Comparison of cell adhesion molecule expression in cutaneous leucocytoclastic and lymphocytic vasculitis

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Abstract

Aims—To compare the expression of the cell adhesion molecules intercellular adhesion molecule-1 (ICAM-1), ELAM-1 (E-selectin), and vascular cell adhesion molecule-1 (VCAM-1) in cutaneous leucocytoclastic and lymphocytic vasculitis.

Methods—Immunohistochemical analysis was performed on early lesional skin biopsy specimens of leucocytoclastic vasculitis (n = 14), lymphocytic vasculitis (n = 10), non-lesional skin (n = 12), and normal skin (n = 5). A standard immunoperoxidase technique was used to detect expression of ICAM-1, E-selectin, VCAM-1, and the cell markers CD11a, CD11b, CD11c, von Willebrand factor, CD3, CD68, and neutrophil elastase (NP57).

Results—Basal keratinocyte intercellular adhesion molecule-1 was expressed in eight (80%) cases of lymphocytic and in only one (7%) case of leucocytoclastic vasculitis, and not in non-lesional skin or control biopsy specimens from normal subjects. E-selectin was expressed on vascular endothelium in eight (57%) cases of leucocytoclastic and in seven (70%) cases of lymphocytic vasculitis. Endothelial vascular cell adhesion molecule-1 expression was seen in three (21%) biopsy specimens of leucocytoclastic and five (50%) of lymphocytic vasculitis.

There were increased numbers of cells in the dermal infiltrate stained for NP57, CD11b, and CD11c in leucocytoclastic compared with lymphocytic vasculitis ($p < 0.001$, $p = 0.013$, $p = 0.009$, respectively); immunoreactive positive cells for CD3 and CD11a were increased in lymphocytic compared with leucocytoclastic vasculitis ($p < 0.001$, $p = 0.011$, respectively).

Conclusions—These observations indicate that upregulation of adhesion molecule expression occurs in both leucocytoclastic and lymphocytic vasculitis. The different patterns of adhesion molecule expression in the two groups of vasculitis may reflect differences in the local release of cytokines. In particular, detection of intercellular adhesion molecule-1 expression by keratinocytes in lymphocytic vasculitis is consistent with an active role for mediators derived from T lymphocytes in the pathogenesis of the lesion.

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Cutaneous vasculitis embraces a group of conditions with characteristic histological features involving small and medium sized blood vessels. Leucocytoclastic vasculitis, which may be associated with several clinical disorders, is characterised by a predominance of perivascular polymorphonuclear cells, nuclear dust, red cell extravasation, and deposition of fibrinoid material around cutaneous blood vessels. In contrast, lymphocytic vasculitis comprises a heterogeneous group of disorders characterised clinically by purpura and histologically by red cell extravasation in association with a perivascular mononuclear cell infiltrate. Controversy still exists about the pathogenesis of cutaneous vasculitis and it is not readily apparent why some patients exhibit a neutrophilic and others a lymphocytic infiltrate.

Several early studies of leucocytoclastic vasculitis have clearly shown the deposition of immunoglobulin and complement within vessel walls.^{1,2} In contrast, lesional deposits of immunoglobulin and complement are rarely found in patients with lymphocytic vasculitis³ and cell mediated immunological mechanisms have been implicated.⁴⁻⁸ Massa and Su⁹ felt that lymphocytic vasculitis is not a specific clinicopathological entity but a combination of histological features seen in a variety of clinical settings which include capillaritis, pityriasis lichenoides, toxic erythema, and drug induced purpura. It has also been suggested that the finding of perivascular lymphocytes in some cases of vasculitis is due to the biopsy specimen being taken at a relatively late stage in the development of a lesion.^{2,10}

It is well recognised that cell adhesion molecules are important in the regulation of normal inflammatory responses.¹¹ ICAM-1 (intercellular adhesion molecule-1) belongs to the immunoglobulin supergene family and is expressed on several different types of cells, including endothelial cells, activated lymphocytes, macrophages and activated keratinocytes.¹² It is constitutively expressed on endothelial cells but it may be upregulated by interleukin-1, tumour necrosis factor, or interferon γ .^{13,14} ICAM-1 binds the leucocyte β_2 integrins lymphocyte function associated antigen-1 (CD11a/CD18) and CD11b/CD18 which mediate leucocyte-endothelial cell adhesion and the transendothelial

