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Expression of vascular addressins and ICAM-1 by endothelial cells in the spinal cord during chronic relapsing experimental allergic encephalomyelitis in the Biozzi AB/H mouse

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SUMMARY

The expression of adhesion molecules on central nervous system (CNS) endothelia was examined during chronic relapsing experimental allergic encephalomyelitis (CREAE) in the Biozzi AB/H mouse. Active disease episodes (acute and relapse) were associated with the up-regulation of MALA-2, the murine homologue of intercellular adhesion molecule-1 (ICAM-1), on CNS endothelia and the infiltration of ICAM-1-positive mononuclear cells. In addition, the high endothelial venule (HEV)-associated MECA-325 antigen was evident in perivascular lesions, particularly in relapsing disease. The peripheral lymph node HEV-associated vascular addressin defined by MECA-79 antibody was not detectable in the CNS during CREAE. However, the mucosal HEV addressin was evident in lesions, which ultrastructurally was found to be expressed on the surface of endothelial cells by immunoelectron microscopy. The expression of adhesion molecules, such as ICAM-1, may provide a means by which both the initial neuroantigen-specific and the subsequent antigen-non specific cells extravasate into the CNS. Such infiltration may induce the expression of the vascular addressins which may then provide a means of site-selective cellular recruitment leading to disease progression.

INTRODUCTION

Lymphocytes continuously recirculate from blood to lymph, extravasating principally via the high endothelial venules (HEV) found in lymphoid tissue.[1] There are at least three distinct specific lymphocyte: HEV recognition systems, one mediating traffic to peripheral lymph nodes (PLN), another directing traffic to the mucosal lymphoid tissue, and a third controlling traffic to the synovium of inflamed joints.[2,3] Monoclonal antibodies (mAb) have been produced which detect such tissue-specific lymphocyte adhesion molecules on HEV, termed vascular addressins.[4] Monoclonal antibodies MECA-367 and MECA-79 recognize functionally associated epitopes, the mucosal and peripheral lymph node vascular addressins, on HEV in mucosal and peripheral lymph nodes, respectively.[5,6] mAb MECA-325 is another marker of HEV in all lymphoid tissues and in sites of chronic inflammation, although its antigen has not been defined.[7] In addition to the HEV-specific antigens, both lymphocytes and endothelial cells express further molecules which are important, albeit in a non-organ-specific manner, leucocyte adhesion to endothelia. One such molecule is LFA-1 (CD11a), whose ligand is intercellular adhesion molecule-1 (ICAM-1; CD54). ICAM-1 is expressed by activated lymphocytes, monocytes and certain non-haematopoietic cells such as some vascular endothelial cells.[8,9] Recently, mAb YN-1/1.7, against the murine lymphocyte activation antigen MALA-2, has been shown to represent the mouse homologue of human ICAM-1.[9]

The blood–brain barrier (BBB) usually serves to limit lymphocyte migration into the central nervous system (CNS). However, in chronic relapsing experimental allergic encephalomyelitis (CREAE), an animal model of multiple sclerosis,[10] disease is characterized by BBB breakdown and the subsequent migration of mononuclear cells into the CNS. As the mechanism of lymphocyte extravasation during disease of the CNS is not clearly defined, this study investigates the vascular expression of ICAM-1 and of the vascular addressins during the course of CREAE in the Biozzi AB/H mouse.[11]

Abbreviations: BBB, blood–brain barrier; CNS, central nervous system; CREAE, chronic relapsing experimental allergic encephalomyelitis; DAB, diaminobenzidine; HEV, high endothelial venule; ICAM-1 intercellular adhesion molecule-1; PLN, peripheral lymph node; mAb, monoclonal antibody; MLN, mesenteric lymph node; PP, Peyer's patch; NMS, normal mouse serum; PBS, phosphate-buffered saline.

