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Cell Adhesion Molecule Expression in Cutaneous Lesions of Dermatomyositis

GISELA HAUSMANN[1], JOSÉ M. MASCARÓ JR[1], CARMEN HERRERO[1], MARIA CINTA CID[2], JOSÉ PALOU[1] and JOSÉ M. MASCARÓ[1]

Departments of [1]Dermatology and [2]Medicine, Hospital Clínic, Barcelona, Spain

Chronic inflammation seems to play a major role in skin and muscle cell damage in dermatomyositis. Adhesion molecules and their ligands are fundamental in regulating inflammation. We have carried out an immunohistochemical analysis of different activation-inducible adhesion markers in 15 biopsy specimens from dermatomyositis skin lesions. Consistent findings were the increased expression of intercellular adhesion molecule-1 (ICAM-1) on endothelial cells, inflammatory cells and focally grouped keratinocytes in contact with subepidermal inflammatory infiltrates. Immunoreactivity for vascular cell adhesion molecule-1 (VCAM-1) was predominant on endothelial cells of the upper reticular dermis and dermal stellate-shaped cells. E-selectin (endothelial leukocyte adhesion molecule-1) immunoreactivity was less extensive, detected mostly on segments of vessels of the papillary dermis and upper reticular dermis, and sometimes independent of inflammation. This pattern of adhesion molecule expression is similar to that described in other immune-mediated dermatoses. The up-regulation of the adhesion molecules appears to play a role in the development and perpetuation of dermatomyositis skin lesions. ***Key words: ICAM-1; VCAM-1; E-selectin; endothelial cells; keratinocytes.***

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C. Herrero, Servicio de Dermatología, Hospital Clínic, Villarroel 170, Barcelona, 08036, Spain.

Dermatomyositis (DM), polymyositis and inclusion body myositis comprise a group of acquired muscle diseases called idiopathic inflammatory myopathies. DM is identified by a characteristic rash accompanying or, more often, preceding muscle weakness (1, 2). The DM myopathy is generally accepted as mediated by immunopathologic events directed against intramuscular microvasculature, but the precise mechanism of vessel injury is unknown (1–5). The skin lesions of DM show histologic similarities to cutaneous lupus erythematosus (6, 7). Dermal inflammatory infiltrates consist predominantly of HLA-DR-expressing macrophages and T cells, especially of the CD4 subset, but B lymphocytes are normally absent (8).

Endothelial cells (EC) are critical elements in the evolution of inflammation (9–11). Regulated expression of cell-surface glycoproteins known as cell adhesion molecules (selectins, immunoglobulin-like molecules and integrins) allows for the precise trafficking of circulating leukocytes to the site of inflammation, injury, or immunologic stimulation. E-selectin (endothelial leukocyte adhesion molecule-1/ELAM-1), a member of the selectin family, is not constitutively expressed by EC, but may be induced within hours by a variety of cytokines (9). E-selectin tethers leukocytes to the EC so as to permit the tighter adhesion mediated by integrin-intercellular adhesion molecule-1 (ICAM-1) interactions (10). ICAM-1 and vascular cell adhesion molecule-1 (VCAM-1) are members of the immunoglobulin gene superfamily. ICAM-1 expression on EC is constitutive but can be markedly increased by exposure to cytokines (9, 10). It is the major ligand for lymphocyte-function-associated antigen-1 (LFA-1), an adhesion molecule on leukocytes (12). Vascular endothelium in most normal tissues expresses little or no VCAM-1 (13, 14). VCAM-1 represents the EC ligand for very late activation antigen-4 (VLA-4), present on lymphocytes, eosinophils and monocytes (10). Interactions between LFA-1/ICAM-1, VLA-4/VCAM-1 are most likely responsible for the accumulation of inflammatory cells in autoimmune diseases (10).

In this study, an immunohistochemical analysis was carried out on biopsy specimens from DM skin lesions in order to investigate the presence and distribution of activation-inducible adhesion molecules on EC and their relation with dermal inflammatory infiltrates.

