Analysis of adhesion molecules in the immunopathogenesis of giant cell arteritis

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Abstract

To explore the role of adhesion molecules in mediating mononuclear cell localisation, development of the granulomatous reaction, and cell mediated damage to the arterial wall in giant cell arteritis, 17 temporal artery biopsy specimens were examined. Eleven showed the histological features of giant cell arteritis and six showed no evidence of arteritis. All were examined for the expression of LFA-3, ICAM-1 and its receptor LFA-1, and HLA-DR. Temporal arteries with early features of arteritis, as well as histologically unaffected skip areas, showed a regional induction of ICAM-1 expression, but not HLA-DR, on smooth muscle cells of the media. ICAM-1 expression was detected in areas where a clinically important mononuclear cell infiltrate had not yet developed. In more florid cases of giant cell arteritis there was an additional widespread induction of ICAM-1 expression on intimal myofibroblasts. Strong expression of ICAM-1, HLA-DR, and LFA-3 was found on macrophages, epithelioid cells, and giant cells comprising the granulomatous lesion.

The pattern of expression of these adhesion molecules suggests that they have a role in leucocyte traffic into the vascular lesion as well as in mediating the intercellular interactions which constitute the granulomatous response.

Giant cell arteritis (temporal arteritis) is a vasculitis of unknown aetiology affecting medium and small sized arteries. Immunophenotypic analysis of the mononuclear cell infiltrate in giant cell arteritis indicates that cell-mediated immune mechanisms may be involved in the pathogenesis of this disease. Lymphocytes found at the site of arterial damage include activated T cells which express the class II major histocompatibility complex (MHC) antigen HLA-DR and the interleukin-2 receptor.¹⁻⁴ The inability to detect these activation markers on peripheral blood T lymphocytes of these patients led investigators to suggest that T cells were activated within the vascular lesions.⁴ Furthermore, expression of HLA-DR by macrophages, epithelioid cells, and multinucleated giant cells, which comprise the granulomatous inflammatory cell infiltrate, suggested that these cells function as antigen

presenting cells and have a role in activating neighbouring T cells.¹⁻⁵ Although no antigen (either foreign or self) has been defined, these cellular interactions are thought to underlie the chronic inflammatory process in this disease.⁵

Conjugate formation between T cells and antigen presenting cells is a prerequisite for antigen specific T cell activation. The MHC class II molecule presents processed exogenous antigen for recognition by the T cell receptor on CD4 positive T cells⁶ and also participates as an adhesion molecule through its interaction with the CD4 molecule.⁷ Several other distinct receptor and ligand interactions contribute towards T cell activation by antigen presenting cells.⁸ Of these, two well characterised accessory adhesion pathways are the LFA-1/ICAM-1 and CD2/LFA-3 interactions.⁹ These adhesion molecules have an important role in mediating the intercellular interactions underlying leucocyte localisation, activation, and effector functions.⁹⁻¹² LFA-1, an $\alpha_1 \beta_2$ heterodimer belonging to the integrin family of molecules,¹³ is expressed on virtually all leucocytes,¹⁰ CD2 has a distribution restricted to T cells.¹⁴ The ligands for both these adhesion receptors are ICAM-1/ICAM-2 and LFA-3, respectively.¹⁵⁻¹⁷ In contrast to the widespread and constitutive expression of LFA-3, an invariable feature of ICAM-1 is its induction on most tissues in response to a range of cytokines.^{12,18} Cytokines are locally released at sites of acute and chronic inflammation and here ICAM-1 may have a role in facilitating interactions among leucocytes and between leucocytes and non-haemopoietic cells. As cytokines play a crucial part in the development of the granulomatous inflammatory process¹⁹ we analysed the distribution of adhesion molecules within the arterial wall and on the granulomatous inflammatory cell infiltrate of giant cell arteritis.