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MATERIALS AND METHODS

Induction of CREAE

Six- to eight-week Biozzi AB/H mice, derived from stock bred at the Royal College of Surgeons,[2] were sensitized by the subcutaneous injection of 1mg autologous spinal cord homogenate emulsified in Freund's incomplete adjuvant supplemented with 60 μg of mycobacteria (*Mycobacterium tuberculosis* H37Ra and *M. butyricum* [8:1]) on Day 0 and Day 7.[11] The acute phase of CREAE was characterized by weight loss and the onset of clinical signs 15–20 days post-inoculation.[11] Following this initial paralytic disease episode the animals then gained weight and the clinical signs abated to a partially limp tail. These remission animals were sampled on Day 27 post-inoculation. A relapse was indicated by a further period of weight loss and an increase in the severity of clinical signs, which occurred generally between Days 33 and 40 post-inoculation. Acute and relapse phase animals had complete hindlimb paralysis at the time of sampling, each group containing a minimum of five animals.

Immunocytochemistry

Five- to eight-micrometre cryostat sections were prepared from peripheral (inguinal) and mesenteric lymph nodes (MLN), Peyer's patch (PP) and spinal cord (cervical) from normal and CREAE animals, fixed in acetone and stored at −20° until used.[11] Cryostat sections were stained with the rat monoclonal antibodies YN-1/1.7 (ICAM-1),[9] MECA-325 (HEV),[5] MECA 79 (against PLN-addressins[7] or MECA-367 (mucosal addressins[6] as described previously.[11] Briefly, endogenous peroxidase blocking was followed by a 1-hr incubation with the primary rat mAb diluted in 5% normal mouse serum (NMS). This was detected by 30 min incubation with biotinylated rabbit anti-rat Ig, avidin:biotin/peroxidase complex and peroxidase-conjugated swine anti-rabbit Ig. The peroxidase was then developed with the chromagen diaminobenzidine (DAB) and the sections were counterstained with haematoxylin. Controls for the staining protocol consisted of omitting the primary antibody, or substituting it with an isotype-matched antibody. These were negative.

Immunoelectron microscopy

Immunoelectron microscopy was performed on spinal cord tissue as described recently.[12,13] Briefly the spinal cord was removed and immersion fixed for 30 min with 1% monomeric glutaraldehyde in Sørensen's buffer. One-hundred micrometre transverse sections were cut under fixative, and immersion fixed for a further 30 min. The unreacted aldehyde groups were quenched with 0·05 M glycine in phosphate-buffered saline (PBS). Following washing the sections were incubated overnight with biotinylated purified monoclonal antibody (YN-1/1.7 or MECA-367) or tissue culture supernatant (MECA-325) diluted in 5% NMS and 0·05% Tween 20 in PBS. Endogenous peroxidase was then blocked. MECA-325 was detected by incubating the sections with biotinylated rabbit anti-rat Ig for 2 hr. This was followed by a 1-hr incubation with avidin:biotin peroxidase complex. The peroxidase was developed with DAB and the section was then osmicated (1% OsO_4 in Sørensen's buffer) overnight. They were then washed, dehydrated and embedded in araldite for electron microscopy. Sections were not counterstained.

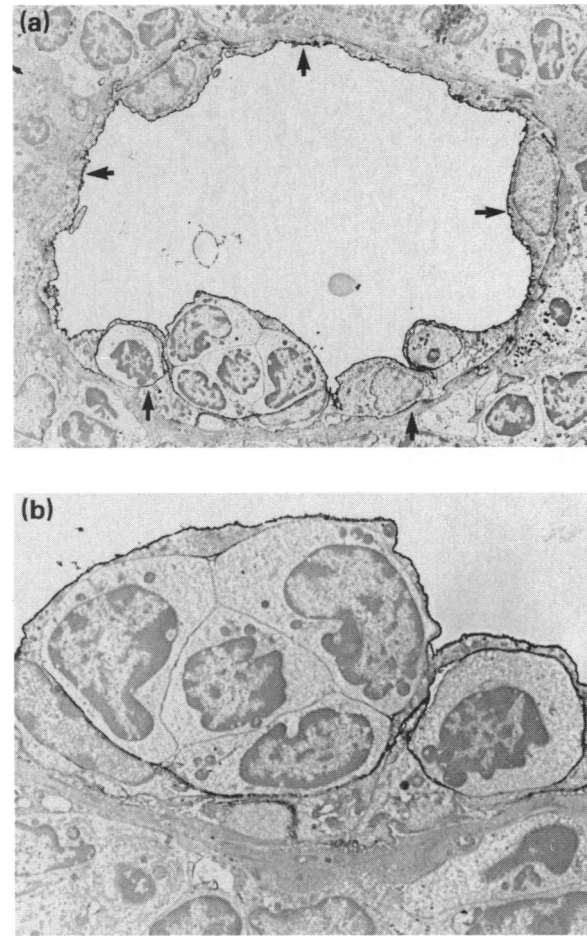

Figure 1. (a) Immunoelectron micrograph of antibody MECA-367 reactivity (→) with a mesenteric lymph node HEV (×2730) (b) High power view of the same section showing leucocytes extravasating across the MEC-367 labelled venule (×9030).

