

Adhesion molecules in atopic dermatitis: VCAM-1 and ICAM-1 expression is increased in healthy-appearing skin

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In the skin of normal and atopic individuals, the expression of E-selectin (ELAM-1), L-selectin (LECAM-1), P-selectin (CD62), CD31 (PECAM), vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and cutaneous lymphocyte antigen (CLA) were compared by immunostaining of skin biopsies which were taken from normal individuals ($n = 17$), the healthy-appearing skin of patients with atopic dermatitis ($n = 10$), and their acute ($n = 5$) and chronic ($n = 6$) skin lesions. In contrast to ELAM-1, the expression of VCAM-1 and ICAM-1 was found to be significantly increased in nonlesional atopic skin in comparison to the skin of normal individuals. Moreover, in contrast to normal skin of healthy individuals, nonlesional atopic skin showed a further increase of VCAM-1, ICAM-1, and ELAM-1 when cultured with medium alone. This suggests that certain adhesion molecules are constitutively upregulated in healthy-appearing skin of patients with atopic dermatitis. In addition, atopic skin appears to respond to nonspecific stimuli (such as culture with medium alone) with upregulation of VCAM-1, ICAM-1, and ELAM-1. It is suggested that the observed upregulation of adhesion molecules is mediated by the release of cytokines such as interleukin-4 from cells which reside in atopic skin. The question of whether the inherent upregulation of adhesion molecules in atopic skin contributes to the development of Th2 cells, which have been found to predominate in atopic inflammation, has to be further investigated.

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The immune system is involved in the pathogenesis of atopic dermatitis (AD) through lymphocyte-mediated inflammation of the skin, and increased incidence of type I allergies induced by environmental allergens (1). In recent years, it has become clear that there is an imbalance in the generation of Th1 and Th2 cytokines in atopic disease which seems to be causally related to disease symptoms (2, 3). Moreover, as shown by cloning lymphocytes, allergen-specific T cells in the skin of AD patients often display a Th2-like cytokine pattern (4–7). This cytokine pattern has been confirmed in early lesions of AD by the polymerase chain reaction technique on skin biopsies (8) and has not been found in normal skin, psoriasis, or lichen planus (9).

The mechanism which leads to the appearance of Th2-predominant, allergen-specific T cells in atopic skin is unknown.

We have recently hypothesized that the skin of patients with AD may provide a special micro-environment which either shifts invading T cells toward a Th2-like secretion pattern or leads to preferential homing of Th2-cells into the skin (10). In a previous study, we also have shown a decreased number of UCHL-1-positive “memory” T cells in the peripheral blood of patients with severe AD, suggesting an increased influx of these cells into the skin (11).

The present work investigated whether a specific pattern of adhesion molecules might be causally

related to the described aberrant findings in atopic skin. The expression of several receptor–ligand pairs known to mediate T-cell adhesion to endothelial and epithelial cells was assessed in uninvolved, healthy-appearing skin of patients with AD and in acute and chronically persistent skin lesions. As it is well-known that interleukin (IL)-4 enhances and interferon-gamma (IFN- γ) inhibits the development of Th2-cells, adhesion molecules, such as VCAM-1 and ICAM-1, which are upregulated by these cytokines were of special interest. VCAM-1, which is strongly induced by IL-4 on human umbilical vein cells (HUVEC) (12–14), binds to the VLA-4 antigen on lymphocytes and is considered to be important for their migration into tissues and for initiation of chronic inflammation (15). ICAM-1, which is strongly upregulated by IFN- γ (16), binds to the LFA-1 molecule on lymphocytes which is expressed in high density on activated T cells. Besides studying these adhesion molecules in fresh-frozen skin biopsies, we also investigated their inducibility by the cytokines in question. Using a skin organ culture system with biopsies of normal and healthy-appearing skin of patients with AD, we compared the effects of IL-4 and IFN- γ on the expression of adhesion molecules. Tumor necrosis factor-alpha (TNF- α) served as a positive control.

Material and methods

Biopsies

Biopsies for cryostat sections were taken from the uninvolved skin of 10 patients with AD, the acute lesions of five patients, and the chronic lesions of six patients with AD. Normal skin was obtained from 17 patients undergoing surgery for primary malignant melanoma. All AD patients had

increased serum-IgE levels and allergic rhinitis, asthma, or food allergy.