MATERIAL AND METHODS

Patients and specimens

Twelve patients with DM were included in this study: 7 idiopathic DM, 3 paraneoplastic DM and 2 juvenile DM (sex 3M/9F, age range 3–79 years, mean: 56 years). Diagnosis was made on the basis of the Bohan/Peter criteria (15). One of the juvenile DM was a 12-year old girl, with severe intestinal vasculitis, refractory to corticosteroids, cyclosporin A, cyclophosphamide and intravenous gammaglobulin. The patients were not receiving systemic therapy when the skin biopsy specimens were taken, with the exception of 2 patients in which cutaneous biopsies were performed prior and following 2 years of systemic therapy with cyclosporin A. All fifteen biopsies were taken from lesional skin: knuckles (Gottron's papules, seven), arm (Gottron's sign, four), anterior chest (erythematous rash, two), and face (heliotrope rash, two). The characteristic pathologic changes included mild vacuolization of the basal keratinocytes, epidermal and dermal colloid bodies, inflammatory exocytosis, and a perivascular lymphocytic infiltrate. The histopathologic skin changes in the three DM subsets showed no significant difference. Three biopsies obtained from healthy subjects were used as controls. All biopsy specimens were obtained under local anaesthesia with 2% mepivacaine hydrochloride, freshly frozen in liquid nitrogen, and stored at −70°C until used.

Monoclonal antibodies and immunohistochemistry

All specimens were serially sectioned with a cryostat (4 μm thick). Primary murine monoclonal antibodies anti-ELAM-1 or E-selectin (clone 1.2B6: cytokine-stimulated EC), anti-ICAM-1 (CD54, clone 84H10: EC, antigen-presenting cells), anti-VCAM-1 (CD106, clone 1G11: cytokine-stimulated EC), anti-VLA-α-4 (CDw49d: leukocytes) were obtained from Immunotech S.A., Marseille. Leu-4 (CD3, pan T-cells), Leu-3a (CD4: helper/inducer T cells), Leu-2a (CD8: cytotoxic/suppressor T cells), Leu-12 (CD19: pan B-cells) were from Becton Dickinson (Mountain View, Ca, USA). EBM-11 (CD68: macrophages), anti-HLA-DR (activated T cells, monocytes, Langerhans'

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cells, B cells) and *Ulex europeus* lectin (vascular endothelium) were obtained from Dako (Copenhagen, Denmark). The immunoreactivity was detected using a two step-immunoperoxidase technique, as previously described (8).

Evaluation of the staining reaction

Staining with *Ulex europeus* lectin was considered as 100% vessel staining and CD3 and CD68 positive cells represented altogether the total number (100%) of inflammatory cells. The percentage of positive cells was calculated semi-quantitatively for EC or inflammatory infiltrates in the following way: (−): less than 5% vessels/inflammatory cells, (+): 5–20% vessels/inflammatory cells, (++): 20–60% vessels/inflammatory cells and (+++): 60–100% staining reactivity. In order to differentiate the localization of endothelial and inflammatory stained cells we separated papillary from reticular dermis. The average score for every localization and antibody was expressed with the calculated median.

RESULTS

Endothelial cells

In DM lesional skin (Table I), a strong ICAM-1 expression (Fig. 1) was found in the papillary dermis and upper reticular dermis. VCAM-1 expression was strong or moderate in the upper reticular dermis (Fig. 2) and weaker in the papillary dermis. E-selectin expression was less extensive than ICAM-1 and VCAM-1 and was detected only on some vessels of the papillary dermis and upper reticular dermis (Fig. 3). Sometimes a strong immunoreactivity of E-selectin was observed only on a fragment of a vessel. Expression of E-selectin did not always correlate with the presence of inflammatory cells in adjacent tissue.

Nearly all vessels were HLA-DR-positive, but the degree of expression was variable in the same specimen. A strong staining of EC with *Ulex europeus* was present in all biopsies. In normal skin, weak ICAM-1 expression was detected on EC.

Table I. *Distribution of cell markers and adhesion molecules in skin lesions of DM (n=15)*

Ulex, Ulex europeus lectin; PD, papillary dermis; RD, reticular dermis. Staining reactivity: (−): less than 5% positive vessels/inflammatory cells, (+): between 5–20% positive vessel/inflammatory cells, (++): between 20 and 60% positive vessels/inflammatory cells and (+++): between 60 and 100%.