RESULTS

In lymph nodes ICAM-1 was expressed by interdigitating cells and on cells in B-cell follicles and on HEV.[9] Similarly, MECA-325 was expressed on PLN-HEV, MLN-HEV and PP-HEV.[5] As previously described, MECA-79 stains at high levels on HEV in both the peripheral and mesenteric nodes and on some Peyer's patch HEV.[7] MECA-367 did not react with PLN-HEV but readily detected the mucosal addressin on PP-HEV and mesenteric lymph node HEV on both the luminal and abluminal surfaces of the venule (Fig. 1). In the spinal cord of normal animals some vessels expressed low levels of ICAM-1 whilst the MECA-325 antigen and the vascular adressins were not detectable (Table 1).

During the acute phase ICAM-1 was expressed at high levels on endothelia in lesions and mononuclear cells in the infiltrate (Fig. 2). In addition many vessels in non-infiltrated areas of the spinal cord expressed ICAM-1 to a higher degree than observed in normal animals. As previously described the remission phase of the disease is characterized by both the abatement of clinical signs and also an almost complete reduction in cellular infiltration.[11] However, more ICAM-1-positive vessels were apparent during remission than in normal animals. In the relapse phase ICAM-1 was readily detectable on endothelia both in lesions

Table 1.

Disease phase	Antibody	Vascular antigen expression: Lesions	Vascular antigen expression: Non-infiltrated
Normal	ICAM-1	N/A	±
	MECA-325	N/A	−
	MECA-79	N/A	−
	MECA-367	N/A	−
Acute	ICAM-1	++	++
	MECA-325	±	−
	MECA-79	−	−
	MECA-367	±	−
Remission	ICAM-1	N/A	+
	MECA-325	N/A	−
	MECA-79	N/A	−
	MECA-367	N/A	−
Relapse	ICAM-1	++	++
	MECA-325	+	−
	MECA-79	−	−
	MECA-367	+	−

Due to the variation in the number, size and form of the lesions in individual animal sections, it was not feasible to quantitate the degree of antigen expression. However, this table gives a representative impression of changes that occur during the different phases of CREAE.

−, not detectable; ±, detectable on a few cells; +, detectable on some cells; ++, detectable on many cells.

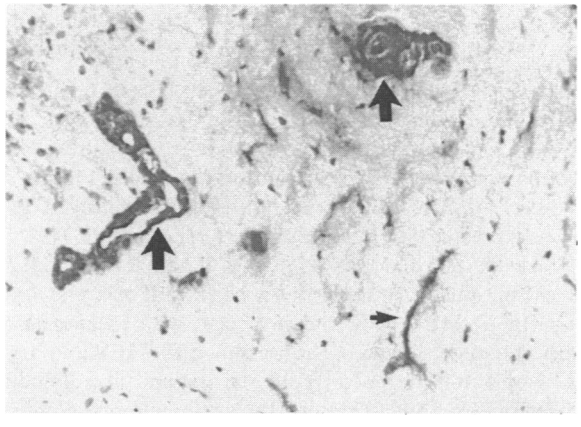

Figure 2. Indirect immunoperoxidase detected ICAM-1 expression in the spinal cord during acute phase CREAE. ICAM-1 is expressed by endothelia and mononuclear cells in lesions (→) and also by small vessels (→) which are not associated with significant cellular infiltration (× 140).

and in uninvolved areas. Ultrastructurally ICAM-1 was detectable on both the luminal and abluminal surfaces of the endothelium (Fig. 3). Macrophages/microglia in lesions and some lymphocytes also expressed high levels of ICAM-1 in the active disease phases.