Human skin organ cultures

Normal skin was obtained from 10 normal individuals undergoing plastic surgery and nonlesional atopic skin from four patients with mild AD, allergic rhinitis, and elevated serum IgE. Both normal skin from healthy controls and biopsies from the uninvolved skin of patients with AD were taken from the back of these individuals. Specimens were immediately cut into 4-mm pieces and placed in 16-mm wells of microtiter plates, epidermal surface up, as described by other authors (17). Sufficient RPMI medium (approximately 200 μ l) covering 80% of the explant (epidermis uncovered) was added, and the explants were incubated at 37°C with 5% CO₂-humidified air for 6 h. The following cytokines were added at the concentrations indicated: 2000 U/ml recombinant human TNF- α expressed in *Pichia pastoris* (Pharma Biotechnology, Hannover, Germany), 1 ng/ml recombinant human IL-4 (Pharma Biotechnology, Hannover, Germany), 100 ng/ml purified recombinant human IFN- γ (Polyferon[®], Rentschler, Germany), and combinations of IL-4 and TNF- α , IFN- γ and TNF- α (concentrations as indicated above). LPS (100 μ g/ml) (*E. coli*-serotype 055:B5, Sigma, Germany) was used as positive control. Working concentrations of cytokines had been previously found to yield optimal results. Biopsies incubated with medium served only as negative controls.

Immunolabeling

Cryostat sections of 6 μ m prepared from biopsies and skin organ culture explants were stained by the indirect peroxidase and alkaline-phosphatase anti-alkaline-phosphatase method (APAAP). Monoclonal antibodies were obtained from Dianova (Table 1) and Dako Diagnostika (both Hamburg, Germany). The 4D10 antibody was a kind gift from S. Goerdts (18), and HECA 452, for detection of the CLA antigen, was a kind gift from S. T. Pals (19). Antimouse immunoglobulin was purchased from Dako Diagnostika. Antirat immunoglobulin labeled with alkaline phosphatase was obtained from Dianova.

Evaluation of slides

The whole epidermis of two sections of the 4-mm biopsies was evaluated for CD3-, CD4-, CD8-, HECA 452-, and LECAM-1-positive lymphocytes. In the dermis, we determined the number of positive cells per 100 mononuclear cells.

List of abbreviations

- AD – atopic dermatitis
- aAD – acute lesion of atopic dermatitis
- cAD – chronic lesion of atopic dermatitis
- uAD – uninvolved nonlesional skin of patients with atopic dermatitis
- CLA – cutaneous lymphocyte antigen
- ELAM-1 – endothelial leukocyte adhesion molecule-1 (E-selectin)
- HUVEC – human umbilical vein endothelial cells
- ICAM-1 – intercellular adhesion molecule-1
- IL-4 – interleukin-4
- IFN- γ – interferon-gamma
- LECAM-1 – leukocyte endothelial cell adhesion molecule-1 (L-selectin)
- LFA-1 – Leukocyte function associated 1-protein
- nc – normal controls
- PECAM – platelet endothelial cell adhesion molecule
- TNF- α – tumor necrosis factor-alpha
- UCHL-1 – clone-producing antibodies to CD45RO molecule
- VCAM-1 – vascular cell adhesion molecule-1.

Table 1. Characteristics of monoclonal antibodies used

CD designation	Name	Specificity	Implicated in (for review see: Mackay & Imhoff [15])
CD54	84H10	ICAM-1	Secondary adhesion
CD62	0767	P-selectin (PADGEM, GMP 140)	Leukocyte rolling
—	1230	L-selectin (LECAM-1, LAM-1, Mel-14, Leu 8)	Lymph-node homing
CD31	0672	135-kDa glycoprotein PECAM	Constitutional antigen of endothelial cells, hematopoietic cells
—	1244	VCAM-1	Homing to inflamed tissues
—	4D10	E-selectin (18)	Leukocyte rolling
—	HECA 452	CLA (19) (sialyl Lewis ^{x/a})	Lymphocyte skin homing
CD3	T3		T cells
CD4	T4		T-helper cells, activated macrophages, Langerhans' cells
CD8	T8		T-suppressor cells
CD45RO	UCHL-1		"Memory" T cells

Staining results for adhesion molecules were scored 0 (negative) to 5 (maximum) according to Brasch & Sterry (20). This evaluation procedure was modified for VCAM-1 and ICAM-1 and for CD31 and CD62, as described below. For calculation of percentages of positive vessels, anti-CD31 was used as a panendothelial cell marker.

Independent microscopic evaluation of all slides by two authors resulted in identical classification in most cases. Discrepancies never exceeded one grade on the scale.

CD31:

- 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.