Methods

Seventeen temporal artery biopsy specimens taken for diagnostic purposes at St Vincent's Hospital and at The Royal Victorian Eye and Ear Hospital were used for this study. Each specimen was received fresh and divided into segments of 3 mm in length. Portions from each specimen were formalin fixed, embedded in paraffin wax, and used for routine histological examination. The remaining portions from each biopsy specimen were placed in OCT Compound (Miles Laboratories, Naperville,

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Table 1 Clinical features of patients with histologically confirmed giant cell arteritis

Case No	Age	Sex	Clinical features	Duration of symptoms	Corticosteroid treatment (days)
1	73	F	Headaches; loss of vision in right eye ESR = 104	5 weeks	1
2	86	F	Headaches; jaw claudication; tender, palpable left temporal artery ESR = 65	2 months	1
3	68	F	Polymyalgia rheumatica, non-tender, palpable left temporal artery ESR = 60	months	0
4	75	M	Headache; malaise; visual loss in the right eye ESR = 23	2 weeks	1
5	78	F	Tender temporal arteries; loss of vision ESR = 110	2 weeks	0
6	66	F	Polymyalgia rheumatica; visual loss in right eye ESR = 49	13 months 3 weeks	1
7	71	F	Visual loss in right eye; bilateral palpable temporal arteries ESR = 14	24 months 1 month	2 years
8	76	M	Headaches; non-tender palpable temporal artery ESR = 80	3 weeks	4
9	73	M	Headaches; jaw claudication; visual loss in right eye ESR = 60	1 month	0
10	74	F	Polymyalgia rheumatica; left left central retinal artery occlusion ESR = 121	11 months 3 days	1
11	73	F	Headaches; blurred vision. ESR = 60	2 weeks	1

Illinois), snap-frozen in liquid nitrogen, and sectioned for immunohistochemistry. Clinical features of all patients with histologically confirmed giant cell arteritis are summarised in table 1.

Cryostat sections (4 µm) were dried overnight at room temperature and fixed in acetone. Cryostat sections were blocked with 0.5% bovine serum albumin, incubated for 30 minutes with primary monoclonal antibodies (table 2), followed by a three-step immunoperoxidase procedure using Dako ABC reagent (Dakopatts, Haegerston, Sweden). Peroxidase activity was detected by a three minute incubation with 3, 3' diaminobenzidine tetrahydrochloride (0.5 mg/ml) (Sigma) containing 0.015% H₂O₂. Sections were counterstained with haematoxylin. All sections were stained at the same time using separate slides for each monoclonal antibody listed in table 2. Negative controls consisted of replacement of primary antibody with phosphate buffered saline or an isotype matched monoclonal antibody, WEHI-B2, which recognises the CD21 antigen on B cells.¹¹

Results

Temporal artery biopsy specimens from six patients lacking the histological features of giant cell arteritis but showing varying degrees of arteriosclerosis were used as controls in this

study. A moderate and uniform staining for LFA-3 was seen on all components of the arterial wall (fig 1). ICAM-1 expression was restricted to endothelial cells (fig 1). Staining for HLA-DR antigen was seen only on the occasional leucocyte present in the adventitia.

Immunohistochemical staining of 11 biopsy specimens with the histological features of giant cell arteritis showed expression of LFA-1 by all mononuclear cells present in the arterial lesions (fig 2). HLA-DR and ICAM-1 showed a similar pattern of immunoreactivity on mononuclear cells, with strong expression on all macrophages and on a proportion of morphologically identified lymphocytes, with a prevalence similar to that previously reported for HLA-DR.¹⁻⁴ HLA-DR and ICAM-1 were also coexpressed on endothelial cells. Staining of selected positive biopsy specimens with a monoclonal antibody against CD 11c (p150/95), an adhesion-associated molecule expressed by monocytic cells,²⁰ showed that mononuclear cells infiltrating between the smooth muscle cells of the media were predominantly macrophages. These cells stained strongly for HLA-DR and ICAM-1 and were also seen in close proximity to the internal elastic lamina (fig 2). In all cases of florid giant cell arteritis there was widespread and strong expression of ICAM-1 on the myofibroblasts of the intima (fig 2). Moderate to strong expression of ICAM-1 was shown by the smooth muscle cells of the media adjacent to infiltrating macrophages and granulomas (fig 2). Biopsy specimens with more focal changes of arteritis also showed a regional expression of ICAM-1 on smooth muscle cells adjacent to the granulomatous inflammatory cells. There was no increased expression of LFA-3 in these same areas of the media when serial sections were examined. HLA-DR showed a contrasting pattern of expression within the intima and media when compared to that for ICAM-1. No expression of HLA-DR was seen on arterial smooth muscle cells nor intimal fibroblasts, despite strong HLA-DR