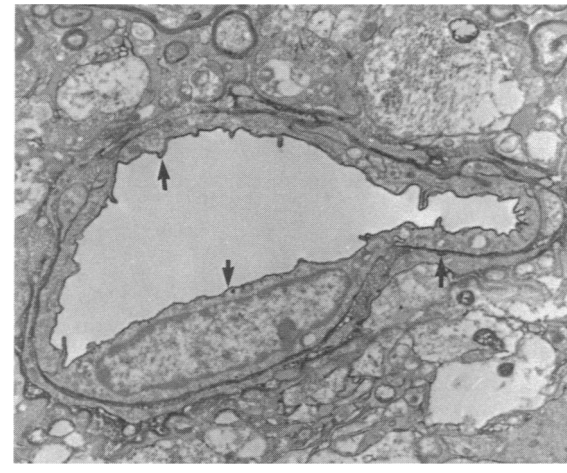

Figure 3. Immunoelectron micrograph of ICAM-1 expression on a vessel in the spinal cord during relapse phase CREAE, showing intense labelling of both the luminal and abluminal surfaces (→) of the endothelia (× 6615).

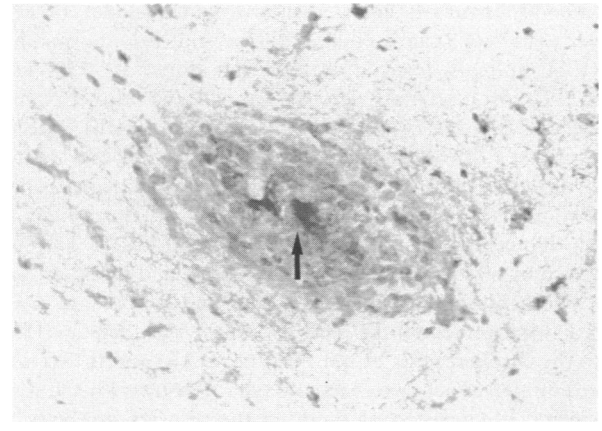

Figure 4. Indirect immunoperoxidase detection of MECA-325 antigen (→) within perivascular lesions in the spinal cord during relapse phase CREAE (counterstained with haematoxylin, × 280).

Although essentially no difference was observed in ICAM-1 expression between the acute and relapse phases, a different profile was observed for MECA-325 antigen expression during the disease (Table 1). The majority of the many lesions apparent in the acute phase of disease expressed either low or undetectable levels of MECA-325 antigen, although some vessels within perivascular lesions did express this antigen. Generally MECA-325 reactivity was not detectable in remission animals. In the relapse phase MECA-325 reactivity was more apparent within the centre of perivascular lesions (Fig. 4), but although more readily detectable than in the acute phase not all vessels in lesions were positive.

In an attempt to further characterize the nature of the high endothelial differentiation implied by reactivity with MECA-325, antibodies to the vascular addressins were used. Surprisingly the peripheral lymph node addressin specific antibody MECA-79 was not detectable in any lesion in the acute or

