

Adhesion molecules in atopic dermatitis: patch tests elicited by house dust mite

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Different T-helper subsets, which are characterized by the secretion of distinct cytokines (Th1, Th2), have been found in house dust mite-exposed skin of sensitized individuals and in nickel-specific T lymphocytes from nickel contact allergic and non-allergic individuals. In order to evaluate the rôle which adhesion molecules may play in the homing of different T-cell subsets into allergen-exposed skin of atopic and normal individuals, we compared the expression pattern of adhesion molecules in patch test reactions to house dust mite antigen (D.pt.), nickel sulfate (Ni) and the irritant anthralin. Biopsies were taken at various time points after application of these agents and studied by immunocytochemistry. To exclude an endogenous difference in adhesion molecule expression in atopic and non-atopic skin, sequential biopsies from Ni patch tests of 2 normal individuals were also included in this study. The expression of E-selectin, P-selectin, CD31, VCAM-1 and ICAM-1 on endothelial cells and other cells in the skin was quantified by microscopic evaluation. Skin homing T cells were also quantified using antibodies to CD3, CD4, CD8, UCHL-1, L-selectin and the cutaneous lymphocyte antigen (CLA). Independent of the eliciting substance, all lesions showed an upregulation of all adhesion molecules tested, with the exception of CD62. The appearance of E-selectin and an increase in ICAM-1 and VCAM-1 expression were first observed at 12 h after application of the various agents. In parallel, the number of CLA+ and L-selectin+ lymphocytes increased steadily. No principle differences could be established between the various types of skin reactions in atopic individuals, nor did the skin of patients with AD differ from normal controls. Our results provide evidence that differential expression of adhesion molecules does not play a major part in observed differential homing of Th1 and Th2-cell subsets into patch test sites provoked by house dust mite and nickel sulfate in atopic and non-atopic individuals.

Key words: anthralin; atopic dermatitis; adhesion molecules; *Dermatophagoides pteronyssinus*; nickel sulfate. © Munksgaard, 1997.

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Patients with atopic dermatitis (AD) may develop delayed-type eczematous skin reactions after epicutaneous application of inhalant allergens such

as *Dermatophagoides pteronyssinus* (D.pt.). (1, 2) Several findings support the assumption that the allergen patch test may be regarded as a model for studying the pathogenesis of AD, as positive patch test reactions to aeroallergens seem to be specific for AD. Moreover, in support of an immunospecific type of skin reaction, these lesions show increased numbers of activated CD4+ T cells and CD1+/CD14+ antigen presenting cells (2, 3).

In fact, recently T cells showing specificity for the eliciting antigens D.pt. or grass pollen but not for unrelated antigens have been cloned from patch test sites of patients with AD (4–6). The frequency of these cells at the test sites compared favourably with the numbers of specific T cells in-

Abbreviations used

AD: atopic dermatitis; CLA: cutaneous lymphocyte antigen; D.pt.: *Dermatophagoides pteronyssinus*; ELAM-1: endothelial leucocyte adhesion molecule-1 (=E-selectin); HUVEC: human umbilical vein endothelial cells; ICAM-1: intercellular adhesion molecule-1; IL-4: interleukin 4; IL-6: interleukin 6; IL-10: interleukin 10; IFN γ : interferon gamma; L-selectin: leukocyte selectin; Ni: nickel sulphate; NS: normal skin; sialyl Lewis x: sialylated Lewis x factor; TGF β : tumor growth factor beta; Th1 cells: T-helper type 1 cells; Th2 cells: T-helper type 2 cells; TNF α : tumor necrosis factor alpha; VCAM-1: vascular cell adhesion molecule-1

volved in delayed-type hypersensitivity reactions (7). However, in contrast to T cells derived from allergic contact dermatitis, a considerable part of the D.pt.-specific skin derived T-helper cells secreted large amounts of interleukin-4 (IL-4), interleukin 10 (IL-10) and no or little interferon-gamma (IFN γ) (4, 5, 7, 8). This secretion pattern resembles that of T-helper 2 (Th2)-cells in mice (9, 10). Furthermore, spontaneously developed skin lesions in AD also harbour Th2 like cells (4, 11, 12). These results, as well as the characterization of cytokines in atopic skin by polymerase chain reaction, suggest (13) that atopic individuals are able to mediate an eczematous delayed-type reaction to inhalant allergens which, early in its development, is characterized by the appearance of allergen-specific Th2-cells.

