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The Expression of Adhesion Molecules on Skin Following Prick Test with *Dermatophagoides Farinae* in Patients with Atopic Dermatitis

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The etiology of atopic dermatitis(AD) is still unknown, but many investigators believe that an allergic reaction to environmental antigens, especially the house dust mite, may be important in pathogenesis of AD. The skin lesion associated with AD is characterized histologically by an infiltration of inflammatory cells which is largely attributed to the expression of adhesion molecules.

We have investigated the changes in the expression of ICAM-1, VCAM-1 and E-selectin on vascular endothelial cells obtained from skin biopsies immediately, 15 minutes and 24 hours after a prick test with *D. farinae* from AD patients.

In clinically normal skin, the expression of ICAM-1 was observed focally on vascular endothelial cells, but VCAM-1 and E-selectin were not expressed. In the skin biopsies obtained 15 minutes after pricking, the expression of ICAM-1 and VCAM-1 was slightly increased while the expression of E-selectin was markedly induced on vascular endothelial cells. 24 hours after pricking, linear and strong expressions of ICAM-1 and E-selectin were observed on the vascular endothelial cells, but the expression of VCAM-1 was only mild.

D. farinae increase the expression of ICAM-1 and induce the expression of VCAM-1 and E-selectin on vascular endothelial cells. It is believed that these processes may play a role in the pathogenesis of AD.

Key words: Atopic dermatitis, Prick test, ICAM-1, VCAM-1, E-selectin

INTRODUCTION

Atopic dermatitis (AD) is a common inflammatory skin disease characterized by several clinical and immunologic alterations. The reason why cells infiltrating the skin are recruited from the circulation rather than from the in situ expansion of resident cells is because few infiltrated inflammatory cells are capable of expressing proliferation markers, such as Ki-67 antigen[1]. It is known that certain soluble mediators, such as the complement component C5a, platelet-activating factor (PAF) and leukotriene B4 (LTB4) are able to exert a chemotactic effect on neutrophils[2], but the action of such mediators may not fully explain the localization of neutrophils at the inflammatory focus. However this is now believed to be largely due to the induced expression of appropriate adhesion molecules on the surface of the vascular endothelium[3,4]. Numerous immunohistochemical studies have demonstrated the expression of adhesion molecules in allergic diseases such as extrinsic allergic asthma and atopic dermatitis[5,6].

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AD may be caused by extrinsic factors including food allergens and common environmental allergens known as inhalant allergens (i.e. house dust mites, pollen, animal dander). Many AD patients with moderate to severe eczema have been found to have higher concentrations of mites in their environment and an immediate reaction to mite allergen in the skin test[7]. Intradermal antigen challenge in sensitized subjects induces the expression of adhesion molecules, in parallel with the development of an inflammatory cell infiltrate made up of eosinophils, neutrophils and mononuclear cells[8].

In the present study we have investigated the changes in the expression of ICAM-1, VCAM-1 and E-selectin on vascular endothelial cells in skin biopsies obtained immediately, 15 minutes and 24 hours after the prick test with *D. farinae*, which is the most common allergen in Korea, amongest AD patients.

MATERIAL AND METHODS

Subjects

Fifteen patients (ages, 6-30 years; mean age, 21.3 years; 9 male and 6 female) who showed positive prick test and specific IgE to *D. farinae* were selected amongest the AD patients who had been diagnosed using the criteria of Hanifin and Rajka[9].

Clinical severity was assessed using the extent of skin lesion, the course of disease, and the intensity of pruritus[10]. Four types of lesions were scored on scale from 0 to 3: erythema, excoriation, lichenification and desquamation.

Informed consent was given by each patient.

Tissue

3 mm punch biopsy specimens were obtained under local anesthesia from the positive reaction sites 15 minutes and 24 hours after pricking with *D. farinae* (Bencard, Brentford, England) from clinically uninvolved skin. For negative control, specimens were obtained from the clinically normal skin of the same patient. The specimens were then bisected. One piece was fixed in formalin for routine histopathologic examination and the other was embedded in Optimum Cutting Temperature compound, frozen in melting isopentane, and stored in liquid nitrogen until required.