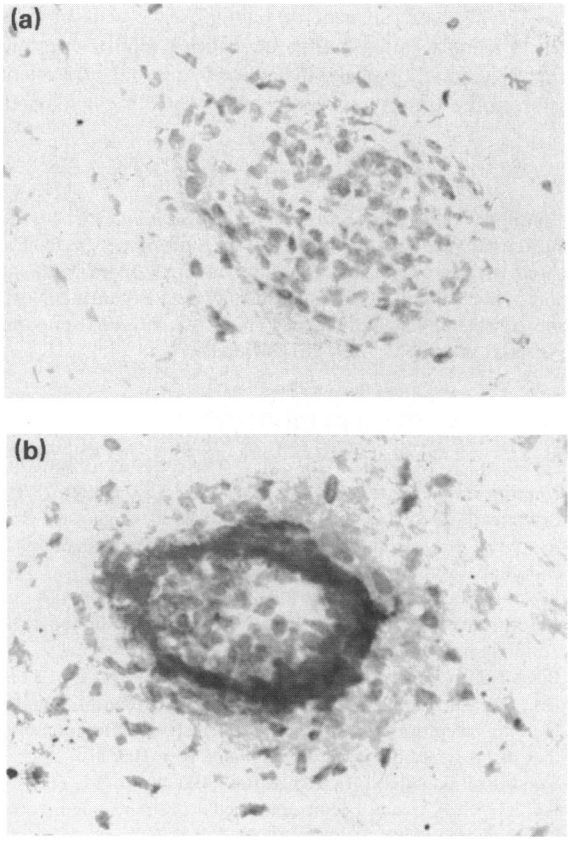

Figure 5. Vascular addressin expression on the same spinal cord lesion during relapse phase CREAE. (a) The PLN addressin MECA-79 antigen was not detectable, whereas (b) the mucosal addressin MECA-367 antigen is evident surrounding and within the perivascular infiltrate in this lesion (counterstained with haematoxylin, × 350).

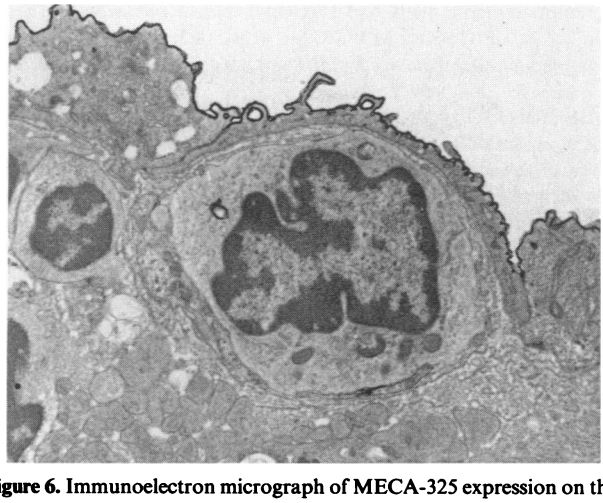

Figure 6. Immunoelectron micrograph of MECA-325 expression on the luminal surface of endothelial cells in the spinal cord during relapse phase CREAE (× 17,700).

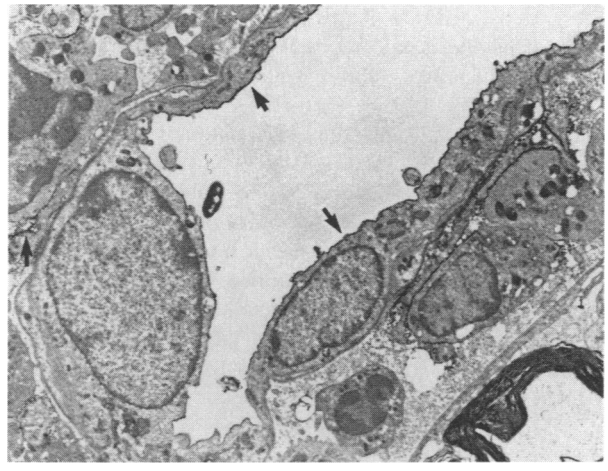

Figure 7. Immunoelectron micrograph of MECA-367 antigen expression in a spinal cord lesion during relapse phase CREAE, MECA-367 antigen (→) is expressed on both the luminal surface of the endothelia and also by components of the extracellular matrix (× 10,800).

relapse animals (Table 1, Fig. 5a). It was found, however, that the mucosal addressin defined by MECA-367 was detectable in the spinal cord of diseased animals (Fig. 5b). Although MECA-367 was generally also reactive in lesions which expressed the MECA-325 antigen, topographically it appeared that whilst MECA-325 was confined to the centre of the lesion, it appeared that localization of MECA-367 staining occurred on both the endothelia and also within and surrounding the perivascular infiltrate (Fig. 5b). Due to the limited resolution afforded by frozen sections immunoelectron microscopy was used to allow a more accurate assessment of the localization of these antigens. It was found that the MECA-325 antigen was restricted to the surface of the endothelium (Fig. 6), whilst MECA-367 reacted with the luminal surface of the endothelium (Fig. 7) and with components of the extracellular matrix including the basement membrane. In the above reactivity of both antibodies was observed on the lumen of the whole vessel or was restricted to individual endothelial cells within such vessels.