Table 2 Monoclonal antibodies used in this study

Monoclonal antibody	Specificity	Dilution	Source/reference
W-CAM-1	ICAM-1 (CD54)	1:500	11
TS2/9-1-1	LFA-3 (CD58)	1:100	14
MHM24	LFA-1, alpha subunit (CD-11a)	1:50	Dakopatts
MHM23	LFA-1, beta subunit (CD18)	1:50	Dakopatts
KB90	p150/95 (CD-11c)	1:50	Dakopatts
3/F10	HLA-DR	1:300	A W Boyd
Wehi-B2	CD21	1:200	11

Figure 1 Distribution of ICAM-1 (A) and LFA-3 (B) expression in a temporal artery biopsy specimen showing mild atherosclerotic degeneration but no evidence of arteritis. In contrast to the widespread expression of LFA-3, ICAM-1 expression is restricted to the endothelium.

expression on inflammatory cells present in all components of the arterial wall (fig 2).

Sectioning through a histologically defined skip area showed regional expression of ICAM-1 on the smooth muscle cells of the media in the absence of a clinically important mononuclear cell infiltrate (fig 3). Expression of ICAM-1 within this region of the media extended over a number of serial sections. Smooth muscle cells showing reactivity for ICAM-1 showed no staining for HLA-DR nor LFA-1 when serial sections were examined.

Macrophages, epithelioid cells, and multinucleated giant cells comprising the granulomas showed strong expression of LFA-1 (fig 3). Both HLA-DR and ICAM-1 were also strongly expressed by multinucleated giant cells and epithelioid cells. HLA-DR, however, showed both diffuse cytoplasmic and membrane staining, ICAM-1 was intensely expressed at the surface membrane with only weak intracellular staining. Granulomas also showed increased expression of LFA-3 compared with the basal level seen on cells of the intima and media. LFA-3 showed a similar pattern of expression on granulomas compared with that seen with ICAM-1.

Discussion

The ICAM-1/LFA-1 interaction plays an important part in facilitating conjugate formation between cytotoxic T cells and target cells.^{11 21 22} Similarly, cell to cell contact between macrophages and target cells is necessary before the macrophage cytotoxic effector response can be activated.²³ LFA-1, expressed by monocytes, is important in mediating the adhesion of monocytes to target cells.²⁴ While the aetiology of giant cell arteritis remains

unclear, several workers have suggested that the disease results from an autoimmune reaction involving arterial elastic tissue.²⁵ Ultrastructural studies have shown that smooth muscle cells of the media act as targets for macrophages in the ensuing immune attack.^{5 26} Smooth muscle cells in the lesions of giant cell arteritis show ultrastructural features of cytolytic damage.^{5 26} It was suggested that antibody dependent, cell mediated cytotoxicity may be responsible for this injury to smooth muscle cells.⁵ Alternatively, smooth muscle cells may act as primary targets by expressing a foreign antigen recognised by cytotoxic T cells. In this study we have shown that ICAM-1 expression is induced on smooth muscle cells adjacent to granulomas and macrophages seen infiltrating within the media (fig 2). Induction of ICAM-1 on smooth muscle cells of the media may facilitate cytolytic damage to this component of the arterial wall by mediating adhesion of macrophages and cytotoxic T cells, both of which express LFA-1, to adjacent smooth muscle cells.

