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Expression of E-Selectin in the Skin of Patients with Atopic Dermatitis: Morphometric Study

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SUMMARY Adhesion molecules may play an important role in the homing of T-cell subsets into allergen-exposed skin of atopic individuals. The aim of this study was to examine the expression of adhesion molecules in atopic dermatitis skin lesions. Biopsies were obtained from lesions in 30 adult patients with atopic dermatitis and 10 healthy adults as controls. Biopsy specimens were studied by immunohistochemistry for the expression of E-selectin in epidermis and dermis cells. Results showed significant changes in the epithelial cell expression of E-selectin, which were especially pronounced in vascular endothelium of the dermis of atopic dermatitis patients.

KEY WORDS: atopic dermatitis, skin, adhesion molecules, E-selectin

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INTRODUCTION

Atopic dermatitis (AD) is a chronic inflammatory skin disease that is characterized by pruritic eczematous lesions and is associated with elevated serum IgE levels, specific IgE environmental allergens such as house dust mites, and tissue and peripheral blood eosinophilia (1-7). AD is characterized by epidermal cells such as keratinocytes, dendritic cells and melanocyte and stromal cells, with predominant infiltration of T cells, eosinophils, macrophages and membrane molecules in the lesional skin. Since immune dysregulation type I and IV is a possible key defect in AD, the expression of different immunologic parameters and co-stimulatory molecules such as adhesion molecules, precisely intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and E-selectin, has been studied in AD patients (8-10). E-selectin is expressed on the surface of activated endothelial cell upon stimulation by inflammatory cytokines. E-selectin is a marker of severity and inflammation in AD and is found to be important in the pathogenesis of AD (10). E-selectin enables adhesion of neutrophil leukocytes and monocytes on activated endothelium of the skin in association with ICAM-1 and keratinocytes (4,7-9).

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PATIENTS AND METHODS

Only patients with clear inflammatory lesions of AD were included in the study. In 30 AD patients, the diagnosis of AD was made according to the criteria of Hanifin and Rajka (6). The patients received no immunosuppressant treatment for at least two weeks before the study. The mean age of study patients was 32 (range, 28-45) years. Skin specimens from ten healthy age-matched donors, stained with the same antibodies were used as control samples.

Skin biopsies were immunohistochemically stained with E-selectin antibody (Dako, Glostrup, Denmark). Slides were stained in an automated immunostainer (TechMate, Dako) using standard avidin-biotin immunoperoxidase staining method. The immunohistochemically stained slides were analyzed by using light microscope. In the skin lesions of AD patients, the expression of adhesion molecule (E-selectin) in the dermis and epidermis was analyzed semiquantitatively.

Morphometric measurements were performed on digital images (Nikon Eclipse E600 light microscope and DXM 1200 digital camera; Nikon, Champigny sur Marne, France) using the Imaging Software Lucia G 4.80 (Laboratory Imaging Ltd., Prague, Czech Republic). The number of epidermal E-selectin positive cells and E-selectin positive epidermal and endothelial areas were measured to quantify changes in the expression of E-selectin in the epidermis and in the vessel wall of dermis in the control group and AD patients. All evaluations (measurements) were made on 6 fields *per* section at a X100 magnification.

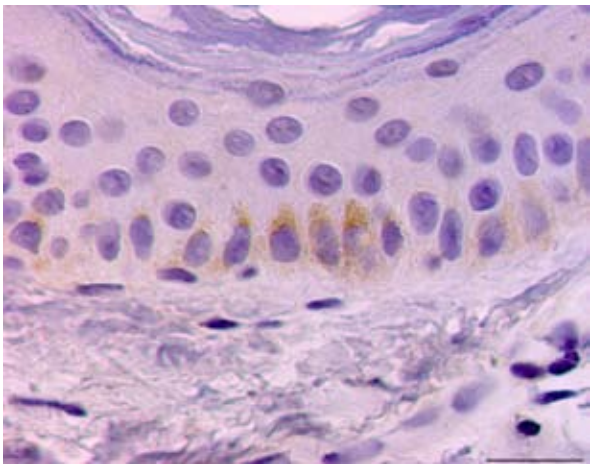

Figure 1. Immunohistochemical detection of E-selectin in the epidermis of healthy skin (control group). Bar = 20 μm.