DISCUSSION

Clinical signs in CREAE are associated with extensive mononuclear cell infiltration of the CNS. However, it seems clear that by the time such clinical signs are manifest, neuroantigen-specific lymphocytes only represent a minority (> 5%) of the inflammatory cell component.[14,15] It is possible that the entry of these cells orchestrates the influx of largely non-specific inflammatory cells that may then mediate demyelination. This study demonstrates the presence of the ICAM-1 and addressin adhesion systems which could facilitate leucocyte extravasation during the course of CREAE.

In keeping with the finding that lymphocyte traffic into the CNS is normally low or absent[16] was the demonstration that the vascular addressins were absent in normal CNS tissue[5,6] and, although present on some endothelial cells, ICAM-1 expression on CNS endothelia was generally low as is the case in other species.[15,16] Similarly ICAM-1 expression by endothelia is increased during inflammation of the CNS.[16,17] The adherence of leucocytes to vascular endothelia is facilitated by the binding of

LFA-1 (α chain CD11a, β chain CD18) to its ligand ICAM-1;[18] as such it has been reported that antibodies to CD18 can, in certain circumstances, inhibit lymphocyte adherence to brain endothelia.[19] The finding that ICAM-1 is expressed, albeit weakly, in normal and remission animals and is readily detectable on the vasculature in the acute and relapse disease phases in this mouse CREAE model could suggest that the LFA-1:ICAM-1 interactions serve as a possible mechanism by which both the initial antigen-specific and the subsequent non-specific inflammatory cells may adhere to endothelia prior to extravasation. However, during active clinical disease it was found that, similar to the guinea-pig,[16] ICAM-1 was expressed by many vessels in uninvolved CNS tissue. In this CREAE model the onset of the post-acute remission is characterized by not only the resolution of clinical signs but also a dramatic reduction in inflammatory cells in the CNS,[11] there it is unlikely that all such ICAM-1-positive vessels will become lesions. Therefore while ICAM-1 may facilitate initial leucocyte:endothelial cell interactions other factors, such as the expression of neurospecific antigen or mediators within the local microenvironment, are probably more important in leucocyte extravasation and the site of lesion formation.[19,20]

This study showed a relative paucity of addressin expression in lesions during the acute phase of CREAE. As these acute-phase lesions generally resolve in remission,[11] this finding would be in keeping with previous reports that vascular addressin expression in non-lymphoid tissue is detectable only at sites of long-standing chronic inflammation, but not in acute or during the initial phase of mononuclear cell recruitment in subacute inflammation.[4,5] Histological examination of the spinal cord from relapse phase CREAE animals, however, more consistently showed the expression of the HEV-associated MECA-325 antigen on endothelium. MECA-325 antigen expression was only detected focally in lesions and is probably a consequence rather than the cause of the initial CNS infiltration. The nature and role of the MECA-325 antigen are unknown. The expression of this marker of HEV-like differentiation within lesions, however, may be associated with a vascular phenotype capable of supporting lymphocyte extravasation into the CNS and contribute to disease progression.

A recent study in passively transfered CREAE in the SJL mouse confirms the findings in the present report, showing MECA-325 antigen in the CNS during active clinical disease.[21] However, in that study the majority of post-capillary venules in lesions in relapsing and also acute EAE phases expressed high levels of MECA-325 reactivity.[21] Whilst the reasons for the apparent difference between the findings in acute and relapse phases of these studies are not clear, they may be due to a difference in the relative degree of inflammation in the spinal cords or the mode of disease induction between these models.

More important is the examination of expression of the vascular addressins, tissue-specific HEV adhesion molecules for lymphocytes. The present study clearly demonstrates the expression of the mucosal vascular addressin defined by mAb MECA-367 during CREAE, whilst the PLN addressin the MECA-79 antigen was not detectable. It has been shown that B cells and CD8-positive T lymphocytes preferentially home to the mucosal (PP-HEV) compared with PLN tissue.[22,23] Similarly, it has been reported that B cells and CD8-positive cells preferentially adhere to activated brain endothelium.[24] As such the expression of the MECA-367 antigen may provide a means for the preferential recruitment of CD8-positive cells in the late stages of clinical disease that have been reported in mouse EAE.[25] The functional significance of mucosal addressin expression in the CNS, however, requires further elucidation.

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