Immunohistochemical Studies

Frozen section (5 μm) were air-dried for 15 min, fixed in acetone at room temperature for 15 min, washed and non-specific blocked with 20% normal swine serum (Blocking solution). Sections were then processed with monoclonal mouse anti-human ICAM-1 (clone 84H10, Immunotech, Westbrook, ME, USA), VCAM-1 (clone 51-10C9, Pharmingen, San Diego, CA, USA) and E-selectin (clone 1.2B6, Immunotech) antibodies. Subsequently, biotinylated rabbit antibody against mouse IgG was added and amplified using streptavidin-peroxidase complex (Dako). Sections were counterstained with haematoxylin.

Assessment of the staining for adhesion molecules on endothelial cells was performed by counting positively stained vessels. The staining intensity was graded as either 0 (absent), 1 + (weak), 2 + (moderate), or 3 + (strong).

Analysis was performed by microscopy at a magnification of $\times$ 400. In the case of the endothelium, a minimum of 15 different vascular sections were studied and the results were expressed as a percentage of stained vascular sections.

RESULTS

Among fifteen patients twelve had moderate and three patients severe atopic dermatitis. The mean clinical score of skin lesions was moderate to severe; erythema: 2.2 $\pm$ 0.9, excoriation: 1.9 $\pm$ 0.7, lichenification: 2.1 $\pm$ 0.8 and desquamation: 2.0 $\pm$ 0.8.

Histologically, little perivascular infiltration of lymphocytes was observed in clinically normal skin. In skin biopsies obtained 15 minutes after pricking, perivascular infiltration of mononuclear cells in the upper dermis was observed. The histologic changes 24 hours after pricking revealed slight hyperkeratosis, spongiosis and exocytosis of the mononuclear cells in the epidermis and perivascular infiltration of mononuclear cells with many eosinophils in the dermis.

In clinically normal skin, the expression of ICAM-1 was observed focally on the vascular endothelial cells, but the expressions of VCAM-1 and E-selectin were absent (Fig. 1). In skin biopsies taken 15 minutes after pricking, the expressions of ICAM-1 and VCAM-1 were slightly increased, while the expression of E-selectin was markedly induced on vascular

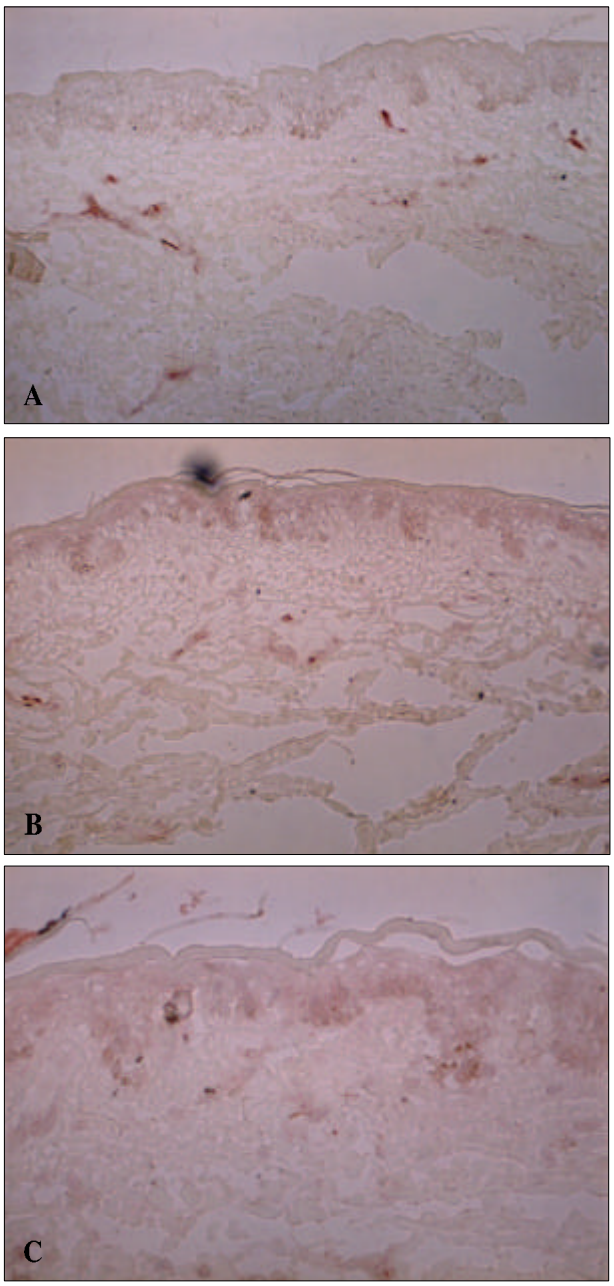

Fig. 1. A. In clinically normal skin, ICAM-1 expression is observed focally on vascular endothelial cells. Magnification × 100. B. Normal skin does not express VCAM-1. Magnification × 100. C. E-selectin expression is not found in normal skin. Magnification × 100.