Lymphocytes, macrophages, epithelioid cells and multinucleated giant cells, which comprise the granulomatous response of giant cell arteritis, have previously been shown to express high concentrations of HLA-DR.²⁻⁴ These studies may suggest that a primary event in the immunopathogenesis of giant cell arteritis is antigen specific activation of T cells by macrophages or other functional antigen presenting cells located within the vascular lesion. The LFA-1/ICAM-1 and CD2/LFA-3 adhesion pathways are crucial in facilitating antigen specific interactions between lymphocytes and antigen presenting cells.^{9 27 28} When we examined epithelioid cells and multinucleated giant cells for the expression of

Figure 2 Serial sections through a temporal artery biopsy specimen showing giant cell arteritis and stained for ICAM-1 expression (A), HLA-DR (B), the α chain of LFA-1 (CD11a) (C), ICAM-1 shows a widespread expression which includes cells comprising the granulomatous infiltrate, fibroblasts of the intima, and smooth muscle cells of the media adjacent to granulomas and macrophages. HLA-DR stains granulomas and inflammatory cells but is not expressed on the intima and media. LFA-1 is expressed on all inflammatory cells.

adhesion molecules in giant cell arteritis, intense expression of ICAM-1 and LFA-3 was found at the surface of these cells. In contrast, HLA-DR showed both a diffuse cytoplasmic distribution as well as expression at the surface membrane. The strong expression of ICAM-1 at the surface of epithelioid cells and multinucleated giant cells, when compared with HLA-DR, indicates a preferential pattern of ICAM-1 distribution within these cells and suggests that cells comprising the granuloma of giant cell arteritis are in a functionally activated state. Expression of adhesion molecules by these cells would facilitate the cell conjugate interactions with T lymphocytes necessary for receptor mediated T cell proliferation.²⁹

In all the cases of giant cell arteritis examined we found a preferential induction of ICAM-1 with no evidence of HLA-DR expression on myofibroblasts of the intima and vascular smooth muscle cells. Evidence that ICAM-1 expression is modulated by cytokines in a different manner to that of HLA-DR has been shown for several tissues.^{30,31} Other workers have also been unable to show HLA-DR expression on smooth muscle cells in giant cell arteritis.⁴ These findings contrast with the observations that vascular smooth muscle cells express HLA class II antigens in atherosclerotic plaques³² and that cytokines such as IFN- γ induce HLA-DR expression on arterial smooth muscle cells in vitro.³³ Analysis of the types of cytokines produced in the vascular lesions may explain these observations as HLA-DR shows variable patterns of expression in response to different cytokines.³³

Locally elaborated cytokines are responsible for the formation and development of the granulomatous response.¹⁹ Recently adhesion molecules such as LFA-1, and to a lesser extent ICAM-1, have also been shown in vitro to mediate the formation of multinucleated giant cells.³⁴ These authors showed that fusion of monocytes in response to IFN- γ could be inhibited by monoclonal antibodies directed against the α or β chains of LFA-1, with some inhibitory effect also shown by a monoclonal antibody against ICAM-1. Cytokines such as IFN- γ or IL-1, in addition to contributing to the development of granulomas,¹⁹ are also probably responsible for inducing ICAM-1 expression on components of the arterial wall adjacent to the granulomatous infiltrate. Studies on a variety of different tissues have shown a close temporospatial relation between induction of ICAM-1 expression and the degree of leucocyte infiltration within those tissues.^{31,35-37} This implies an important role for the LFA-1/ICAM-1 interaction in leucocyte trafficking at sites of inflammation. The demonstration that within a skip area ICAM-1 was expressed on regions of the media suggests that expression of ICAM-1 on the smooth muscle cells of the media may precede heavy leucocyte infiltration into those regions of the media. As biopsy confined to "skip areas" and routine staining can result in false negative diagnosis³⁸ the detection of ICAM-1 expression within regions of the media may improve the sensitivity of detecting early arteritis. Our

Figure 3 Sectioning through a skip area from a case of giant cell arteritis shows expression of ICAM-1 by smooth muscle cells in regions of the media. There is minimal inflammatory cell infiltrate into these areas of the arterial wall.

finding of strongly induced ICAM-1, as well as LFA-1, on the surface of cells which comprise the granulomas of giant cell arteritis suggests that these adhesion molecules are important in the formation of multinucleated giant cells seen in this disease. Together with HLA-DR expression, these molecules can then enable multinucleated giant cells, as well as epithelioid cells and macrophages, to function as highly efficient antigen presenting cells.

