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The Role of Adhesion Molecules in Atopic Dermatitis

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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 pilot study was to examine the expression of adhesion molecules in atopic dermatitis (AD) skin lesions. Biopsies were taken at acute skin lesions from 10 AD patients and 5 healthy controls, and were studied by immunohistocytochemistry for the expression of E-selectin, L-selectin, ICAM-1 and ICAM-3 on cells in the epidermis and dermis. Study results revealed all AD patients to express ICAM-1 (10/10) and ICAM-3 (10/10) in the dermis, and most of them to express E-selectin (9/10) and L-selectin (6/10) in the dermis, without expression of E- and L-selectins in the epidermis. Our results revealed a high expression of adhesion molecules, especially ICAM-1 and ICAM-3, in the skin lesions of AD patients, which may play an important role in the pathogenesis of AD, and these preliminary results may be of clinical relevance for the treatment of AD.

KEY WORDS: atopic dermatitis, skin, adhesion molecules, selectins

INTRODUCTION

Atopic dermatitis (AD) is a chronic relapsing inflammatory skin disorder associated with erythema, scaly and oozing plaques on the forehead and face, which spread to the neck, hands and flexural areas, and severe pruritus (1-5). AD is characterized by the predominant infiltration of T cells, eosinophils and macrophages in the lesional skin. It appears that immediate, late and delayed allergic reactions are invovled. Since immune dysregulation is a possible key defect in AD, the expression of different immunologic parameters and costimulatory molecules such as integrins and selectins have been studied in AD patients (6). Adhesion molecules E-selectin, P-selectin, ICAM-1 and VCAM-3 are membrane-bound molecules that mediate the attachment of leukocytes to endothelial cells and are preferentially expressed on activated endothelium (7,8). The selectin family of membrane glycoproteins are responsible for the initial stickiness of leukocytes to vascular endothelium and include three molecules designated as L, E and P. It has been observed that most circulating leukocytes express L-selectin, whereas E-selectin and P-selectin are expressed on vascular endothelial cells (8). Analysis of AD skin for the expression of VCAM-1, ICAM-1, E-selectin and P-selectin on endothelium showed a significantly higher VCAM-1 expression in erythrodermic AD than in lesional

Acta Dermatovenerol Croat
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skin of AD, without differences in the expression of other adhesion molecules. In atopic diseases L-selectin is involved in the extravasation of monocytes towards local inflammatory sites, and high serum sL-selectin level in children suggested its presence in the pathogenesis of the disease (9).

PATIENTS AND METHODS

Only patients with clear clinical signs of AD were included in the study. The diagnosis of AD was made by using the criteria of Hanifin and Rajka (10). The patients received no treatment for at least one week before the study. Biopsy specimens were obtained from acute skin lesions of ten AD patients (three females and seven males). The patients were aged 25-50 (mean age 34) 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 anti-ICAM-1, ICAM-3, E-selectin and L-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 molecules (ICAM-1, ICAM-3, E-selectin and L-selectin) in the dermis and epidermis was analyzed semiquantitatively. Total cell count *per* mm^2 of dermis and total cell count *per* mm epidermis length were determined.

RESULTS

The results obtained from AD patients in comparison to healthy controls yielded a greater expression of adhesion molecules in AD skin lesions than in healthy controls (Fig. 1). Study results showed the majority of AD patients to express adhesion molecules (ICAM-1, ICAM-3, E-selectin and L-selectin) in the dermis of skin lesions (Figs. 1 and 2). ICAM-1 was expressed in the epidermis of six AD patients (high in two and moderate in four patients), but all patients expressed ICAM-1 in the dermis (high in four and moderate in six patients). ICAM-3 was moderately expressed in the epidermis of six AD patients, but all AD patients expressed ICAM-3 in the dermis (high in four and moderate in six patients). E-selectin in the dermis was expressed in nine patients (moderate in five and rare in four patients), whereas the expression of E-selectin in the epidermis was not observed.