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migration of leucocytes into the tissues.¹¹⁻¹⁵ VCAM-1 (vascular cell adhesion molecule-1) also belongs to the immunoglobulin supergene family. Expression of VCAM-1 on large vessel endothelial cells is induced by interleukin-1, tumour necrosis factor, or interleukin-4,¹⁶⁻¹⁸ whereas induction on microvascular endothelial cells is restricted to tumour necrosis factor in vitro.¹⁹ Expression of VCAM-1 in the skin is not restricted to endothelial cells but is also seen on some perivascular and dermal dendritic cells.²⁰ VCAM-1 binds to VLA-4 (CD49d/CD29) found on lymphocytes, monocytes, eosinophils, and Langerhans' cells.²¹ E-selectin (endothelial leucocyte adhesion molecule-1) was first identified as an adhesion molecule for neutrophils.²² It is now recognised as also binding to eosinophils, basophils, monocytes, and a subpopulation of memory T cells (CD4+, CD45RO).²³⁻²⁴ The neutrophil ligand for E-selectin has been identified as a carbohydrate, sialyl-Lewis X,²⁵ whereas the cutaneous lymphocyte associated antigen (CLA), recognised by the monoclonal antibody HECA-452, is the ligand present on memory T cells.²⁶ E-selectin is minimally expressed on unstimulated endothelial cells but is induced by interleukin-1 or tumour necrosis factor, but not directly by interferon γ or interleukin-4.¹⁸⁻²⁷

There is evidence that the patterns of cell adhesion molecule expression may reflect the nature of the leucocyte infiltrate present in different forms of cutaneous inflammation.²⁰ To throw further light on the pathogenesis of vasculitis, we therefore investigated whether leucocytoclastic and lymphocytic vasculitis are associated with differential expression of these cell adhesion molecules.

Methods

Hospital ethical committee approval was obtained to perform lesional and, where possible, site matched non-lesional elliptical biopsies in patients with cutaneous vasculitis. Site matched skin biopsy specimens were also taken from five age and sex matched healthy volunteers. The biopsies were performed under 2% lignocaine anaesthetic injected into the periphery of the area to be excised.

The skin specimens were bisected and one half was fixed in formalin and paraffin wax embedded for histological examination. The other half was immediately fixed for immunohistochemistry by immersion in Zamboni's fluid for six hours at room temperature, then transferred into a 0.1M phosphate buffered saline (PBS, pH = 7.2) containing 15% sucrose and 0.1% sodium azide. Frozen sections (10 μ m) were cut, collected onto poly-L-lysine coated glass slides, and left to dry at room temperature for one hour before being immunostained.

Twenty four patients with active cutaneous vasculitis were recruited from the dermatology outpatient department. The patients with vasculitis were subdivided, according to established histological criteria, into leucocy-

Table 1 Clinicopathological diagnoses

Leucocytoclastic vasculitis (n = 14)	Lymphocytic vasculitis (n = 10)
Urticarial vasculitis (n = 7)	Capillaritis (n = 6)
Cryoglobulinaemia (n = 2)	Pityriasis lichenoides chronica (n = 1)
Henoch Schönlein purpura (n = 2)	Idiopathic (n = 3)
Drugs (n = 1)	
Idiopathic (n = 2)	

toclastic or lymphocytic vasculitis (table 1).²⁸

The antibodies used for staining are listed in table 2. Sections were stained using the three-step peroxidase-antiperoxidase (PAP) immunohistochemical method.²⁹ Endogenous peroxidases were inhibited by soaking the sections in 0.3% hydrogen peroxide in methanol for 15 minutes. Diaminobenzidine was used as a substrate to visualise von Willebrand factor, ICAM-1, E-selectin, and VCAM-1; the sections were counterstained with Mayer's haematoxylin. To visualise leucocyte markers and integrin expression, the peroxidase was developed according to the glucose oxidase/nickel enhancement reaction.³⁰ Negative controls were stained by omission of the primary antibody.

Two sections from each biopsy specimen were stained and analysed for expression of ICAM-1, E-selectin, VCAM-1, and von Willebrand factor. The numbers of immunoreactive cells within the dermal infiltrate were manually counted in six random fields ($\times 40$ magnification, within a 1 mm² grid) from two sections per biopsy specimen stained for CD11a, CD11b, CD11c, CD3, NP57, and CD68. The results were then averaged for each group.

The expressions of antigens (excluding NP57 staining) were analysed separately using analysis of variance. The subjects were treated as a random factor, the condition (control, lymphocytic, and leucocytoclastic vasculitis) as a between-subjects factor and the lesion state (lesional or non-lesional) as a within-subjects factor. The residuals were checked for normal distribution using the Shapiro-Francia W test and the data were log transformed when necessary to produce normality. When log data were used, the difference between groups was presented as a ratio of the geometric means. It was necessary to compare NP57 staining using the Mann-Whitney U

Table 2 Primary antibodies used for immunohistochemistry

Antibody	Antigen	Reference
6-5B5	ICAM-1	36
1-4C3	VCAM-1	36
1-2B6	E-selectin (ELAM-1)	36
MHM24	CD11a	37*
44	CD11b	38†
3-9	CD11c	39†
A542	CD3 (pan T cell)	40*
NP57	Neutrophil elastase	41*
EBM11	CD68 (mononuclear phagocytes)	42*
A082	Von Willebrand factor	43*

* Purchased from Dakopatts.