Although several cytokines are known to either positively or negatively regulate the development of Th1- and Th2-cells (10, 14), the reason for this imbalanced development of Th2-cells in AD is presently not known. In contrast to AD, in non-atopic individuals contact allergens have been described to elicit a Th1 response which is characterized by the secretion of IFN γ and IL-2 (15, 16). It thus may be hypothesized that the skin of patients with AD provides a special environment either for homing or for *in loco* differentiation of Th2 cells. It is well-known that allergen exposure of the skin results in the upregulation of various membrane molecules which are related to T-cell migration (17–21). Endogenous differences in the release of cytokine response from keratinocytes and other resident skin cells in response to allergens in normal and atopic individuals, as well as differences in the expression of receptor-ligand pairs known to mediate T-cell adhesion, may be responsible for the observed differential development of T-cell sub-

sets in allergen-exposed skin. In order to answer the question whether a differential regulation of adhesion molecules occurs in the skin of normal individuals and of patients with AD, we performed an immunocytochemical study on patch test reactions and irritant reactions in both groups.

Patients and Methods

Biopsies

5 patients with long-standing AD, as defined by the criteria of Hanifin et Rajika (22), were patch tested with 20 μ l D.pt.-antigen (500 μ g/ml in 0.9% NaCl) on healthy appearing back skin of their back without prior scarification of the skin (5). All patients had a positive prick test and a RAST class >2 to D.pt. of their serum (Ciba Corning, Fernwald, Germany). 2 patients (RAST class 4) developed papules and erythema starting at 24 h after application of D.pt. 1 patient (RAST class 3) developed erythema at 24 h which persisted for 2 days. All patients with RAST class >3 had a weal and flare reaction at 30 min. 4-mm punch biopsies were taken before and 12, 24, 48 and 72 h after allergen application and were subsequently snap frozen in liquid nitrogen.

Sequential biopsies were also taken from 4 atopic and 2 non-atopic individuals with positive test reactions to nickel sulfate (5% pet.). Erythema, papules and vesicles appeared 24 h after application of the antigen to the back skin in all these individuals.

Furthermore, anthralin in yellow soft paraffin (0.125%) was applied to the healthy appearing back of 6 individuals with AD. Maximal irritant contact dermatitis was observed between 3 and 7 days. From each patient skin punch biopsies

Table 1. Characterization of monoclonal antibodies

Designation	Name	Specificity	Implicated in
CD54	84H10	ICAM-1	secondary adhesion
CD62P	0767	P-selectin (PADGEM, GMP 140, CD62)	leucocyte rolling
CD62L	1230	L-selectin (LECAM-1, LAM-1, Mel-14, Leu8)	lymph node homing
CD31	0672	PECAM, 135 kD glycoprotein	constitutional antigen of endothelial cells, haematopoietic cells
CD106	1244	VCAM-1	homing to inflamed tissues
CD62E	4D10	E-selectin (23)	leukocyte rolling
–	HECA 452	CLA (24)	lymphocyte skin homing
CD3	T3		T cells
CD4	T4		T-helper cells, activated macrophages, Langerhans cells
CD45RO	UCHL-1		"memory" -T cells
CD8	T8		T-suppressor cells

(4mm) were taken at 12 h, 24 h, 48 h and 72 h after anthralin had been applied.

6- μ m cryostat sections were stained by the indirect peroxidase and alkaline-phosphatase anti-alkaline-phosphatase-method (APAAP). Monoclonal antibodies were obtained from Dianova (Hamburg, Germany) (Table 1) and Dako Diagnostika (Hamburg, Germany). The 4D10 antibody was a kind gift of S. Goerdts, (Münster, Germany) (23). The HECA 452 antibody was a kind gift of S. T. Pals (University Amsterdam, Netherlands) (24). The anti-mouse immunoglobulin was purchased from Dako Diagnostics (Hamburg, Germany). The anti-rat immunoglobulin labeled with alkaline phosphatase was obtained from Dianova (Hamburg, Germany).

Evaluation of slides

Staining results for adhesion molecules were scored 0 (negative) to 5 (maximum) as described above. Independent microscopical evaluation of all slides by 2 authors resulted in identical classification in most cases. Discrepancies never exceeded 1 grade on the scale.

CD 31:

- 1=staining of all endothelial cells, no other cells positive
- 2=additional staining of a few dermal cells
- 3=additional staining of numerous dermal cells and a few intraepidermal cells
- 4=additional staining of numerous dermal cells and more than 10 intraepidermal cells per section

CD 62:

- 1=endothelial staining in less than 25% of dermal microvessels (as identified by CD31)
- 2=endothelial staining in 25–50% of dermal microvessels
- 3=endothelial staining in 50–75% of dermal microvessels
- 4=staining of all endothelial cells

VCAM-1:

- 1=weak endothelial staining in less than 25% of dermal microvessels
- 2=Weak endothelial staining in 25–50% of dermal microvessels
- 3=moderate endothelial staining in 50–75% of dermal microvessels; occasional perivascular cells positive
- 4=strong endothelial staining in more than 75% of dermal microvessels; clusters of perivascular cells positive; occasional dermal and intraepidermal cells positive