CD62:

- 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 keratinocyte 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.

Values obtained were compared by the U-Wilcoxon 2-sample test for $\alpha < 0.05$.

Results

Adhesion molecules in fresh-frozen biopsies of healthy-appearing skin of patients with AD

Although apparently healthy, the nonlesional skin of patients with AD showed significantly more often VCAM-1-positive dermal microvessels than the healthy skin of normal controls (Figs. 1 and 2). There was also increased staining of perivascular cells and basal epidermal cells for ICAM-1 (Fig. 3). There was no increase in either E-selectin or CD62, or CD31, a glycoprotein which is produced by endothelial cells and hematopoietic cells as well (21–24). Surprisingly, enhanced expression of VCAM-1 and ICAM-1 was not accompanied by increased numbers of CD3-positive or UCHL-1 positive T cells. The number of CD3-positive cells in the epidermis of nonlesional atopic skin was 3.9 compared to 2.6 in normal controls, and 18.4 in the

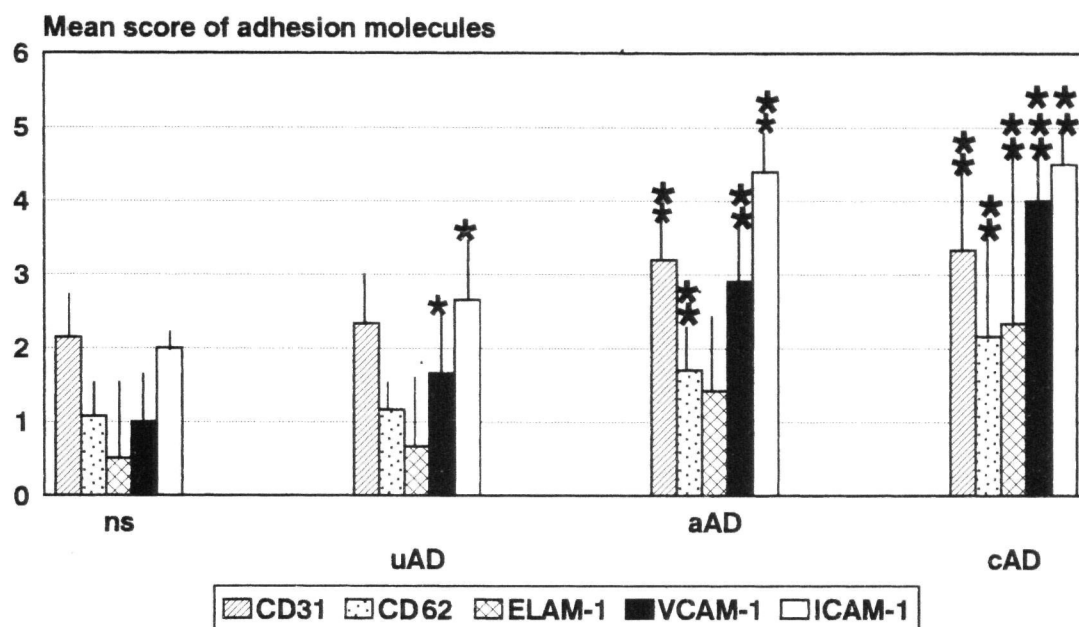


Fig. 1. Expression of CD31, CD62, ELAM-1, VCAM-1, and ICAM-1 in skin of patients with AD. Results are presented as mean values $\pm$ standard deviation calculated from staining scores of different patient groups. *Significant difference in comparison to normal skin; **significant difference in comparison to uninvolved skin of AD; ***significant difference in comparison to acute lesions of AD; ns: normal controls ($n = 17$); uAD: uninvolved skin of individuals with AD ($n = 10$); aAD: acute lesions of AD ($n = 5$); cAD: chronic lesions of AD ($n = 6$).

dermis compared to 22, respectively (Table 2). Moreover, the number of HECA-positive cells did not differ significantly in those groups, indicating that an isolated rise of VCAM-1 and ICAM-1 in the clinically healthy-appearing skin of patients with AD is not sufficient to cause an increase in the number of resident skin T cells.