endothelial cells. 24 hours after pricking, linear and strong expressions of ICAM-1 and E-selectin were observed on the vascular endothelial cells, but the expression of VCAM-1 was mild (Table 1, Fig. 2-4).

There was no correlation between clinical severity and degree of expression of ICAM-1, VCAM-1 and E-selectin.

Table 1. Mean Values of ICAM-1, VCAM-1, and E-selectin Expression on Endothelial Cells in Skin Biopsies from Positive Prick Test Reaction to *D. farinae* of Atopic Dermatitis Patients

	No. of patients	Times after skin test with *D. farinae*		
		Control	15 min	24 hrs
ICAM-1	15	1.3±0.5	1.7±0.5	2.4±0.5
VCAM-1	15	0.2±0.4	1.0±0.5	1.2±0.4
E-selectin	15	0.3±0.5	2.4±0.7	2.8±0.4

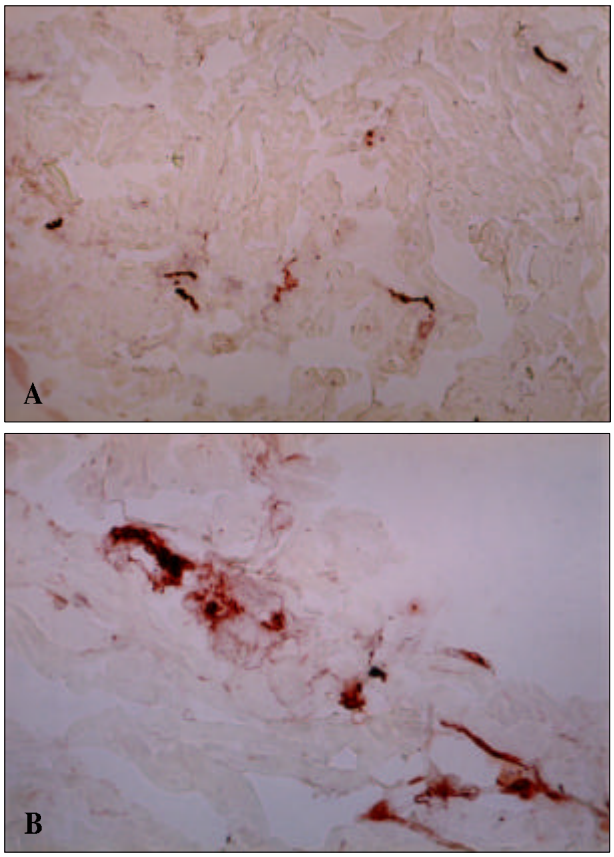

Fig. 2. A. In skin biopsies taken 15 minutes after pricking, the expression of ICAM-1 is slightly increased. Magnification × 200. B. 24 hours after pricking, linear and strong expression of ICAM-1 is observed. Magnification × 400.

DISCUSSION

The adhesion of leukocytes to microvascular endothelium is essential to facilitate their migration into inflamed tissues. Endothelial cells lining the postcapillary venules and microcirculation produce leukocyte-specific adhesion molecules both constitutively and in response to a wide range of inflammatory mediators[11]. Currently endothelial leukocyte receptors