† A kind gift from Dr Nancy Hogg, Imperial Cancer Research Fund, London.

Table 3 Numbers of cells stained positively for leucocyte integrins and cell markers in lesional and control biopsy specimens

Antigen	Mean number of immunoreactive positive cells with 95% confidence intervals*			p Value between I and II
	(I) Leucocytoclastic vasculitis (n = 4)	(II) Lymphocytic vasculitis (n = 10)	(III) Control† (n = 17)	
CD11a	114 (85–152)	213 (147–308)	11 (7–16)	0.01
CD11b	21 (11–39)	6 (3–13)	1 (0–3)	0.013
CD11c	112 (76–166)	47 (28–79)	12 (6–21)	0.009
CD3	44 (30–65)	242 (148–396)	18 (10–33)	< 0.001
CD68	113 (83–153)	106 (72–154)	27 (18–42)	0.79
NP57	245 (165–311)	6 (1–12)	0	< 0.001

* Confidence intervals and p values were derived from analysis of variance on log transformed data apart from NP57 which was compared using the Mann-Whitney U test and is therefore expressed as median values.
† Included specimens from normal individuals and non-lesional skin from patients with vasculitis.

test due to the absence of staining in a large proportion of biopsy specimens. The BMDP package (program 3V with REML option) was used to analyse the data.

Results

There was no difference in staining patterns for each antibody between skin from normal subjects and non-lesional skin specimens from patients with vasculitis. These biopsy specimens are referred to collectively below as control biopsy specimens.

A significant increase in the numbers of

Table 4 Numbers of biopsy specimens stained positively for ICAM-1, E-selectin, and VCAM-1 in lesional skin of leucocytoclastic and lymphocytic vasculitis

	Leucocytoclastic vasculitis	Lymphocytic vasculitis
Keratinocyte ICAM-1	1 (7.0%)	8 (80%)
Endothelial ICAM-1	14 (100%)	10 (100%)
Endothelial E-selectin	8 (57%)	7 (70%)
Endothelial VCAM-1	3 (21%)	5 (50%)

CD3+, NP57+, CD68+ and β_2 leucocyte integrin positive cells was seen in all lesional biopsy specimens compared with those from controls. Table 3 shows that there was an increase in NP57+, CD11b+, and CD11c+ cells in leucocytoclastic compared with lymphocytic vasculitis ($p < 0.001$, $p = 0.013$, $p = 0.009$, respectively), whereas CD11a+ and CD3+ cells were increased in lymphocytic vasculitis ($p = 0.01$, $p < 0.001$, respectively). There was no significant difference in the numbers of CD68+ cells between the two vasculitic groups ($p = 0.79$).

ICAM-1 was expressed constitutively on endothelial cells in control biopsy specimens, precluding meaningful comparisons between lymphocytic and leucocytoclastic vasculitis. Keratinocyte ICAM-1 expression was absent in control biopsy specimens, and the proportion of specimens with detectable keratinocyte staining for ICAM-1 was significantly increased in lymphocytic vasculitis (eight of 10, 80%) compared with leucocytoclastic vasculitis (one of 14, 7%), $p = 0.0013$ (table 4). This ICAM-1 staining was almost exclusively confined to basal keratinocytes (fig 1A) and was seen close to the sites of CD11a (lymphocyte function associated antigen-1) positive cells, either in the upper dermis or within the epidermis (fig 1B). Greater numbers of ICAM-1 positive dermal dendritic cells were seen in lymphocytic vasculitis, although the number of positive biopsy specimens involved were too small for statistical comparisons.

E-selectin expression (figs 2A and B) was not present in control skin specimens but was seen in postcapillary venules in both leucocytoclastic (eight cases, 57%) and lymphocytic vasculitis (seven cases, 70%) (table 4).

VCAM-1 expression, which was absent in control biopsy specimens, was more frequently observed on endothelial cells in

Figure 1 Lymphocytic vasculitis. (A) Strong ICAM-1 expression confined to basal keratinocytes (immunoperoxidase); (B) same biopsy specimen as in fig 1A showing CD11a (lymphocyte function associated antigen-1) positive cells in the upper dermis and epidermis (immunoperoxidase).

