charbonnier AS, Kohrgruber N, Kriehuber E, Stingl G, Rot A, Maurer D. Macrophage inflammatory protein 3α is involved in the constitutive trafficking of epidermal Langerhans cells. *J Exp Med* 1999;190:1755-68.
- Wollenberg A, Haberkost J, Schuller E, Teichmann B, Bieber T. Upregulation of Fcγ receptors on epidermal dendritic cells is specific for Psoriasis vulgaris. *Arch Dermatol Res* 1999;291:153.
- Brand CU, Hunger RE, Yawalkar N, Gerber HA, Schaffner T, Braathen LR. Characterization of human skin-derived CD1a-positive lymph cells. *Arch Dermatol Res* 1999;291:65-72.
- Morhenn VB, Chang EY, Rheins LA. A noninvasive method for quantifying and distinguishing inflammatory skin reactions. *J Am Acad Dermatol* 1999;41:687-92.
- Baadsgaard O, Gupta AK, Taylor RS, Ellis CN, Voorhees JJ, Cooper KD. Psoriatic epidermal cells demonstrate increased numbers and function of non-Langerhans antigen presenting cells. *J Invest Dermatol* 1989;92:190-5.