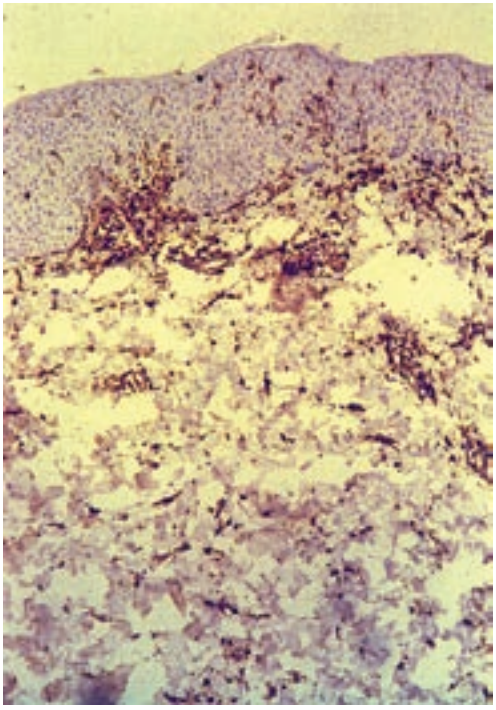

Figure 1. Skin specimen of atopic dermatitis immunohistochemically stained with anti-VCAM antibody, showing strong intensity staining on vascular endothelial cells in the dermis and on some leukocytes in the dermis and epidermis.

The expression of L-selectin in the dermis was moderate in six patients, whereas another four patients did not express it. The expression of E-selectin and L-selectin was not observed in the epidermis of AD patients.

The expression of ICAM-1, ICAM-3, E-selectin and L-selectin adhesion molecules was not observed in the skin of healthy controls.

Study results revealed the majority of AD patients to express adhesion molecules (ICAM-1, ICAM-3, E-selectin and L-selectin) in their skin lesions.

DISCUSSION

Various studies have indicated the importance of cell adhesion molecules (CAMs) and their soluble forms (sCAMs) in the interactions between vascular endothelium and activated leukocytes in various inflammatory skin diseases including AD (6,11). It has been observed that selectins and CAMs such as E-selectin, P-selectin, ICAM-1 and VCAM-1 are preferentially expressed on activated endothelium mediating attachment of leukocytes to endothelial cells, and expression of selectins (E-selectin, L-selectin and P-selectin), vascular

Acta Dermatovenerol Croat
2006;14(1):2-7

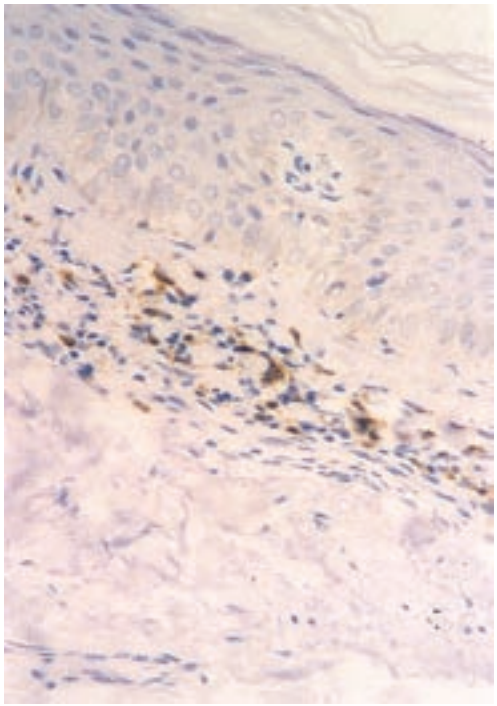

Figure 2. L-selectin expression on some leukocytes in the dermis of a patient with atopic dermatitis. Immunohistochemistry with anti-L-selectin antibody, counterstaining with hematoxylin, x400.

and intercellular adhesion molecules, cutaneous lymphocyte antigen (CLA) have been examined in AD skin. Comparison of the skin of normal and atopic individuals for the expression of E-selectin (ELAM-1), L-selectin (LECAM-1), P-selectin (CD62), CD31 (PECAM), VCAM-1 and ICAM-1 showed a significantly increased expression of VCAM-1 and ICAM-1 (in contrast to ELAM-1) in nonlesional atopic skin as compared with the skin of healthy controls (12). These data revealed constitutive upregulation of certain adhesion molecules in healthy-appearing skin of AD patients, mediated by the release of cytokines such as interleukin-4 (IL-4) from Th2-cells that reside in atopic skin.

Our study results, similar to those reported by Jung *et al*., also confirmed a greater expression of adhesion molecules in AD skin lesions than in the skin of healthy controls, revealing expression of ICAM-1, ICAM-3, E-selectin and L-selectin in the dermis of skin lesions of the majority of AD patients (12). Most prominent was the expression of ICAM-1 and ICAM-3 in the dermis of all AD patients, and of E-selectin in the dermis of the majority of them. The expression of L-selectin in the dermis was found in about half of AD patients, without expression of E-selectin and L-selectin in the epidermis of these patients. The expression of adhesion molecules (ICAM-1, ICAM-3, E-selectin and L-selectin) in the skin lesions of the majority of AD patients supports their role in cell interactions. However, another examination of E-selectin, P-selectin, CD31, VCAM-1 and ICAM-1 on endothelial and other skin cells revealed no major differences between various types of skin reactions in atopics, nor did the skin of AD patients differ from that of healthy controls (13).