Figure 2 E-selectin staining confined to vascular endothelial cells. (A) Leucocytoclastic vasculitis (immunoperoxidase); (B) lymphocytic vasculitis (immunoperoxidase).

lymphocytic (five cases, 50%) than leucocytoclastic vasculitis (three cases, 21%), although this was not clinically important (table 4). As with ICAM-1, VCAM-1 immunoreactive dermal dendritic cells were more pronounced in lymphocytic compared with leucocytoclastic vasculitis, although the number of positive biopsy specimens were too small for statistical analysis.

Reduced or absent staining of all three endothelial adhesion molecules was seen within small areas of many lesional biopsy specimens. This was associated with a diffuse, weak perivascular staining by von Willebrand factor, suggesting antigen loss from the endothelial cytoplasm. These observations corresponded to sites of pronounced fibrinoid necrosis of blood vessels shown by haematoxylin and eosin staining.

Discussion

The pathophysiological mechanisms which underlie the development of cutaneous vasculitic lesions are still poorly understood. Some of the events may be common to inflammatory responses in general, but others are likely to determine the specific characteristics of a lesion. In this study we investigated the presence of three cell cytokine mediated adhesion molecules in leucocytoclastic and lymphocytic vasculitis. We found that lymphocytic vasculitis is associated, in most cases, with a pattern of adhesion molecule expression that distinguishes the pathology of this lesion from that of leucocytoclastic vasculitis.

In contrast to lesional skin, we were unable to detect E-selectin or VCAM-1 expression on blood vessels in biopsy specimens taken from non-lesional skin, indicating that endothelial activation occurs in both forms of

vasculitis. Judging from in vitro experiments, the likely cytokines involved are either interleukin-1 or tumour necrosis factor,²⁷ which could be released from a number of cells in the vicinity of the blood vessel, including macrophages, lymphocytes, keratinocytes, smooth muscle cells, and endothelial cells themselves.³¹

E-selectin was detected on endothelium of lesional skin in eight of 14 cases of leucocytoclastic vasculitis and in seven of 10 cases of lymphocytic vasculitis. The absence of E-selectin staining in six of the leucocytoclastic biopsy specimens may in part be related to endothelial injury as four showed pronounced fibrinoid necrosis. The varying ages of the vasculitic lesions are also likely to be important, although whenever possible early (less than 24 hours' duration) lesions were biopsied. E-selectin is dependent on gene transcription and de novo protein synthesis and it is possible that the biopsy specimens might have been taken at a time point preceding E-selectin expression. More likely, however, E-selectin expression may have been missed as it is seen maximally two to four hours after cytokine stimulation.³²

Endothelial VCAM-1 expression was seen in three of 14 cases of leucocytoclastic vasculitis and in five of 10 cases of lymphocytic vasculitis. Although there was no difference between leucocytoclastic and lymphocytic vasculitis in the expression of VCAM-1 by endothelium, endothelial VCAM-1 expression tended to be associated with greater numbers of T cells in the tissues, implicating VCAM-1 in lymphocyte recruitment.

Although both leucocytoclastic and lymphocytic vasculitis were characterised by endothelial expression of E-selectin, the two forms of lesion could largely be distinguished

by the presence or absence of ICAM-1 expression by keratinocytes. Previous studies have shown that expression of ICAM-1 by keratinocytes is a feature of T lymphocyte mediated responses^{20 33 34} and is not an inevitable feature of cutaneous inflammation.²⁰ Our observations are therefore consistent with the lymphocytes emigrating into the tissues in lymphocytic vasculitis and playing an active part in the evolution of the lesions, perhaps by responding to antigen. Further studies examining cell surface markers of T cell activation and the presence of T cell derived cytokines, such as interferon γ , which are known to induce ICAM-1 expression by keratinocytes,^{14 35} should throw further light on this matter.

Leucocytoclastic vasculitis, by definition, exhibits a perivascular neutrophil infiltrate and it was therefore anticipated that neutrophil elastase (NP57), CD11b, and CD11c would be found in the dermal infiltrate. Likewise, the inflammatory cells in lymphocytic vasculitis stained positively for CD3 and CD11a. These results reflect cell lineage and no inference can be drawn from this study regarding their role in cutaneous vasculitis.

In conclusion, this study has shown that endothelial cell activation occurs in both leucocytoclastic and lymphocytic vasculitis, as demonstrated by the presence of E-selectin expression. Keratinocyte expression of ICAM-1 was also more common in lymphocytic vasculitis, suggesting an active role for T cells in the pathogenesis of this form of vasculitic lesion.

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