ELAM-1:

- 1=weak endothelial staining in less than 10% of dermal microvessels
- 2=moderate endothelial staining in 10–25% of dermal microvessels
- 3=strong endothelial staining in 25–50% of dermal microvessels
- 4=strong endothelial staining in 50–75% of dermal microvessels
- 5=strong endothelial staining in 100% of dermal microvessels

ICAM-1:

- 1=a few endothelial cells and a few perivascular cells positive
- 2=homogeneous endothelial staining; clusters of 5–10 perivascular cells positive; single keratinocytes staining positive
- 3=homogeneous endothelial staining; moderate cuff-like perivascular cells positive; staining of groups of basal epidermal cells
- 4=strong homogeneous endothelial staining; dense perivascular cuffs of positive cells; clusters of positive basal and suprabasal keratinocytes
- 5=strong homogeneous endothelial staining; dermal infiltrate intensely positive; whole epidermis staining positively

The cellular infiltrate was scored as following:

- 0=a few perivascular cells
- 1=10–50 perivascular cells
- 2=50–100 perivascular cells
- 3="lichenoid" dermal infiltrate
- 4="lichenoid" dermal infiltrate with epidermotropism

The epidermis was evaluated for CD3, CD4, CD8, HECA 452 and LECAM-1-positive cells per 2 successive 3 mm sections. In the dermis we evaluated the percentage of positive cells per 100 mononuclear cells.

Results

Mean scores for the expression of CD31 (Fig. 1), CD62 (Fig. 2), ELAM-1 (Fig. 3, 4), VCAM-1 (Fig. 5) and ICAM-1 (Fig. 6) in the different test groups were calculated. Independently of the eliciting substance, all lesions showed a marked upregulation of all adhesion molecules tested, with the exception of CD62. CD62 was found to be only slightly elevated above the baseline level over the whole observation period in patch tests provoked by nickel sulfate and D.pt., and was found to be unchanged in the anthralin reaction.

No difference in the pattern of adhesion molecules was observed in atopic skin between the

CD31 expression during patch test reactions

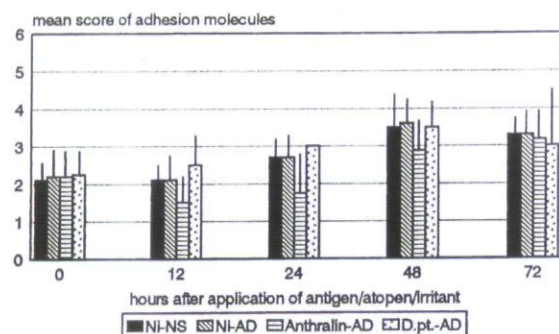


Fig. 1. Time course of CD31 expression in various patch test reactions: Nickel sulfate was applied to healthy normal skin of volunteers (Ni-NS) ($n=2$) and to patients with AD (Ni-AD) ($n=4$). Anthralin (Anthralin-AD) ($n=6$) and D.pt. were applied to patients with AD (D.pt.-AD) ($n=5$). Results are presented as mean values of scores.

CD62 expression during patch test reactions

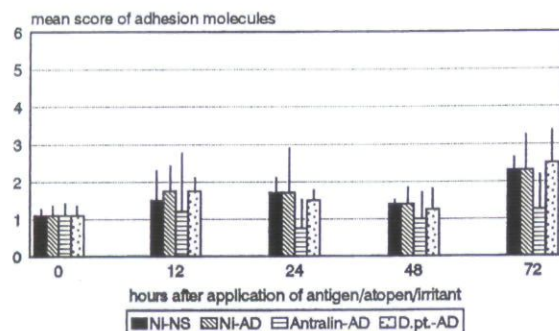


Fig. 2. Time course of P-selectin (CD62) expression in various patch test reactions: Nickel sulfate was applied to healthy normal skin of volunteers (Ni-NS) ($n=2$) and to patients with AD (Ni-AD) ($n=4$). Anthralin (Anthralin-AD) ($n=6$) and D.pt. were applied to patients with AD (D.pt.-AD) ($n=5$). Results are presented as mean values of scores.