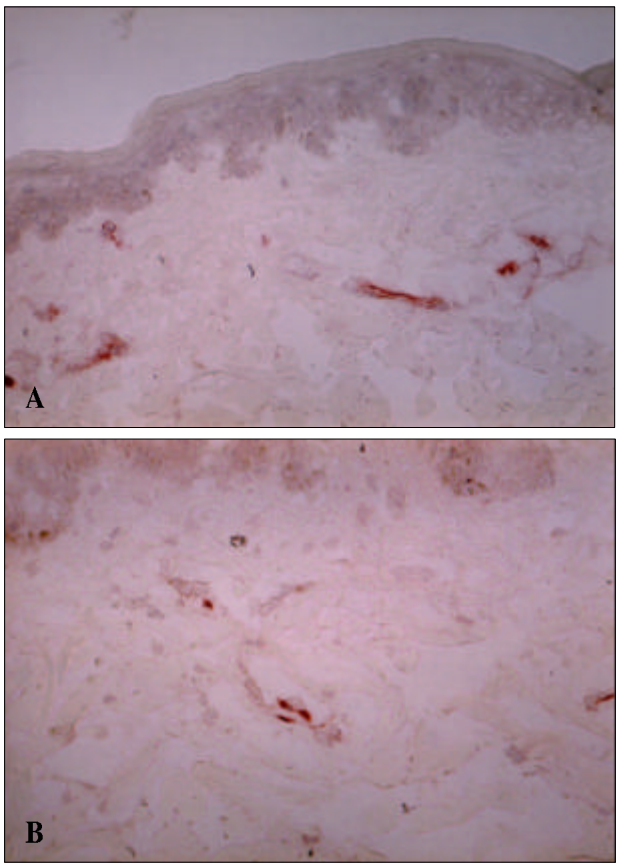

Fig. 3. A. VCAM-1 expression is slightly increased 15 minutes after pricking Magnification × 200 B. Only mild expression is observed 24 hours after pricking. Magnification × 200.

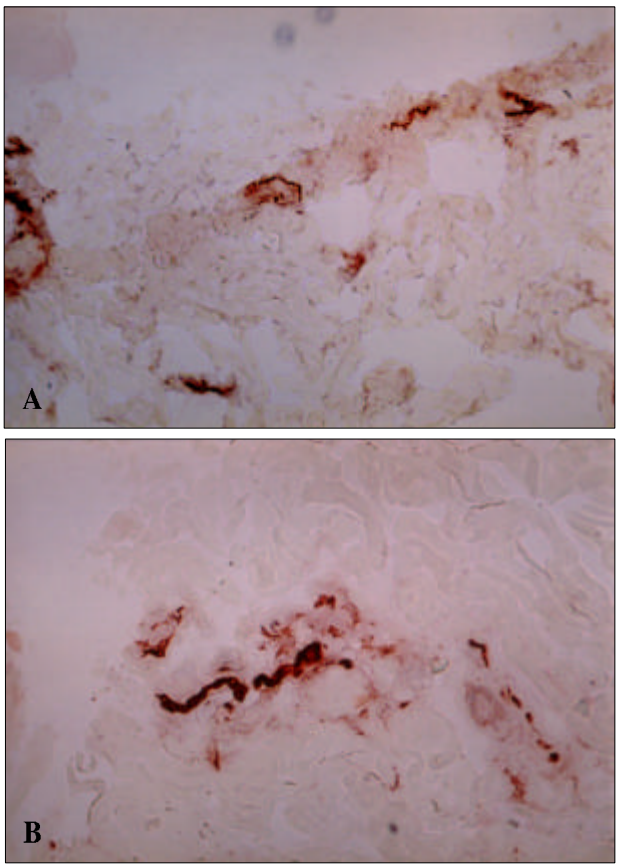

Fig. 4. A. E-selectin expression is induced markedly on vascular endothelial cells 15 minutes after pricking. Magnification × 200. B. After 24 hours, linear and strong expression of E-selectin is observed on the vascular endothelial cells. Magnification × 400.

are classified as belonging to three structural groups, the immunoglobulin gene superfamily, the integrin family, and a newly discovered family of proteins called selectins or LECCAMs[3].

The immunoglobulin gene superfamily encompasses a large group of molecules, which are involved in recognition and adhesion. Members are characterized by the presence of one or more immunoglobulin homology units. Ligands of ICAMs and VCAM-1 are members of the widely distributed integrin family of adhesion molecules, which mediate cell-extracellular matrix and cell-to-cell binding. In contrast to the multifunctional immunoglobulin and integrin families, the known members of the LECCAM or selectin family all function in the area of leukocyte-endothelial adhesion.

ICAM-1 is constitutively expressed on endothelial cells and can be upregulated by IL-1, TNF, or IFN-γ[12]. The receptor on leukocytes for ICAM-1 is the β2integrin LFA-1 (CD11a/CD18), which is present on virtually all circulating white cells[13]. ICAM-1 expression is enhanced on resident cells in cutaneous inflammation characterized by the presence of T cells in the tissues, such as allergic contact dermatitis, lichen planus, psoriasis, and atopic dermatitis[14].