Besides the role of membrane-bound molecules, the soluble forms of CAMs (sCAMs) have also been detected in the sera of AD patients, resulting from shedding, and some may reflect AD activity (7). In severe AD patients markedly elevated levels of E-selectin were found, VCAM-1, and ICAM-1, and significantly elevated sE-selectin in severe AD over the levels in mild AD (11). The levels of sE-selectin also showed positive correlation with sVCAM-1 levels, serum IgE levels and eosinophil number, thus being a sensitive clinical parameter in monitoring the clinical course in AD patients (11). A study including children with AD showed a significantly increased sE-selectin in children with specific IgE and correlation with AD activity, also confirming their use in monitoring the disease activity (7).

Examination of the roles of E- and P-selectin in eosinophil recruitment in inflamed AD skin, and the expression of sialyl-Lewis x (sLex) structures and selectin ligands on eosinophils revealed blood eosinophils bound to sP-selectin, however, eosinophils had little avidity for sE-selectin (14). A significantly greater amount of P-selectin bound to eosinophils was observed in AD patients than in healthy controls, indicating eosinophil interaction with endothelial P-selectin, important for recruitment into the inflamed AD skin (14). Several laboratory markers including sCAMs and eosinophil granular proteins have been described to correlate positively with the disease activity of AD, but the assessment of different objective and subjective parameters (E-selectin, sVCAM-1) revealed that only sE-selectin and subjective parameters statistically significantly correlated with the Scoring AD (SCORAD) score (15). We found E-selectin in the dermis of almost all AD patients, and L-selectin in the dermis of about half of the patients, without E-selectin and L-selectin expression in the epidermis.

ICAMs are involved in AD pathogenesis, including repeated antigen stimulation and contact hypersensitivity responses, which cause chronic inflammatory responses with infiltrating leukocytes. A study in mice has revealed that L-selectin and

ICAM-1 cooperatively regulate the induction of immediate-type response by mediating mast cell accumulation into inflammatory sites, with a potential use as targets for regulating human allergic reactions (16). Migration of cells into the skin lesions is connected with skin-homing receptor CLA, a carbohydrate epitope expressed on memory/effector T cells, which infiltrate inflamed skin, mostly allergen-specific Th2 cells that generate exacerbated responses and induce skin inflammation (17). Namely, naive Th cells with receptors for cutaneous antigens become activated in skin-draining lymph nodes and express CLA, confering the capacity to migrate into the skin. E-selectin is a ligand for CLA, which is induced under inflammation on endothelial cells, and targeting CLA/E-selectin interactions prevents recruitment of Th2 memory cells to the skin, which can be efficiently suppressed by using a low molecular weight E-selectin antagonist that may be of clinical relevance for the treatment of AD (Fig. 3). This selective transendothelial migration of memory/effector T cells by interaction with E-selectin on endothelial cell layers involves activation of proinflammatory cytokines and receptor-ligand pairs VLA-4/VCAM-1 and LFA-1/ICAM-1 (18). Our previous study has confirmed that AD skin lesions are predominantly infiltrated by CD3+ with high expression of CD45Ro (memory T cells), CD1a, CD29, IFNγ+cells, as supported by other authors (5). Analysis of CD45RO+ T cells of AD patients and specific *in vitro* response to skin-associated allergens has revealed enhanced migration of CLA+ T cells through activated human umbilical vein endothelial cell monolayers compared to CLA- T cells. The CLA binding to E-selectin is initially responsible for the extravasation that also involves VLA-4/VCAM-1 and LFA-1/ICAM-1 interactions (18). Migration of CLA+ T cell is connected with IL-8 as an endothelial cell-derived chemokine and is under the control of IL-8 receptor type B, which localizes memory/effector T cells that respond to antigens and reach the body through inflamed skin (18). This regionalization of the skin immune system may allow for an efficient distribution of immune defense to different sites of the body. On the other hand, the examination of cord blood T cells in infants with AD stimulated by milk allergen has shown that the gut-homing receptor integrin alphaEbeta7 but not CLA precedes the development of milk-induced eczema in early infancy, providing a clue to the avoidance of specific allergens and potential therapy targeting homing receptors in food allergy (19).