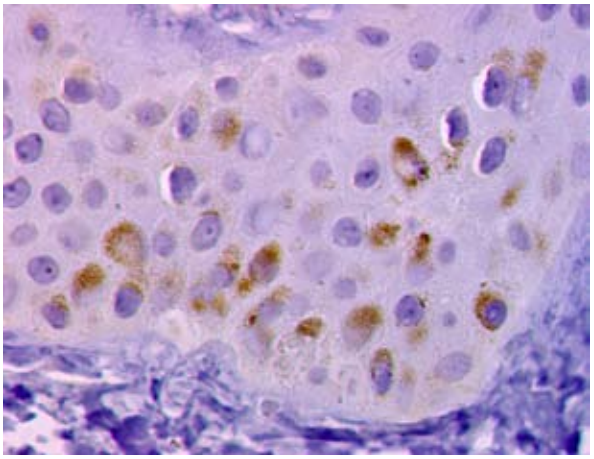

Figure 2. Increased expression of E-selectin in the epidermis of AD patients. Bar = 20 μm.

All values in the graphs are expressed as mean±SEM. The results on study parameters obtained in both AD and control groups showed normal distribution. Statistical significance of differences between the groups was assessed by t-test.

RESULTS

Research of adhesion molecules expression in bioptic samples of healthy and inflammatory changed skin was performed by immunohistochemical analysis. Immunohistochemical analysis of skin tissue obtained from control group and AD patients showed E-selectin to be expressed in epidermal cells (Figs. 1 and 2) and endothelial cells of dermal vessels (Figs. 3 and 4). Morphometric measurements revealed the number of E-selectin positive epidermal cells to be slightly lower in control group than in AD patients (Fig. 5), where-

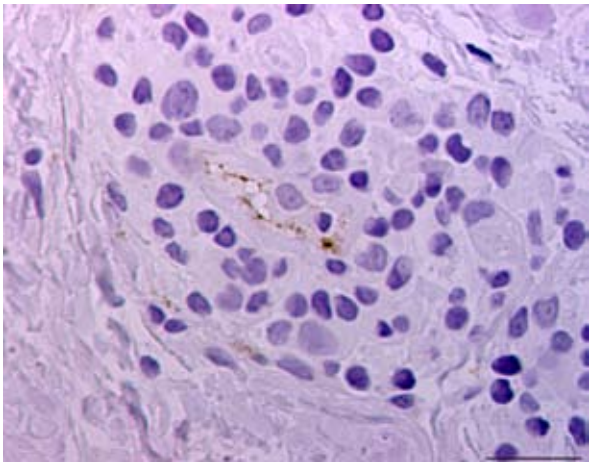

Figure 3. Weak expression of E-selectin in endothelial cells of dermal vessel in healthy skin (control group). Bar = 20 μm.

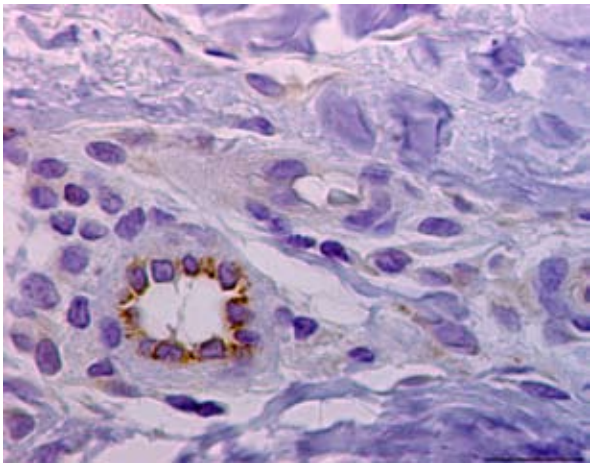

Figure 4. Expression of E-selectin in endothelial cells is significantly enhanced in AD patients. Bar = 20 μm.