ELAM-1 expression during patch test reactions

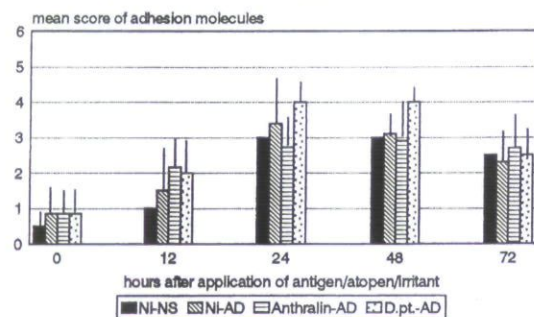
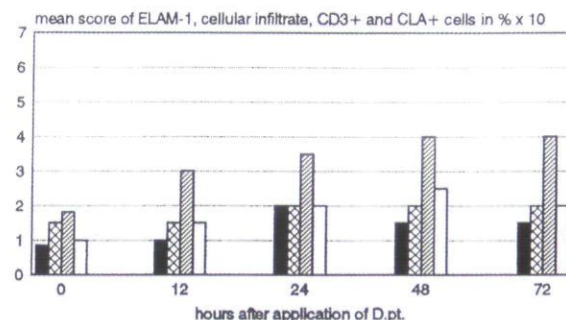


Fig. 3. Time course of ELAM-1 expression in various patch test reactions: Nickel sulfate was applied to healthy normal skin of volunteers (Ni-NS) ($n=2$) and to patients with AD (Ni-AD) ($n=4$). Anthralin (Anthralin-AD) ($n=6$) and D.pt. were applied to patients with AD (D.pt.-AD) ($n=5$). Results are presented as mean values of scores.

Negative D.pt.patch tests ($n=2$)



Positive D.pt.patch tests ($n=3$)

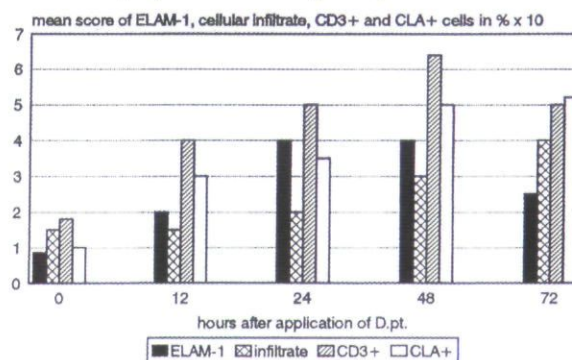


Fig. 4. Expression of ELAM-1 and characterization of the cellular infiltrate during negative and positive D.pt. patch tests in patients with AD: Results are presented as mean values of ELAM-1 scores, infiltration scores and the numbers of CD3 and CLA positive cells per 100 mononuclear cells evaluated in the dermis.

VCAM-1 expression during patch test reactions

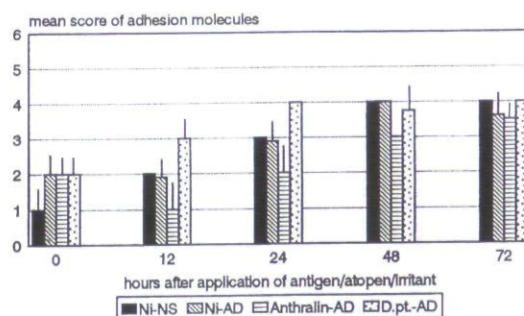


Fig. 5. Time course of VCAM-1 expression in various patch test reactions: Nickel sulfate was applied to healthy normal skin of volunteers (Ni-NS) ($n=2$), nickel sulfate to patients with AD (Ni-AD) ($n=4$), anthralin to patients with AD (Anthralin-AD) ($n=6$) and D.pt. to patients with AD (D.pt.-AD) ($n=5$). Results are presented as mean values of scores.

D.pt. and Ni patch tests. Already 24 h after application of D.pt. and Ni to atopic skin, staining for VCAM-1 and ICAM-1 had increased and constantly continued to increase thereafter. At 48 h, besides positive endothelial cells and dermal leuko-

ICAM-1 expression in patch test reactions

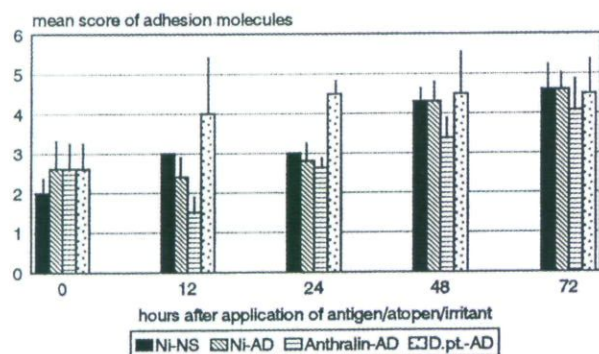


Fig. 6. Time course of ICAM-1 expression in various patch test reactions: Nickel sulfate was applied to healthy normal skin of volunteers (Ni-NS) ($n=2$), nickel sulfate to patients with AD (Ni-AD) ($n=4$), anthralin to patients with AD (Anthralin-AD) ($n=6$) and D.pt. to patients with AD (D.pt.-AD) ($n=5$). Results are presented as mean values of scores.