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- Banks PM, Cohen MD, Ginsburg WW, Hunder GG. Immunohistologic and cytochemical studies of temporal arteritis. *Arthritis Rheum* 1983;26:1201-7.
- Andersson R, Jonsson R, Tarkowski A, Bengtsson B-A, Malmvall B. T cell subsets and expression of immunological activation markers in the arterial walls of patients with giant cell arteritis. *Ann Rheum Dis* 1987;46:915-23.
- Cid MC, Campo E, Ercilla G, et al. Immunohistochemical analysis of lymphoid and macrophage cell subsets and their immunologic activation markers in temporal arteritis. *Arthritis Rheum* 1989;32:884-93.
- Andersson R, Hansson GK, Söderström T, Jonsson R, Bengtsson B-A, Nordburg E. HLA-DR expression in the vascular lesion and circulating T lymphocytes of patients with giant cell arteritis. *Clin Exp Immunol* 1988;73:82-7.
- Shiiki H, Shimokama T, Watanabe T. Temporal arteritis: cell composition and the possible pathogenetic role of cell-mediated immunity. *Hum Pathol* 1989;20:1057-64.
- Moller G. Antigen requirements for activation of MHC restricted responses. *Immunol Rev* 1987;98:1-18.
- Doyle C, Strominger J. Interaction between CD4 and Class II MHC molecules mediates cell adhesion. *Nature* 1987;330:256-9.
- Shimizu Y, van Seventer GA, Horgan KJ, Shaw S. Roles of adhesion molecules in T-recognition: fundamental similarities between four integrins on resting human T cells (LFA-1, VLA-4, VLA-5, VLA-6) in expression, binding and costimulation. *Immunol Rev* 1990;114:109-43.
- Makgoba MW, Saunders ME, Shaw S. The CD2-LFA-3 and LFA-1-ICAM pathways: relevance to T cell recognition. *Immunol Today* 1989;10:417-22.
- Springer TA, Dustin ML, Kishimoto TK, Marlin SD. The lymphocyte function-associated LFA-1, CD2, and LFA-3 molecules: cell adhesion receptors of the immune system. *Ann Rev Immunol* 1987;5:223-52.
- Boyd AW, Wawryk SO, Burns GF, Fecondo JV. Inter-cellular adhesion molecule 1 (ICAM-1) has a central role in cell-cell contact-mediated immune mechanisms. *Proc Natl Acad Sci USA* 1988;85:3095-9.
- Wawryk SO, Novotny JR, Wicks IP, et al. The role of the LFA-1/ICAM-1 interaction in human leukocyte homing and adhesion. *Immunol Rev* 1989;108:135-61.
- Hynes RO. Integrins: a family of cell surface receptors. *Cell* 1987;48:549-54.
- Krensky AM, Sanchez-Madrid F, Robbins E, Nagy JA, Springer TA, Burakoff SJ. The functional significance, distribution, and structure of LFA-1, LFA-2, and LFA-3: cell surface antigens associated with CTL-target interactions. *J Immunol* 1983;131:611-6.
- Marlin SD, Springer TA. Purified intercellular adhesion molecule 1 (ICAM-1) is a ligand for lymphocyte function-associated antigen (LFA-1). *Cell* 1987;51:813-9.
- Staunton DE, Dustin ML, Springer TA. Functional cloning of ICAM-2, a cell adhesion ligand for LFA-1 homologous to ICAM-1. *Nature* 1989;339:61-3.
- Selvaraj P, Plunkett ML, Dustin M, Sanders ME, Shaw S, Springer TA. The lymphocyte glycoprotein CD2 binds the cell surface ligand LFA-3. *Nature* 1987;326:400-3.
- Dustin ML, Staunton DE, Springer TA. Supergene families meet in the immune system. *Immunol Today* 1988;9:213-5.
- Sheffield EA. The granulomatous inflammatory response. *J Pathol* 1990;160:1-2.
- Kiezer GD, Te Velde AA, Schwarting R, Figdor CG, De Vries JE. Role of p150, 95 in adhesion, migration, chemotaxis and phagocytosis of human monocytes. *Eur J Immunol* 1987;17:1317-22.