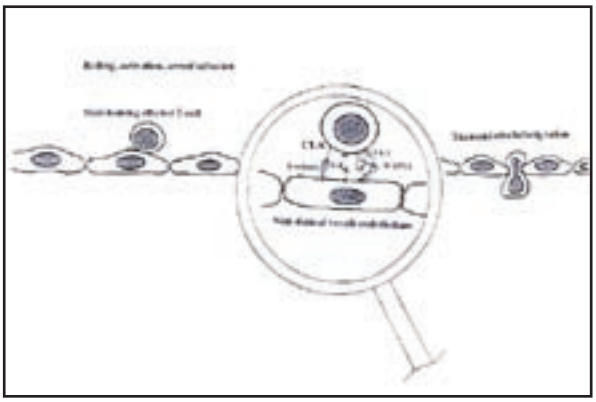

Figure 3. A scheme of cell interactions in cell adhesion to the endothelium in atopic dermatitis skin (modified from Goldsby RA, Kindt TJ, Osborne AO) (8).

It is also important that adhesion, chemotaxis, and activation of many types of leukocytes are under the control of various chemokines such as CCR3/CCR4 and CCR5/CXCR3, expressed on the infiltrating Th2-cells in skin lesions. Chemokines are a superfamily of small polypeptides, which selectively and often specifically control the adhesion, chemotaxis and activation of leukocytes, which are separated, based on the position of the four invariant cysteine residue, into C-C subgroup and C-X-C subgroup (7). It has been observed that IL-8 and related Glu-Leu-Arg (ELR+) CXC chemokines are potent chemoattractants for neutrophils and accumulation and positioning of mononuclear phagocytes in Th2-dominated responses (19). Examination of peripheral blood from AD patients has shown a significantly higher percentage of CCR4+ cells in CD4+ CD45RO+ T cells than in healthy controls, which correlated positively with total serum IgE, eruption score, eosinophil number, lactate dehydrogenase (LDH), IL-4 and IL-13 secretion in CD4+ T cells, and inversely with IL-2 and IFNγ secretion in CD4+ T cells (20). AD patients had a significantly decreased percentage of CCR5+ or CXCR3+ cells in CD4+ CD45RO+ T cells, which correlated positively with the eruption score, without detection of CCR3 on circulating CD4+ T cells in AD patients (21).

Two CC chemokines have also been identified: macrophage-derived chemokine (MDC)/CCL22, a selective chemoattractant for CCR4-expressing cells, and thymus and activation-regulated chemokine (TARC), its serum levels correlating with AD severity (3). Serum MDC levels significantly correlated with the SCORAD index, serum sE-selectin, sIL-2Rs, TARC and eosinophils (3).

Table 1. Results of immunohistochemical analysis of ICAM-1, ICAM-3, E-selectin and L-selectin expression in the skin of atopic dermatitis patients and healthy controls

		Atopic dermatitis patients					Healthy controls				
		No	Yes/	Rare	Moderate	High	No	Yes/	Rare	Moderate	High
ICAM-1	**E**	4	6/	0	4	2	10	0/	0	0	0
	D	0	10/	0	6	4	10	0/	0	0	0
ICAM-3	**E**	4	6/	0	6	0	10	0/	0	0	0
	D	0	10/	0	6	4	10	0/	0	0	0
E-selectin	**E**	10	0/	0	0	0	10	0/	0	0	0
	D	1	9/	4	5	0	10	0/	0	0	0
L-selectin	**E**	10	0/	0	0	0	10	0/	0	0	0
	D	4	6/	0	6	0	10	0/	0	0	0

Important roles of some other adhesion molecules have also been noticed on various cells such as dendritic cells (DC) and Langerhans cells (LCs), and homing receptors for T cells that are selective for skin localizations (22). It is important not to forget various molecules expressed on LCs such as E-cadherin, a homophilic adhesion molecule, etc.

CONCLUSION

Our results revealed the expression of ICAM-1 and ICAM-3 in the dermis of all AD patients, and expression of E-selectin and L-selectin in the dermis of most of them, which may be the result of enhanced cell activation and migration to skin lesions. Although many new information regarding the pathogenesis of AD have evolved over the past several years, the basic underlying etiology of this disorder remains elusive. We hope that the coming years will witness the development of new strategies for the treatment of AD, aimed at specific targets based on a thorough understanding of its pathogenesis.