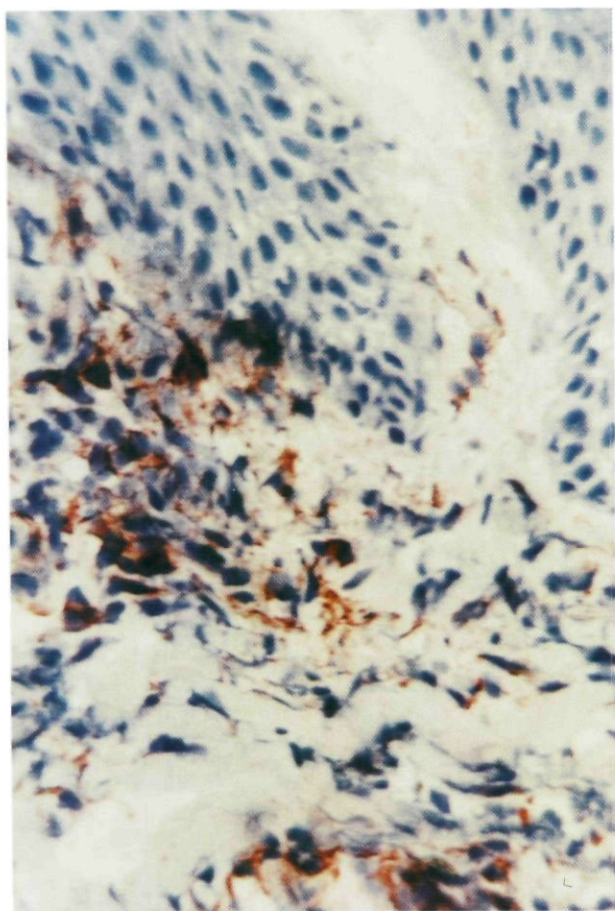


Fig. 7. Expression of VCAM-1 after D.pt. application in atopic skin: The skin biopsy was taken 48 h after epicutaneous application of D.pt. to healthy appearing skin of the back of a patient with AD (RAST-class 4 for D.pt.). 6 µm cryostat section was stained with the monoclonal antibody 1244 directed against VCAM-1 (magnification, $\times 100$).

cytes, single mononuclear intraepidermal cells also strongly expressed the VCAM-1 molecule (Fig. 7). 24 to 48 h after application of both antigens, a maximum of ICAM-1 and VCAM-1 expression with respect to the number of positive cells as well as staining intensity was reached in the skin. Moreover, at 12 h ICAM-1 started to appear on keratinocytes and was strongly expressed in areas of spongiosis after 48 h (Fig. 8).

Among all adhesion molecules tested, ELAM-1 showed the strongest (>3 -fold) increase above the baseline level. ELAM-1 staining (Fig. 3) on endothelial cells increased after 12 h and reached a maximum after 24 h in D.pt. as well as Ni patch tests in atopic skin, decreasing thereafter. ELAM-1 staining was stronger in biopsies which were de-

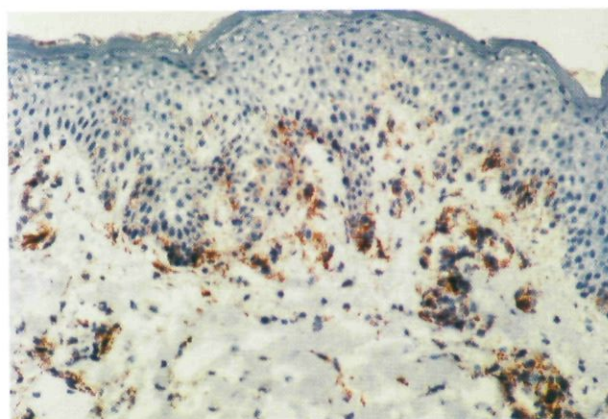


Fig. 8. Expression of ICAM-1 after D.pt. application in atopic skin: The skin biopsy was taken 72 h after epicutaneous application of D.pt. to healthy appearing skin of the back of a patient with AD (RAST-class 4 for D.pt.). 6 µm cryostat section was stained with the monoclonal antibody 84H10 directed to ICAM-1 (magnification, $\times 50$).

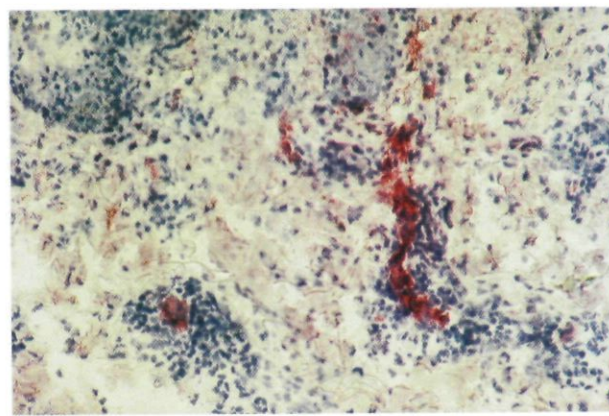


Fig. 9. Expression of ELAM-1 after anthralin application in atopic skin: The skin biopsy was taken 72 h after epicutaneous application of D.pt. to healthy appearing skin of the back of a patient with AD. 6 µm cryostat section was stained with the monoclonal antibody 4D10 directed to ELAM-1 (magnification, $\times 50$).

rived from positive D.pt. patch test sites (Fig. 4, 9).