- Makgoba MW, Sanders ME, Luce GEG, Gugel EA, Dustin ML, Springer TA, Shaw S. Functional evidence that intercellular adhesion molecule-1 (ICAM-1) is a ligand for LFA-1-dependent adhesion in T cell-mediated cytotoxicity. *Eur J Immunol* 1988;18:637-40.
- Weetman AP, Freeman M, Borysiewicz LK, Makgoba MW. Functional analysis of intercellular adhesion molecule-1-expressing human thyroid cells. *Eur J Immunol* 1990;20:271-5.
- Higuchi M, Higashi N, Taki H, Osawa T. Cytolytic mechanisms of activated macrophages. *J Immunol* 1990;144:1425-31.
- Strassman G, Springer TA, Somers SD, Adams DO. Mechanisms of tumor cell capture by activated macrophages: evidence for involvement of lymphocyte function-associated (LFA)-1 antigen. *J Immunol* 1986;136:4328-33.
- Wilkinson IMS, Russell RWR. Arteries of the head and neck in giant cell arteritis: a pathological study to show the pattern of arterial involvement. *Arch Neurol* 1972;27:378-91.
- Reinecke RD, Kuwabara T. Temporal arteritis. I. Smooth muscle cell involvement. *Arch Ophthalmol* 1969;82:446-53.
- Dougherty GJ, Murdoch S, Hogg N. The function of human intercellular adhesion molecule-1 (ICAM-1) in the general of an immune response. *Eur J Immunol* 1988;18:35-9.
- Altman DM, Hogg N, Trowsdale J, Wilkinson D. Co-transfection of ICAM-1 and HLA-DR reconstitutes human antigen-presenting cell function in mouse L cells. *Nature* 1989;338:512-4.
- Kawakami K, Yamamoto Y, Kakimoto K, Onove K. Requirement for delivery of signals by physical interaction and soluble factors from accessory cells in the induction of receptor-mediated T cell proliferation. *J Immunol* 1989;142:1818-25.
- Griffiths CEM, Nickoloff BJ. Keratinocyte intercellular adhesion molecule-1 (ICAM-1) expression precedes dermal T lymphocytic infiltration in allergic contact dermatitis (Rhus dermatitis). *Am J Pathol* 1989;135:1045-55.
- Tabibzadeh SS, Poubouridis D. Expression of leukocyte adhesion molecules in human endometrium. *Am J Clin Pathol* 1990;93:183-9.
- Jonasson L, Holm J, Skalli O, Gabbiani G, Hansson GK. Expression of class II transplantation antigen on vascular smooth muscle cells in human atherosclerosis. *J Clin Invest* 1985;76:125-9.
- Stemme S, Fager G, Hansson GK. MHC class II expression in human vascular smooth muscle cells is induced by interferon-gamma and modulated by tumour necrosis factor and lymphotoxin. *Immunol* 1990;69:243-9.
- Möst J, Neumayer HP, Dierich MP. Cytokine-induced generation of multinucleated giant cells in vitro requires interferon-γ and expression of LFA-1. *Eur J Immunol* 1990;20:1661-7.
- Munro JM, Pober JS, Cotran RS. Tumour necrosis factor and interferon-γ induce distinct patterns of endothelial activation and associated leukocyte accumulation in skin of PAPIO ANUBIS. *Am J Pathol* 1989;135:121-33.
- Sobel R, Mitchell ME, Fondren G. Intercellular adhesion molecule-1 (ICAM-1) in cellular immune reactions in the human central nervous system. *Am J Pathol* 1990;136:1309-16.
- Volpes R, van Oord JJ, Desmet VJ. Immunohistochemical study of adhesion molecules in liver inflammation. *Hepatology* 1990;12:59-65.
- Klein R, Campbell RJ, Hunder GG, Carney JA. Skip lesions in temporal arteries. *Mayo Clin Proc* 1976;51:504-10.