VCAM-1 is minimally expressed on cultured endothelial cells but induced in response to IL-1 or TNF[15]. The leukocyte receptor for VCAM-1 is the β2integrin VLA-4 (CD49d/CD29), which is found on lymphocytes and monocytes[16]. Consistent with this distribution, *in vitro* experiments indicate that VCAM-1 is a selective adhesion molecule for mononuclear cells[15].

E-selectin is expressed by cultured endothelial cells by IL-1 and TNF[17]. *In vitro* experiments indicate that E-selectin selectively binds neutrophils and perhaps monocytes but not the majority of lymphocytes[18]. E-selectin has been found to be induced on endothelial cells in situ in human skin following

intradermal injection of streptokinase-streptodornase[19] and during IL-2 immunotherapy[20].

The soluble forms of the adhesion molecules (sE-selectin, sICAM-1, sVCAM-1) also exist and they originate from membrane-bound molecules due to shedding or proteolytic cleavage[21]. The amount of sE-selectin and sICAM-1 released was found to correlate directly with cell surface expression *in vitro*[22]. Increased concentrations of soluble forms of adhesion molecules may reflect the increased expression of these molecules on the endothelial cell surface. The mechanism which facilitates the release of cell adhesion molecules into the circulation remains unclear.

The level of soluble E-selectin in the sera of patients with AD correlates with the disease activity[23], for example soluble VCAM-1 concentrations are increased in atopic dermatitis patients, and may be used as an index of atopy if their values are above normal. However, it is doubtful whether they are indicators of disease activity, since there is no correlation between their concentrations and clinical severity[24].

The skin lesion of atopic dermatitis is characterized by the infiltration of CD4+ T cells and antigen presenting cells. This suggests that AD represents a delayed-type hypersensitivity reaction[25]. In recent years, it has become evident that there is an imbalance in the generation of Th1 and Th2 cytokines in AD[26].

The mechanism which leads to the appearance of Th2-predominant, allergen-specific T cells in atopic dermatitis is unknown. In addition, Langerhans cells have the high-affinity Fc receptor for IgE[27], the patient with AD have high levels of IgE for allergens, and show positive prick test results for allergens. Therefore it is believed that IgE-related immune reactions participate in the formation of AD lesions. Many patients with atopic dermatitis show an immediate reaction to mite allergen in the skin test. Histologic changes of positive prick test responses do not become dramatic, but studies have shown that when these antigens are applied under patch tests, a proportion of subjects develop spongiotic lesions, which resemble the lesions of allergic contact dermatitis[8].

Numerous immunohistochemical studies have demonstrated the significant expression of E-selectin, ICAM-1 and VCAM-1 in allergic diseases such as extrinsic allergic asthma and AD[5,6]. It has been demonstrated that various stimuli, including chemicals or UV, induce the production of several cytokines and the expression of cell adhesion molecules by keratinocytes[28,29]. Furthermore, intradermal antigens challenge in sensitized subjects induces significant E-selectin expression and the upregulation of ICAM-1, in parallel with the development of an inflammatory cell infiltrate made up of eosinophils, neutrophils and mononuclear cells[6,7].

In the present study we investigated the changes of the expression of ICAM-1, VCAM-1 and E-selectin on vascular endothelial cells in skin biopsies obtained immediately, 15 minutes and 24 hours after a prick test with *D. farinae* upon patients with AD. Our study suggests that *D. farinae* increase the expression of ICAM-1 and induce the expression of VCAM-1 and E-selectin on vascular endothelial cells. Histologic changes may be related to changes of these adhesion molecules.

Jung et al.[30] reported that VCAM-1 and ICAM-1 expression is increased in AD skin compared to the skin of normal controls and even in the healthy-appearing skin of AD patients. This result may explain the high skin irritability of these patients but it is not certain whether the inherent upregulation of adhesion molecules in atopic skin contributes to the development of Th2 cells.

Our results suggest that *D. farinae* increases the adhesion molecules and thus, induces the inflammatory reaction. Persistent expression of adhesion molecules may be related to the prolongation of the skin lesion in AD. Furthermore therapeutic approaches based upon blocking adhesion pathways presents us with the possibility of new modalities of AD treatment.

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