In general, the staining pattern provoked by anthralin in atopic skin showed a similar amount of upregulation of adhesion molecules as was observed with allergens. However, the increase of VCAM-1 and ICAM-1 seems to be delayed in irritant reactions to anthralin compared to patch test reactions provoked by D.pt. and Ni (Figs. 5, 6). This corresponded to the rather late and variable appearance of the anthralin reaction.

In order to exclude an endogenous difference responsible for a different regulation of adhesion molecules of atopic and non-atopic skin, Ni patch tests were compared in both groups (Figs. 1–3, 5–6). Interestingly, Ni patch tests showed a similar expression pattern of adhesion molecules in patients with AD as in non-atopic individuals.

The enhanced expression of adhesion molecules during the course of patch test reactions correlated positively with the immigration of CLA+ UCHL-1+ "memory" T cells into the dermis and epidermis (Figs. 4, 10). This occurred independently of the eliciting substance. Most of these T cells stained positive for CD4 and L-selectin. With in-

creasing numbers of CD3+ cells in the epidermis and dermis, CLA+ cells also increased proportionally. Until 48 h after antigen/irritant application, the number of CLA+ cells in the epidermis and dermis never exceeded 50% of CD3+ cells. In the dermis about 80–90% of CD3+ cells were CLA+ at 72 h (Fig. 10). Staining intensity of CLA at a single cell level was strongest in the epidermis, while UCHL-1 staining of single intraepidermal T cells was very weak when compared to the dermis. In the epidermis the number of CLA+ cells did not exceed 60% of CD3+ cells.

As early as 12 h after application of D.pt., a small number of L-selectin+ cells had invaded the upper dermis. The number of L-selectin+ cells (and their staining intensity) increased until 72 h. L-selectin+ cells were observed in close contact with the basal layer of the epidermis and within the epidermis at 24 h after antigen challenge. Interestingly, patch test reactions that were negative to D.pt. also showed a similar pattern of migration of CLA+ and L-selectin+ cells into the skin (Fig. 10). No principle differences in CLA and L-selectin expression could be established between