Adhesion molecules in fresh-frozen biopsies of lesional skin

In acute (aAD) and chronic (cAD) lesions of AD, the expression of all adhesion molecules studied (with the exception of ELAM-1 in aAD) had significantly increased above the level of the healthy-appearing skin of the patients. Moreover, Fig. 1 shows that VCAM-1 expression was significantly higher in cAD than in aAD. Increased expression of these adhesion molecules in lesional atopic skin was paralleled by increased numbers of mononuclear cells which had migrated into the dermis and the epidermis (Table 2). T cells, which are known to bind to ICAM-1 via LFA-1 molecules, had increased twofold in the dermis of aAD and cAD and 7–11-fold in the epidermis. The most prominent rise was seen with UCHL-1-positive, HECA-positive, and LECAM-1-positive cells in the epidermis. Since UCHL-1-positive “memory” T cells exhibit a much higher density of LFA-1 on their membranes than “naive” T cells (25), ICAM-1 expression in basal and suprabasal keratinocytes of AD seems to be responsible for the binding of UCHL-

Table 2. Immunocytochemistry of skin-infiltrating mononuclear cells. Results are presented as mean values $\pm$ standard deviation of positively stained cells in epidermis and dermis

Skin lesions	CD3	UCHL-1	HECA 452	LECAM-1
Epidermis				
ns	2.6 $\pm$ 1.8	2.25 $\pm$ 2	4.4 $\pm$ 3.1	0
uAD	3.87 $\pm$ 7.5	0.6 $\pm$ 1.3	0.4 $\pm$ 0.7	0
aAD	28 $\pm$ 44	7.6 $\pm$ 15	14 $\pm$ 17.6	0.8 $\pm$ 1.8
cAD	45.2 $\pm$ 14	22 $\pm$ 13.8	23 $\pm$ 8.8	1.2 $\pm$ 2.0
Dermis				
ns	22 $\pm$ 3.8	22 $\pm$ 8.3	8.3 $\pm$ 1.5	0.9 $\pm$ 1.3
uAD	18.4 $\pm$ 18	14 $\pm$ 19	7.6 $\pm$ 8.4	2.3 $\pm$ 3.2
aAD	48 $\pm$ 16.7	33 $\pm$ 18.2	21 $\pm$ 8.5	7.9 $\pm$ 3.5
cAD	49 $\pm$ 30.6	56 $\pm$ 11.3	34 $\pm$ 27	10 $\pm$ 13.4

ns: normal skin of nonatopic individuals ($n = 17$).

uAD: uninvolved skin of individuals with AD ($n = 10$).

aAD: acute lesions of patients with AD ($n = 5$).

cAD: chronic lesions of patients with AD ($n = 6$).

1-positive skin T cells. These cells are known to coexpress mostly the CLA-antigen (26). Since ELAM-1 is considered to be the ligand for CLA-positive, skin-seeking lymphocytes (27, 28), it is not surprising that a coincidence of high ELAM-1 expression and HECA-positive cells was observed in the dermis.

Expression of adhesion molecules in skin cultures

In biopsies from the healthy skin of normal individuals which were cultured with the cytokines TNF- α , IL-4, and IFN- γ , expression of VCAM-1