References

1. Galli E, Cicconi R, Rossi P, Casati A, Brunetti E, Mancino G. Atopic dermatitis: molecular mechanisms, clinical aspects and new therapeutical approaches. Curr Mol Med 2003;3:127-38.
2. Braun-Falco O, Plewig G, Wolff HH, Burgdorf WHC, eds. Dermatology. 2nd Completely Revised Edition. Berlin: Springer-Verlag, 2000.
3. Kakinuma T, Nakamura K, Wakugawa M, Mitsui H, Tada Y, Saeki H, *et al*. Serum macrophage-derived chemokine (MDC) levels are closely related with the disease activity of atopic dermatitis. Clin Exp Immunol 2002;127:270-3.
4. Lugovic L, Lipozencic J, Jakic-Razumovic J. Atopic dermatitis: immunophenotyping of inflammatory cells in skin lesions. Int J Dermatol 2001;40:489-94.
5. Lipozencic J, Bobek D, Jakic-Razumovic J. The presence of surface CD30 on T cells in atopic dermatitis. Acta Dermatovenerol Croat 2003;11:145-52.
6. Oberwalleney G, Henz BM, Worm M. Expression and functional role of co-stimulatory molecules in CD40+IL-4-stimulated B cells from atopic and non-atopic donors. Acta Derm Venereol 2000;80:287-91.
7. Wolkerstorfer A, Laan MP, Savelkoul HF, Neijens HJ, Mulder PG, Oudesluys-Murphy AM, *et al*. Soluble E-selectin, other markers of inflammation and disease severity in children with atopic dermatitis Br J Dermatol 1998;138:431-5.
8. Goldsby RA, Kindt TJ, Osborne AO. Leukocyte migration and inflammation. In: Kuby J, ed. Immunology, 4th ed. New York: WH Freeman and Company, 2002;371-9.
9. Yamashita H, Tanno Y. L-selectin expressed on peripheral monocytes and soluble L-selectin in sera in atopic diseases. Arerugi 2000;49:397-403.

10. Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. Acta Derm Venereol (Stockh) 1980;92:44-7.
11. Koide M, Furukawa F, Tokura Y, Shirahama S, Takigawa M. Evaluation of soluble cell adhesion molecules in atopic dermatitis. J Dermatol 1997;24:88-93.
12. Jung K, Linse F, Heller R, Moths C, Goebel R, Neumann C. Adhesion molecules in atopic dermatitis: VCAM-1 and ICAM-1 expression is increased in healthy-appearing skin. Allergy 1996;51:452-60.
13. Jung K, Linse F, Pals ST, Heller R, Moths C, Neumann C. Adhesion molecules in atopic dermatitis: patch tests elicited by house dust mite. Contact Dermatitis 1997;37:163-72.
14. Satoh T, Kaneko M, Wu MH, Yokozeki H, Nishioka K. Contribution of selectin ligands to eosinophil recruitment into the skin of patients with atopic dermatitis. Eur J Immunol 2002;32:1274-81.
15. Gutgesell C, Heise S, Seubert A, Stichtenoth DO, Frolich JC, Neumann C. Comparison of different activity parameters in atopic dermatitis: correlation with clinical scores. Br J Dermatol 2002;147:914-9.
16. Shimada Y, Hasegawa M, Kaburagi Y, Hamaguchi Y, Komura K, Saito E, *et al.* L-selectin or ICAM-1 deficiency reduces an immediate-type hypersensitivity response by preventing mast cell recruitment in repeated elicitation of contact hypersensitivity. J Immunol 2003;15;170:4325-34.
17. Biedermann T, Schwarzler C, Lametschwandtner G, Thoma G, Carballido-Perrig N, Kund J, *et al.* Targeting CLA/E-selectin interactions prevents CCR4-mediated recruitment of human Th2 memory cells to human skin in vivo. Eur J Immunol 2002;32:3171-80.
18. Santamaria LF, Perez Soler MT, Hauser C, Blaser K. Allergen specificity and endothelial transmigration of T cells in allergic contact dermatitis and atopic dermatitis are associated with the cutaneous lymphocyte antigen. Int Arch Allergy Immunol 1995;107:359-62.
19. Kohno Y, Shimojo N, Kojima H, Katsuki T. Homing receptor expression on cord blood T lymphocytes and the development of atopic eczema in infants. Int Arch Allergy Immunol 2001;124:332-5.
20. Bonecchi R, Facchetti F, Dusi S, Luini W, Lissandrini D, Simmelink M, *et al.* Induction of functional IL-8 receptors by IL-4 and IL-13 in human monocytes. J Immunol 2000;164: 3862-9.
21. Okazaki H, Kakurai M, Hirata D, Sato H, Kamimura T, Onai N, *et al.* Characterization of chemokine receptor expression and cytokine production in circulating CD4+ T cells from patients with atopic dermatitis: up-regulation of C-C chemokine receptor 4 in atopic dermatitis. Clin Exp Allergy 2002;32:1236-42.
22. Cantani A. Pathogenesis of atopic dermatitis (AD) and the role of allergic factors. Eur Rev Med Pharmacol Sci 2001;5:95-117.