	Endothelial cells		Infiltrating cells		Keratinocytes
	PD	RD	PD	RD	
Cell markers					
CD3	...	...	++	++	...
CD4	...	...	++	++	...
CD8	...	...	+/−	+	...
CD19	...	...	−	−	...
CD68	...	...	+++	+++	...
HLA-DR	+++	+++	++	++	−
Ulex	+++	+++	...	...	+++
Adhesion molecules					
ICAM-1	+++	+++	+/++	++	−/+
VCAM-1	++	++	+/++	++	...
VLA-4	...	...	+/++	++	...
E-selectin	+/++	+/++	...	...	...

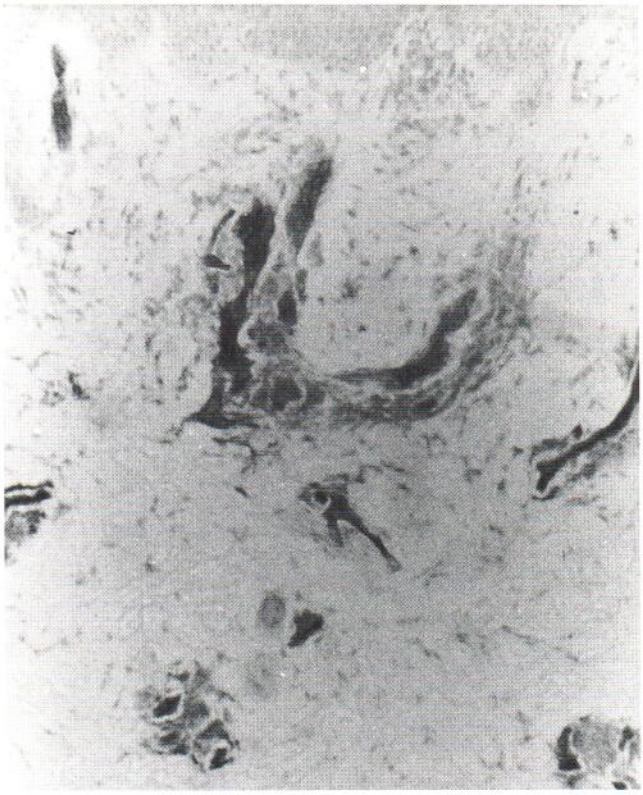

Fig. 1. ICAM-1 expression in lesional DM skin. Note the expression of ICAM-1 on vascular endothelium, perivascular inflammatory cells and interstitial dermal cells.

VCAM-1 positivity was absent. E-selectin expression was undetectable or seen only on a few EC in the control specimens.

Infiltrating inflammatory cells

T lymphocytes (CD3+cells) were usually sparse, with a perivascular and subepidermal distribution. T helper cells were predominant with a CD4/CD8 ratio>3. B lymphocytes (CD19+cells) were usually absent. Macrophages (CD68+cells) were abundant and outnumbered T lymphocytes. They were scattered through the papillary dermis and reticular dermis, surrounding or mixed among perivascular T lymphocyte infiltrates, or in subepithelial accumulations, sometimes boarding the epidermis. Almost all infiltrating cells (lymphocytes and macrophages) were strongly HLA-DR-positive. Only some small groups or scattered cells were clearly HLA-DR-negative. ICAM-1 was expressed by the majority of the perivascular and subepidermal inflammatory cells and dermal interstitial cells, especially in the upper reticular dermis. Staining intensity of the perivascular infiltrates in the same specimen was not uniform. VCAM-1 was expressed also on some inflammatory cells and on isolated stellate-shaped cells in the dermal interstitium. A strong to moderate expression of VLA-4 was observed on the majority of inflammatory cells (Fig. 4).