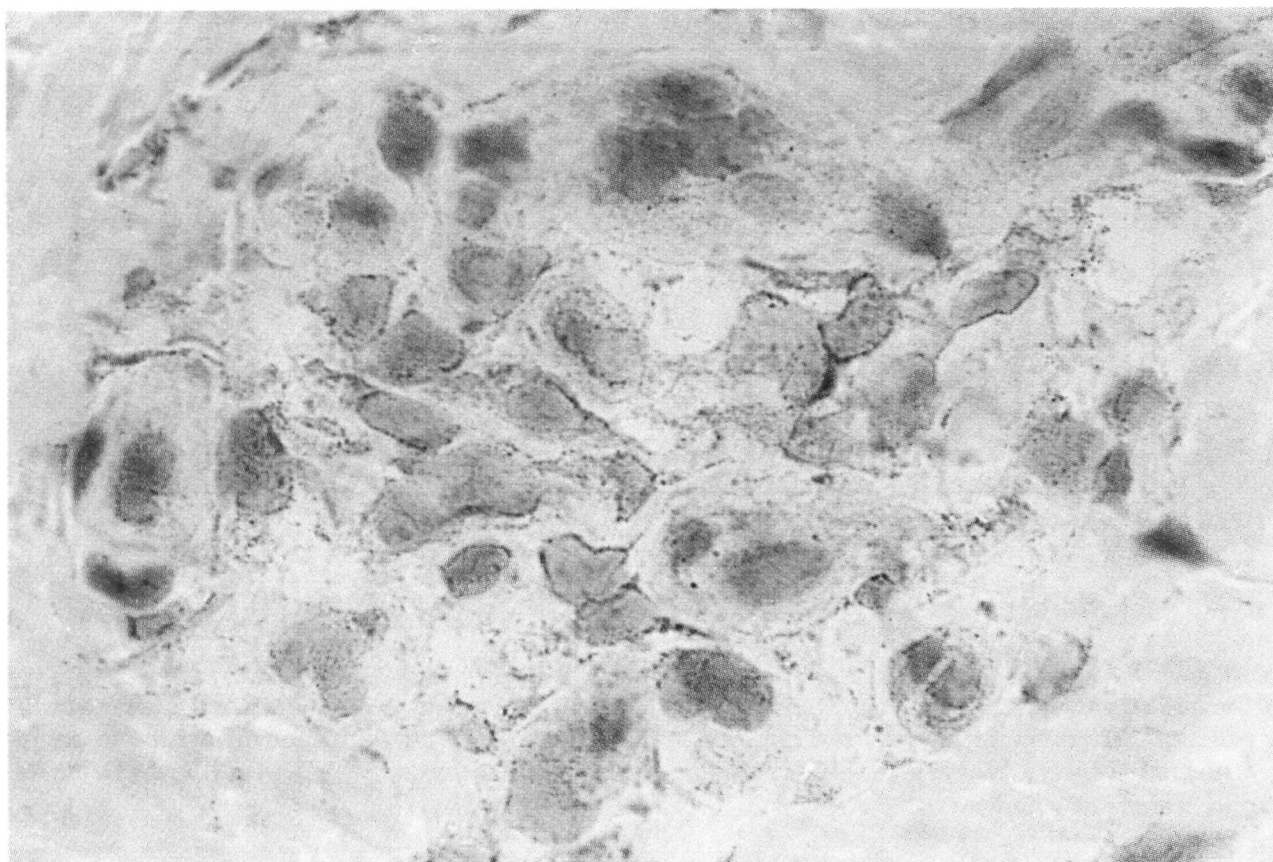


Fig. 2. VCAM-1 expression in nonlesional atopic skin ($\times 250$).