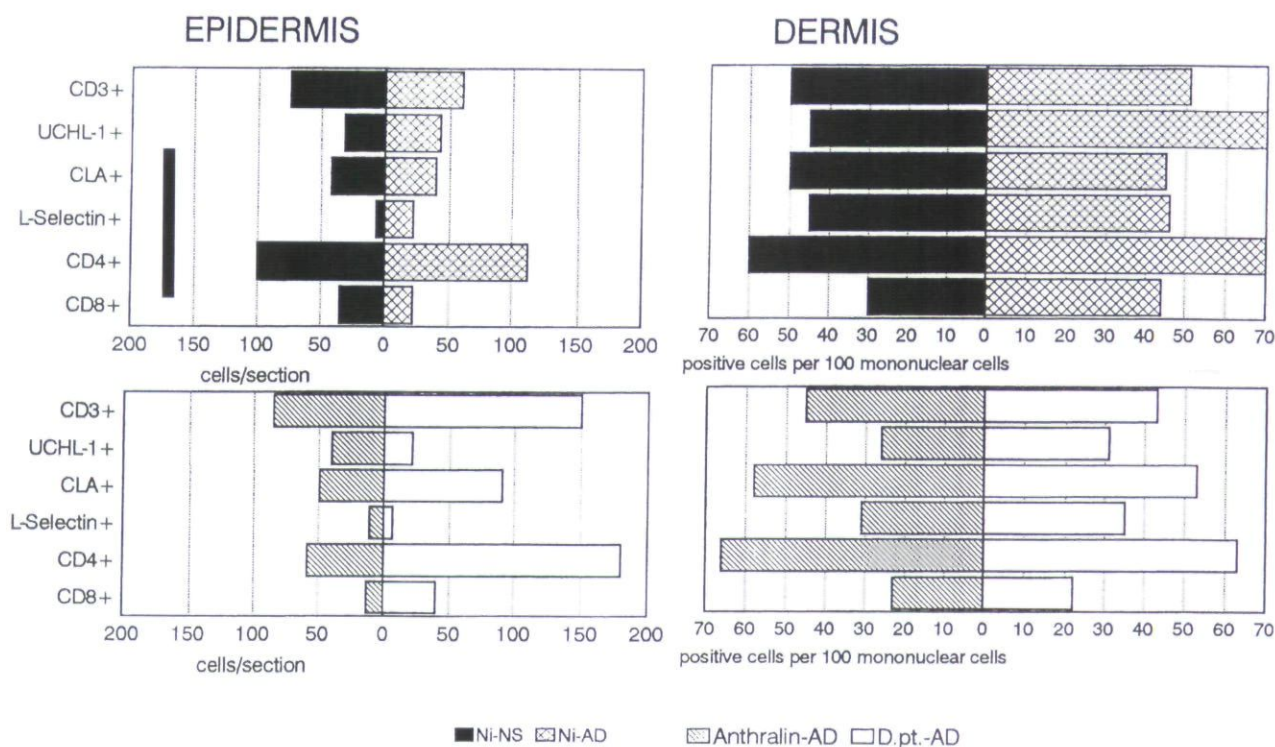


Fig. 10. Characterization of the mononuclear infiltrate in the epidermis and dermis 72 h after skin challenge: Numbers of positive cells were determined per section in the epidermis and per 100 mononuclear cells in the dermis. Mean values of the obtained results in different groups are presented:

Ni-NS=nickel sulfate patch tests in healthy individuals ($n=2$)

Ni-AD=nickel sulfate patch tests in patients with AD ($n=4$)

Anthralin-AD=anthralin patch tests in non-lesional skin in patients with AD ($n=6$)

D.pt.-AD=Dermatophagoides pteronyssinus patch tests in patients with AD ($n=5$)

patch tests to D.pt., Ni and anthralin in atopic patients and to Ni in normal individuals.

Discussion

Skin lesions of allergic contact dermatitis from nickel sulfate and certain chronic inflammatory diseases such as psoriasis vulgaris and lichen ruber planus seem to be dominated by an infiltration with Th1-cells that produce large amounts of IFN γ but no IL-4 (25). Patients with AD, however, harbour Th2-cells that are characterized by the prevalence of IL-4 in their skin lesions. They may also show a delayed-type hypersensitivity skin reaction initiated by the Th2-cells that are specific to inhalant allergens (4–6, 8, 11). The mechanisms that lead to the accumulation of different T-helper subsets in diseased skin are still unknown. Changes between atopics and non-atopics in T-cell subpopulations in the skin may be influenced by any circulating T cells. To our knowledge there is presently no unequivocal evidence that Th1- and Th2-cells show differential expression of homing receptors. It therefore might be speculated that inherent differences may exist in atopic and non-atopic skin in the capacity to express adhesion molecules that direct the homing of T-cell subsets.

Consequently, we studied the expression of a series of receptor-ligand pairs in sequentially biopsied patch test reactions to D.pt., nickel sulfate and the irritant anthralin. Interestingly, during the eliciting phase the expression of ELAM-1, ICAM-1, VCAM-1, CD31, CLA and L-selectin increased in all these types of skin reactions. In general, there were no significant qualitative or quantitative differences between the reaction patterns caused by the various substances. Minor differences in the time course of VCAM-1 and ICAM-1 as observed for D.pt. (early expression), and anthralin (late expression) may correlate with the onset of clinically perceptible erythema. The reaction to anthralin showed great variations, reaching its maximum at 3–7 days. Other authors comparing allergic patch test reactions and irritant reactions in non-atopic individuals for ELAM-1 expression have found no quantitative differences, but described kinetic differences of ELAM-1 expression in irritant and allergic skin reactions (26).

The pattern of adhesion molecules that we observed in the elicitation phase in the skin of patients with AD does not seem to differ from the pattern others have described in allergic contact dermatitis of non-atopic individuals (21, 27, 28). Also, a similar pattern of VCAM-1, ICAM-1 and ELAM-1 expression was previously found in delayed-type skin reactions to tuberculin (29). As we recently demonstrated that healthy appearing skin of atop-

ic individuals shows higher baseline expression of VCAM-1 and ICAM-1 than the skin of controls (30), quantitative differences might also have been expected after application of eliciting substances. However, investigating sequential biopsies from Ni patch tests of patients with AD and 2 non-atopic individuals, we have obtained identical results. Differences between atopic and non-atopic skin seem rather to imply prestimulation (as evidenced by an elevated baseline expression of certain adhesion molecules) than a general abnormality in the capacity to upregulate these molecules. In summary, our results provide evidence for a stereotypic pathway, upregulating adhesion molecules on endothelial and epithelial cells of atopic and normal skin after the application of inhalant allergens, metal haptens and the irritant anthralin as well. However, it should be mentioned that the number of patients in the study is rather small, and that the results therefore have to be interpreted with some caution. Differential action of chemokines on T-cell subsets rather than differential expression of adhesion molecules could be responsible for a preferential homing of Th2-cells to atopic skin (31). A similar upregulation of adhesion molecules was also observed by others in negative patch tests to antigens (28). The present work seems to confirm these findings in an albeit small number of negative patch tests to D.pt. (Fig. 4).