Keratinocytes

In lesional DM skin ICAM-1+ focally grouped keratinocytes were observed, frequently near focal subepidermal mono-

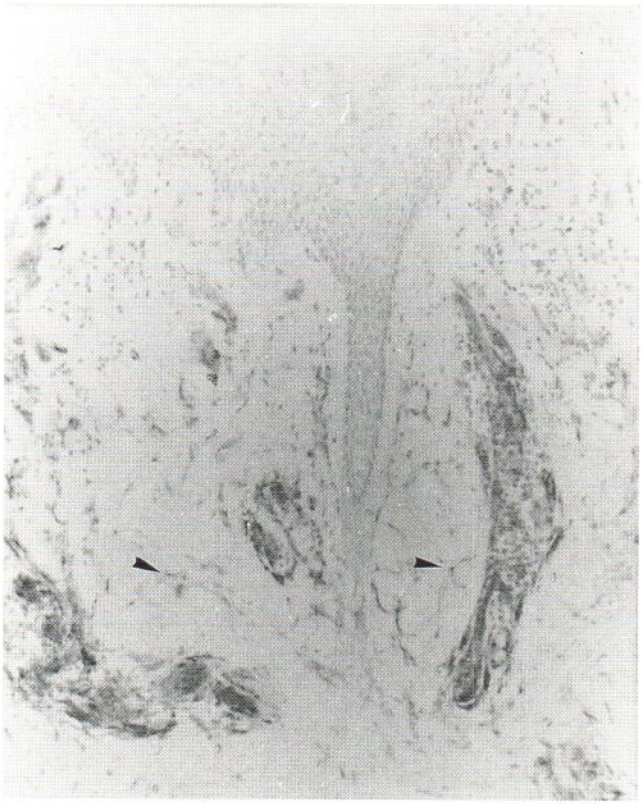

Fig. 2. VCAM-1 expression on vascular endothelium of upper reticular dermis, some perivascular infiltrating cells and on isolated stellate-shaped cells (*arrows*) in the dermal interstitium.

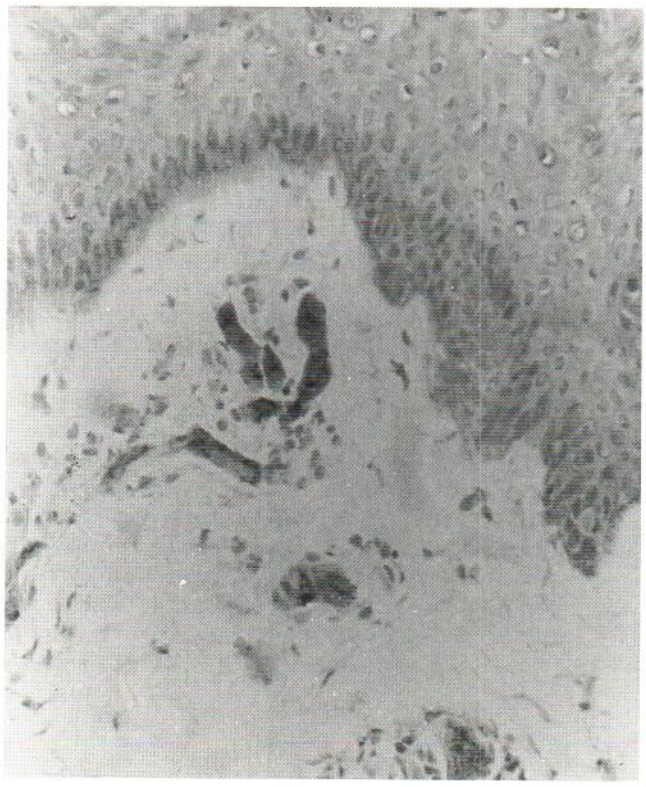

Fig. 3. E-selectin reactivity on endothelial cells in the papillary dermis and the upper reticular dermis in DM lesional skin.

nuclear infiltrates. VCAM-1, E-selectin or VLA-4 expression on keratinocytes was not detected.

In normal skin, no ICAM-1 expression was found on keratinocytes. Staining of the keratinocytes with *Ulex europeus* was seen in the granular layer of control specimens and in the entire epidermis in sections of DM lesional skin, as has been reported previously (16).

DISCUSSION

The current study shows that the activation-inducible adhesion receptors E-selectin, VCAM-1 and ICAM-1 are all expressed in DM lesional skin, although to a varying degree. ICAM-1 expression was present on dermal EC, inflammatory cells and focally grouped keratinocytes. VCAM-1 staining was predominant on EC of the upper reticular dermis and a minority of inflammatory cells. E-selectin positivity was less extensive and maximally expressed on segments of vessels of the papillary and upper reticular dermis.