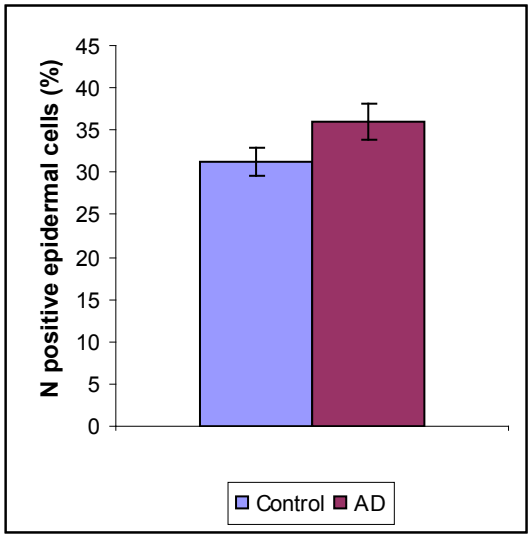

Figure 5. E-selectin positive epidermal cells in control group and AD patients. Data are percentage of positive epidermal cells in total epidermal cells and expressed as mean±SEM.

as positive immunoreaction in the epidermal area was higher in AD patients (Figs. 2 and 6). Morphometric measurements also revealed significant changes in endothelial expression of E-selectin in the vessel wall, in the form of an enlarged positive endothelial area (Figs. 4 and 7); in AD group, the mean value was 3.5-fold higher that in control group. According to t-test results, this difference was statistically significant at $P<0.05$ (Table 1). A statistically significant difference between the control and AD groups was also found for E-selectin expression in the epidermis and dermis (Table 2).

DISCUSSION

The results of the present study indicated E-selectin to be a marker of AD severity. Similar results with soluble E-selectin and ICAM-1 have been reported by Wolkerstorfer *et al*. (10). It has been suggested that soluble adhesion molecules may have physiological implications, such as inhibition of adhesion by competitive binding. It has also been suggested that these molecules may act in a proinflammatory manner by chemoattraction and by activating leukocyte and E-selectin may be a more stable underlying systemic representation of AD (10).

Many authors studied the expression of adhesion molecules in skin biopsies from patients with atopic dermatitis (4,7-9). Inhibition of adhesion molecule expression may represent another selective mechanism of action of immunosuppressive treatment in AD, e.g., tacrolimus (8).

The expression of adhesion molecules is up-regulated in the skin of AD patients. Although E-selectin and vascular endothelial adhesion molecule-1 are expressed on endothelial cells only, they accelerate inflammation of the skin in association with intercellular adhesion molecule-1 on endothelial cells and keratinocytes (9). Histologic changes from eczematous AD skin lesions vary according to patient age. Biopsy of urticarial erythema shows superficial perivascular infiltration of lymphocytes with a few eosinophils and occasional infiltration of these cells into the epidermis. These changes are similar to those seen in contact hypersensitivity (9). Cellular hypersensitivity against various environmental substances which easily penetrate the epidermis and dermis is important in the formation of eczematous lesion in AD. It has been suggested that characteristic Th2 cells which

Table 1. Results of t-test for incidence of E-selectin expression in the epidermis and dermis in control (C) and AD group

Parameter tested in	Statistical parameter										
	X_{AD}	X_C	t	df	p	N_{AD}	N_C	SD_{AD}	SD_C	F	*P*
Epidermis	0.99	0.54	3.88	52	0.000294*	36	18	0.47	0.20	5.86	0.000308*
Dermis	14.32	3.99	8.85	63	0.000000*	48	17	4.58	2.39	3.67	0.006400*

*Statistically significant difference ($P<0.05$)

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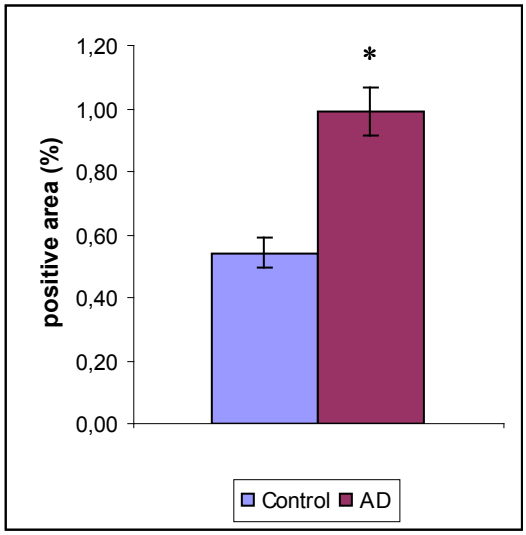

Figure 6. E-selectin positive epidermal area in control group and AD patients. Data are expressed as positive area in percentage of the total epidermal surface area and expressed as mean±SEM. *Statistically significant difference.