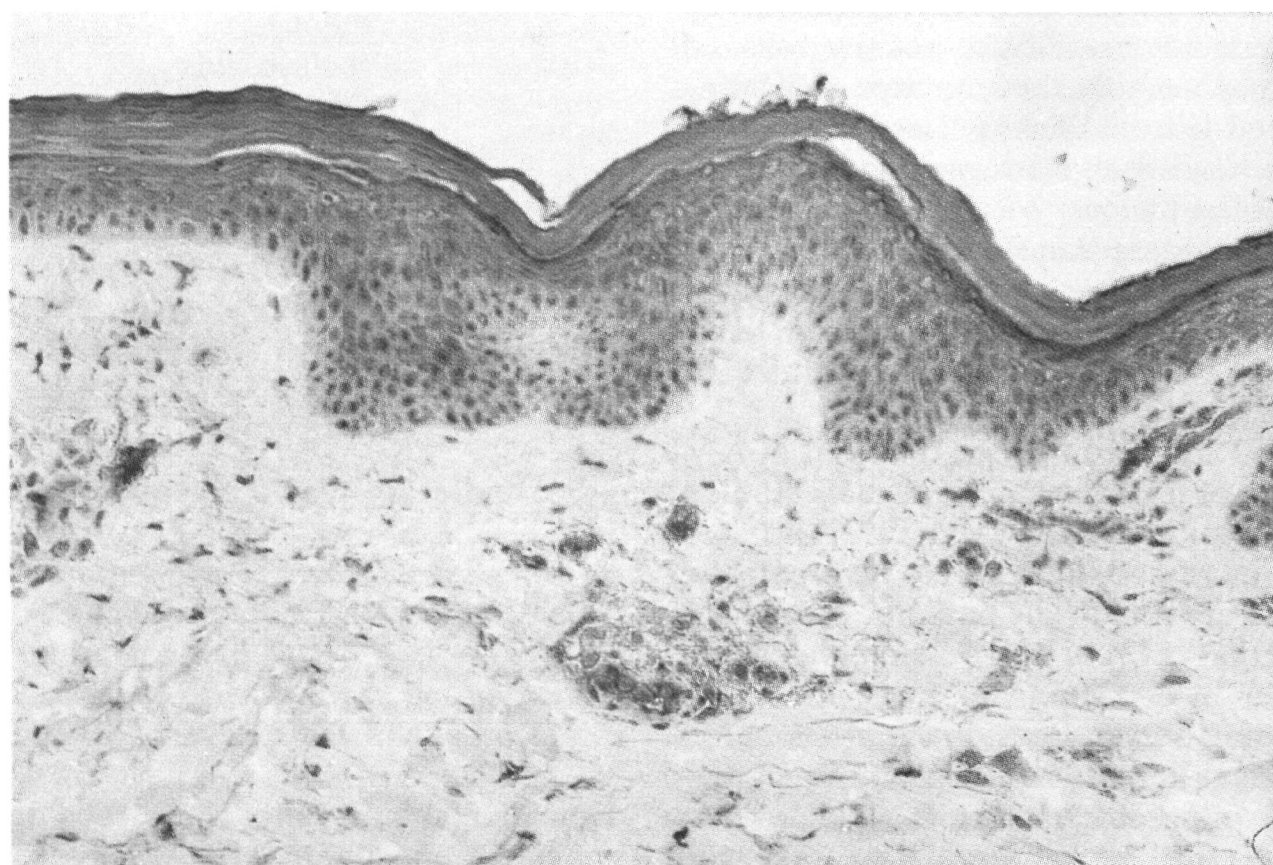


Fig. 3. ICAM-1 expression in nonlesional atopic skin ($\times 50$).

and ICAM-1 was significantly upregulated after 6 h of incubation (Fig. 4A). In the presence of $\text{TNF-}\alpha$ and $\text{IFN-}\gamma$, no significant increase of ELAM-1 was observed, while lipopolysaccharide (used as a control) induced a dramatic increase. In contrast, HUVEC, which we also used as a control, responded vigorously to $\text{TNF-}\alpha$ with increased ELAM-1 expression (results not shown). $\text{IFN-}\gamma$,

which led to a weak but not statistically significant effect, probably acted by releasing other cytokines, as it is known to have no direct effect on the expression of E-selectin in HUVEC (29). IL-4, which has been found to induce ELAM-1 in HUVEC, was also not active on the dermal microvasculature.

Fig. 4B also shows that the healthy-appearing skin of patients with AD, when cultured with