Dermal infiltrates in DM are mainly composed by HLA-DR-expressing macrophages and T lymphocytes, especially of the CD4 subset (8). The observation of E-selectin expression in DM lesional skin confirms that this inducible adhesion molecule is not restricted to neutrophilic dermatoses (17, 18). E-selectin expression in normal skin is minimal, but a variety of inflammatory skin disorders, including contact dermatitis, atopic dermatitis, psoriasis and lichen planus (17), demonstrate strong EC expression of this molecule. In vivo experiments have shown that neutrophil adhesion may be predominantly associated with de novo expression, while memory T cells have been found to adhere later to E-selectin during sustained expression (18, 19). Our observation of E-selectin positivity only on segments of the microvasculature agrees with a ultrastructural study of the same phenomenon in other T cell-dominated skin diseases. Structural features of these E-selectin-positive vessel segments appeared similar to lymph node high endothelial venules (20). We found sporadic E-selectin-positive endothelium without inflammatory infiltrates in the surrounding tissue. This may indicate that E-selectin expression on EC is up-regulated before inflammatory cells leave the circulation. The final arrest of leukocyte travel enabling transendothelial diapedesis is the result of integrin-immunoglobulin superfamily interactions (17).

Unlike E-selectin, ICAM-1 is expressed by a wide variety of cells. ICAM-1 is constitutively expressed on EC and is also found on activated keratinocytes, lymphocytes and macrophages (9–12). Compared with healthy skin, in lesional DM skin we found an up-regulation of ICAM-1 on EC. ICAM-1 expression was also detected on the majority of inflammatory cells, although to a varying degree. The varying expression of ICAM-1 may indicate the influence of locally produced proinflammatory cytokines. Comparing HLA-DR with ICAM-1 immunoreactivity on parallel sections, we found that ICAM-1 staining was less extensive. We also observed a focal expression of ICAM-1 on keratinocytes, frequently overlying areas of focal subepidermal inflammatory infiltrates. Among the factors that control induction of ICAM-1 expression in human keratinocytes are TNF-α and IL-1 (21, 22). It is also evident that ultraviolet radiation modulates ICAM-1 expression of keratinocytes (23). In DM, ultraviolet radiation or cytokines released by inflammatory cells might enhance ICAM-1 expression in the epidermis.

The VLA/VCAM-1 pathway is considered to be important for transendothelial migration of immunocompetent cells into tissue (13, 14). A consistent finding of the present study was endothelial VCAM-1 expression predominantly in the upper recticular dermis in contact with perivascular inflammatory infiltrates. Interestingly, the VCAM-1 positivity of EC in the papillary dermis seemed to be less intense. In inflamed dermis, VCAM-1 was expressed by some mononuclear cells with the morphologic appearance of dendritic cells, surrounding perivascular infiltrates as well as scattered throughout the dermis. Similarly, other authors found that VCAM-1 reacted strongly with dendritic cells in lymphoid tissue of tonsil, peripheral lymph nodes and spleen (13, 14). It has been postulated that VCAM-1 on dendritic cells and macrophages supports lymphocyte adhesion and thus may play a role in antigen presentation and lymphocyte activation (13). When VCAM-1 staining of inflammatory cells was compared with HLA-DR and ICAM-1 staining in parallel sections, reactivity for VCAM-1 was less extensive. VLA-4, an integrin found on lymphocytes, mononuclear cells and eosinophils (10), has been shown to interact with VCAM-1 in mediating lymphocytic adhesion (14). In fact, we found a strong or moderate expression of VLA-4 in the majority of inflammatory cells.

Our findings support the idea that EC activation facilitates the local accumulation of inflammatory cells in DM skin lesions. The pattern of expression of adhesion molecules we found is similar to that reported in different cutaneous inflammatory disorders (ultraviolet B-induced erythema, psoriasis, scleroderma, systemic lupus erythematosus, etc.) (23–27). In all these conditions adhesion and homing receptors seem to play an essential role during lymphocyte recirculation. However, the clinical and histologic appearance of the skin lesion that provides disease specificity must clearly depend upon additional actually unknown factors (25).

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