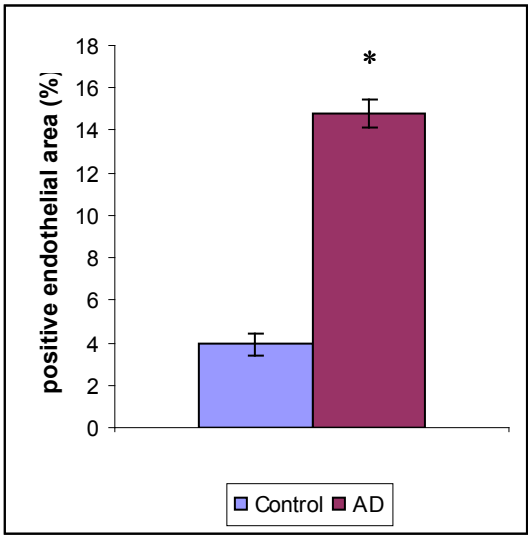

Figure 7. E-selectin positive endothelial area of dermal vessel in control group and AD patients. Data are expressed as positive area in percentage of total vessel surface area and expressed as mean±SEM. *Statistically significant difference.

Table 2. Basic statistical parameters of the incidence of E-selectin expression in the epidermis and dermis in control (C) and AD group

Group	Statistical parameter	Epidermis	Dermis
C	N	18	17
	X	0.54	3.99
	SD	0.20	2.39
	RSD	0.36	0.60
	SEM	0.05	0.58
	M	0.52	3.34
	Minimum	0.24	0.84
	Maximum	0.91	8.73
	Normal Distribution	0.62	0.43
AD	N	36	48
	X	0.99	14.32
	SD	0.47	4.58
	RSD	0.48	0.32
	SEM	0.08	0.66
	M	0.94	13.94
	Minimum	0.28	5.74
	Maximum	1.94	23.13
	Normal Distribution	0.34	0.36

N = number of measured fields; X = mean value; SD = standard deviation; RSD = relative standard deviation; SEM = standard error of mean; M = median

produce various cytokines including interleukin-4 (IL-4) and IL-5 are found in the lesional skin of AD. Such cytokines may influence the expression of cell adhesion molecules either on endothelial cells or keratinocytes in acute AD (9).

Our results in biopsy specimens of AD patients showed increased expression of E-selectin in the epidermis and significantly enhanced expression in endothelial cells. Expression of adhesion molecule E-selectin is present in epidermis of healthy and inflammatory changed atopic skin in basal and lower layers of stratum spinosum. Morphometric measurement revealed significant changes in endothelial expression of E-selection in the vessel wall, with the mean value in AD group being 3.5-fold that recorded in control group, yielding a statistically significant difference at $P<0.05$. A strong reaction was found also in macrophages and in some perivascular cells. A statistically significant difference between the AD and control group was also found for E-selectin expression in the epidermis. Jung *et al*. also proved the appearance of E-selectin and an increase in ICAM-1 and VCAM-1 expression after various agents in AD skin biopsies (7).

In the skin of atopic individuals, the expression of E-selectin was found by immunostaining skin biopsies in a similar manner as in Jung *et al*. study (7). It is suggested that the observed up-regulation of E-selectin is mediated by the release of cytokines such as interleukin-4 from cells that reside

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in atopic skin (7). This finding contributes to the development of Th2 cells, which predominate in atopic inflammation.

Huang *et al*. found the E-selectin (ELAM-1) serum levels to be significantly higher in children with AD than in normal control infants and conclude that determination of adhesion molecules (ICAM-1 and ELAM-1) may be useful in monitoring disease activity of AD in children (11).

Our results proved that the expression of adhesion molecule E-selectin is important in the interaction with invading T lymphocytes in the epidermis and dermis.Thus, it may be hypothesized that the skin of AD patient provides a special environment either for homing or for *in loco* differentiation of Th-2 cells. Cytokines released by keratinocytes may provide the first signal for the induction of adhesion molecules in AD lesional skin and concomitant increase in E-selectin expression on dermal cells (7).

CONCLUSION

The AD skin lesions are related to differences of microenvironmental factors provided by keratinocytes, dendritic cells and melanocytes as well as stromal cells, which may provide different cytokines, chemokines and adhesion molecules for the interaction with lymphocytes. E-selectin is an important adhesion molecule in inflamed AD skin lesions.

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