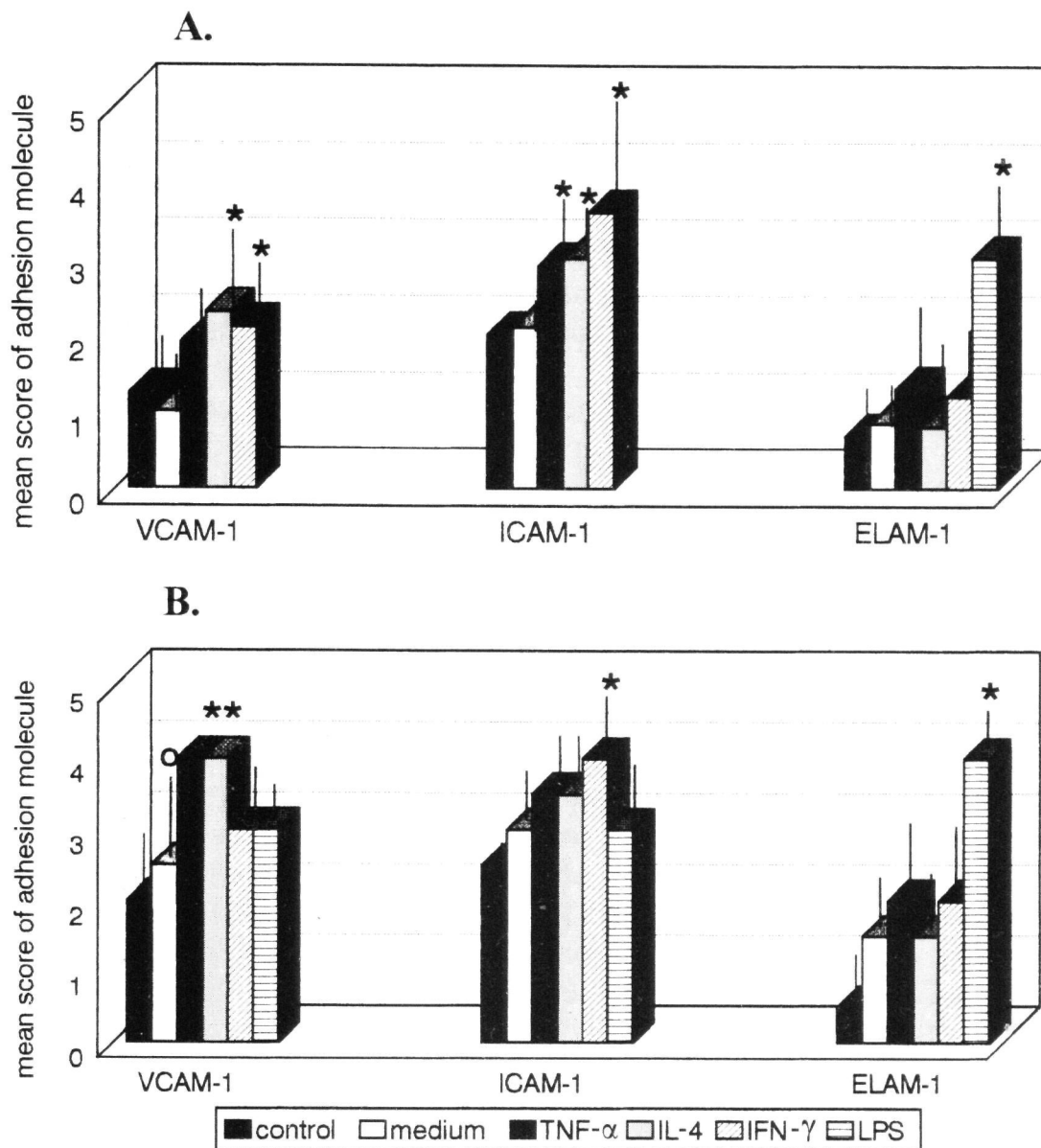


Fig. 4. Short-term skin organ culture with various cytokines: effect on expression of VCAM-1, ICAM-1, and ELAM-1. A) Biopsies from normal skin of nonatopic individuals ($n = 10$); B) biopsies from uninvolved skin of patients with AD ($n = 4$). Results are presented as mean values $\pm$ standard deviation calculated from staining scores. Control: snap-frozen biopsy without culture; medium: culture with medium only; TNF- α : culture with 2000 IU TNF- α /ml; IL-4: culture with 1 ng IL-4/ml; IFN- γ : culture with 100 ng IFN- γ /ml; LPS: culture with 100 μ g LPS/ml (positive control). o – Significant difference of mean value obtained with normal skin in comparison to mean value obtained with uninvolved atopic skin (panel A). *Significant difference of mean value obtained in culture with cytokine in comparison with mean value obtained in culture with medium alone.

medium alone, expressed higher levels of adhesion molecules than the normal skin of healthy individuals. This was more pronounced with VCAM-1 ($|Z| = 0.0146$; significantly) than with ELAM-1 ($|Z| = 0.0978$; NS) and ICAM-1 ($|Z| = 0.1366$; NS) and may have been caused by increased susceptibility to endogenous cytokines or increased release of cytokines from various resident skin cells in the organ culture. A pronounced further increase of VCAM-1 was seen in nonlesional AD skin stimulated with TNF- α ($|Z| = 0.0211$; significantly) or IL-4 ($|Z| = 0.0211$, significantly) and of ICAM-1 with IFN- γ ($|Z| = 0.0491$; significantly). Interestingly, neither in atopic skin nor in normal skin did IL-4 lead to an increase of ELAM-1 expression. This was in contrast to its significant effect on VCAM-1 expression. As a similar pattern of high VCAM-1 and low ELAM-1 expres-

sion was seen in the fresh-frozen biopsies of nonlesional atopic skin, these results may point to a chronic endogenous release of IL-4 and/or other VCAM-1-inducing cytokines in healthy-appearing skin in AD.

Discussion

Comparing the expression of a series of adhesion molecules in snap-frozen normal skin and healthy-appearing skin of patients with AD, we found a significant increase of VCAM-1 and ICAM-1 expression in atopic skin. This may be caused by the release of cytokines from endogenous skin cells. This hypothesis is supported by our finding that skin organ culture without exogenous cytokines upregulates all adhesion molecules in healthy-appearing skin of AD, but not in normal skin

(Fig. 4). When nonlesional skin of patients with AD was cultured with IL-4 or TNF- α , VCAM-1 was strongly upregulated, indicating that these cytokines may be responsible for the *in situ* upregulation of VCAM-1 observed in fresh-frozen lesions. Increased VCAM-1 expression in non-lesional atopic skin and in acute lesions may be responsible for the increased number of eosinophils and basophils binding to dermal endothelial cells via the VLA-4 antigen (30–32).