Cytokines released by keratinocytes may provide the first signals for induction of adhesion molecules in patch test reactions. Keratinocytes damaged by irritants, protein denaturing and other substances may elaborate cytokines such as IL-1 α (32–34) and TNF α (35, 36). It is conceivable that protease activity in the house dust mite extract may induce cytokine release in keratinocytes (37). D.pt. has no ability to directly upregulate endothelial adhesion molecules, as we saw in HUVEC (data not shown). On the other hand, as shown for nickel, allergens are able to release IL-1 from keratinocytes without demonstrable cytotoxicity (38). Clearly, an impaired epidermal barrier occurs in D.pt. patch test reactions, as evidenced by trans-epidermal water loss (39). On the other hand, Willis et al. (40) have shown that different irritants or sensitizing agents are able to cause different patterns of keratinocyte damage, which might be followed by distinct patterns of cytokine release from keratinocytes (41). Such mechanisms may be responsible for the observed kinetic difference in VCAM-1 and ICAM-1 upregulation in the anthralin type of skin reaction.

Concomitantly with the increase in ELAM-1 expression on dermal endothelial cells, an influx of mononuclear, mainly CD3+, UCHL-1+, CD4+ and CLA+ cells was observed. At day 3

after allergen challenge or application of anthralin, about 80%-90% dermal T cells stained strongly positive for CLA. ELAM-1 is considered to be a ligand for the CLA-molecule recognized by the monoclonal antibody HECA 452 (42). The sugar moieties sialyl Le (a) and sialyl Le (x) of the CLA are the possible epitopes recognized by HECA 452 (43). It is well-known that in normal human skin about 40% of CD3+ T cells express CLA (44) while 80-90% are positive in chronic inflammatory diseases of the skin (45) and in cutaneous T cell lymphoma (46). These findings and the fact that only 10-25% of circulating CD3+ peripheral blood lymphocytes are CLA+ emphasize the importance of this molecule for various types of cutaneous inflammation.

Also, using flow cytometry, others have shown before that skin-derived T cells, cloned T cells derived from D.pt. patch tests (47) and T cells from skin blister fluids overlaying delayed-type hypersensitivity reactions to mumps and candida antigens (48, 49) are strongly and homogeneously CLA+. In this context, a recent observation made on the correlation of allergen specificity and CLA expression is of great interest. In peripheral blood lymphocytes the specificity to Ni in individuals with contact sensitivity to Ni (and to D.pt. in sensitized patients with AD) was found to be largely confined to the CLA+ cell subset (50). This explains why these cells, upon allergen application to the skin, preferentially migrate into the skin. Interestingly, we found that epidermal T cells were less often CLA+ than dermal T cells (about 50%), compatible with the assumption that only the smaller part of T cells that invade the epidermis after allergen challenge are specific. Mechanisms other than CLA-expression must be operative in intraepithelial invasion (51), as we constantly found a larger part of CLA negative epidermal T cells in various types of skin inflammation. This does not seem to depend on the inflammatory state of the skin (30).

In peripheral blood, L-selectin is uniformly expressed on phenotypic virgin T cells (CD45RA high/CD45RO low/CD58 low), whereas memory T cells (CD45RA low/CD45RO high/CD58 high) are bimodal (49). It was suggested that predominantly a CLA+/L-selectin+ subset of CD45RO+ memory/effector T cells is recruited from the blood into the inflamed skin (48). However, in the present study, although there was a marked increase in CLA+ T-lymphocytes, only a few L-selectin+ -cells were found in the epidermis after antigen challenge and after the application of anthralin (Fig. 9). This dissociation of CLA and L-selectin on T cells was not anticipated, since in vitro-systems CLA induction (on "naive"

and "memory/effector" T cells) and the upregulation of L-selectin have been shown to be mediated by the same cytokines (TGF- β 1, IL-6) (48, 49). Sialyl Lewis x on keratinocytes (52) and Langerhans cells (53) may be possible ligands for L-selectin+ cells in the epidermis. Taken together, these findings suggest that in both irritant and allergic patch test reactions T cell subpopulations invading the epidermis and the dermis are different. This may be related to differences in the microenvironment provided by epidermal cells such as keratinocytes, dendritic cells and melanocytes on the one hand and stromal cells on the other, which certainly may provide different cytokines, chemokines and membrane molecules for interaction with invading lymphocytes (31, 54).

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