Recently, mast cells residing in uninvolved atopic skin have been found to contain significant amounts of IL-4 (12). Mast cells may be a cellular source for cytokines which induce the high levels of VCAM-1 and ICAM-1 observed in the skin biopsies taken from patients with AD. It has been shown that the expression of VCAM-1 on HUVEC could be enhanced by IL-4 (12), while IL-4 did not promote ICAM-1 expression on HUVEC (29). Interestingly, it could be shown that IL-4 and IFN- γ regulate ICAM-1 transcription by the same promoter gene (34). While IL-4 and TNF- α seem to play an important role in the upregulation of VCAM-1 in atopic skin, their effect on ICAM-1 and ELAM-1 expression seems to be of minor importance. Endogenous IFN- γ may be responsible for the observed upregulation of ICAM-1, as it led to an increase of this adhesion molecule in skin organ culture.

The observation that nonlesional, fresh-frozen skin of AD shows upregulation at least of VCAM-1/ICAM-1 and that culturing the skin without the addition of further cytokines increases substantially the level of VCAM-1 might explain the high skin irritability observed in these patients. Interestingly, the increase in VCAM-1 and ICAM-1 without an increase in ELAM-1 (as observed in the fresh-frozen biopsies of patients with AD) was not accompanied by an influx of T cells either into the epidermis or into the dermis (Table 2). This agrees with the observation that ELAM-1 is a ligand for the CLA molecule expressed on the membrane of skin-seeking T cells and is essential for binding these T cells to the endothelial wall. Our results show that ELAM-1 increased coincidentally with the number of HECA-positive cells in aAD and cAD. This supports the assumption that, in order to complete extravasation of skin-seeking T cells, not only cytokines which upregulate VCAM-1 and ICAM-1 but also ELAM-1 on endothelial cells have to be active. Such cytokines may be released when atopic skin is exposed to irritants or allergens. Taken together, our results confirm that, besides endogenous upregulation of VCAM-1 and ICAM-1, cytokines which upregulate ELAM-1 appear to be crucial in initiation and possibly perpetuation of inflammatory reaction in atopic skin.

Further work has to clarify whether the enhanced reactivity of the microvasculature in the skin of AD, which we have documented here, may play a role in directing preferential migration of Th2 cells to the skin. Our own preliminary studies with a panel of Th2 and Th1 clones did not detect differences in the adhesiveness of these subtypes to skin sections of AD (results not shown). We therefore conclude that resident cells in non-lesional atopic skin constitutively release cytokines which upregulate certain adhesion molecules, leading to an increased susceptibility to inflammatory reactions, and that this is not dependent on the mechanisms which direct skin-invading CD4+ T cells toward the Th2-differentiation pathway.

In aAD and cAD, besides the increase in ICAM-1, VCAM-1, and ELAM-1, the expression of CD62 and CD31 also significantly increased in comparison to uninvolved atopic skin. This was accompanied by an influx of HECA-positive lymphocytes and LECAM-positive mononuclear cells (Table 2), as well as CD4-positive cells in the epidermis and dermis (not shown). Studies with cultured lymphocytes have shown that CD62 (P-selectin) binds to chronically stimulated, but not to resting, CD4-positive T cells and that CD62 regulates their production of proinflammatory cytokines (35). Thus, P-selectin, besides controlling adhesion and migration of granulocytes (36) may also participate in the process of lymphocyte migration (26) to atopic skin. However, its ligand on lymphocytes is not known at present.

Human CD31 is a recently described glycoprotein which, under certain circumstances, can be produced by endothelial and/or hematopoietic cells, except mature erythrocytes (21–24). In mice, its role in recruitment and transmigration of lymphocytes through the vessel walls has been recently demonstrated (22). Expression of CD31 has recently been proposed to be characteristic of CD4-positive suppressor T cells (24). In lesional atopic skin, CD31 was expressed not only on endothelial cells, but also on a few mononuclear cells in the dermis, and on single keratinocytes. CD31-positive mononuclear cells were often seen in close contact to the basal layer of the epidermis. The presence of CD31 on both mononuclear leukocytes and endothelium suggests that this molecule may interact homotypically. CD31-transfected cells spontaneously aggregate in culture and localize CD31 to sites of contact with other CD31-expressing cells (21). To our knowledge, CD31 has not been described on keratinocytes before. There is evidence that besides the ICAM-1/LFA-1 pathway other mechanisms of keratinocyte/lymphocyte adhesion may exist (37–39). It may be speculated that CD31 acts as a receptor for T cells on keratinocytes of atopic skin

and amplifies the β 1-integrin-mediated adhesion to keratinocytes (40).

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