Brief Definitive Report

Upregulation and Coexpression of Adhesion Molecules Correlate with Relapsing Autoimmune Demyelination in the Central Nervous System

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Summary

The expression of adhesion molecules on central nervous system (CNS) vessels was examined during chronic relapsing experimental autoimmune encephalomyelitis in the SJL mouse. Two molecules associated with cell adhesion were studied: MECA-325, a murine lymph node high endothelial venule marker; and MALA-2, the murine homologue of intercellular adhesion molecule 1. During initial disease, upregulated coexpression of these two molecules occurred in the CNS. This correlated with inflammatory cell invasion. During remission, expression was downregulated, and each subsequent relapse was accompanied by corresponding upregulation. Thus, up- and downregulation of adhesion molecules in the target organ appeared to form an integral part of the inflammatory process in this autoimmune condition and support a role for receptor-mediated inflammatory cell invasion of relevance to the pathogenesis of multiple sclerosis.

The mechanisms by which circulating immune cells recognize a target organ are not well understood, but in the case of the central nervous system (CNS), where the blood brain barrier (BBB) prevents free interchange between blood and CNS parenchyma, such mechanisms are probably fundamental to inflammation. In diseases of the CNS like multiple sclerosis (MS) and its laboratory model, experimental autoimmune encephalomyelitis (EAE), initiating events are believed to involve changes at the BBB resulting in increased vascular permeability. These events may facilitate the adhesion of lymphocytes to CNS endothelium, after which cells invade the target organ and initiate T cell–mediated demyelination (1). Similar molecular events occur in lymph nodes, where, at the sites of lymphocyte extravasation, blood vessels have endothelial cells (EC) of a distinct cuboidal morphology: high endothelial venules (HEV) (2). In rheumatoid arthritis, EC acquire properties of HEV at sites of lymphocyte adhesion and invasion (3). In adoptively transferred EAE, EC have been described with HEV morphology to which mononuclear cells were attached during lesion formation (4, 5). The present study has examined within the CNS during chronic relapsing adoptively transferred EAE, the expression of two molecules associated with cell adhesion: MECA-325, a murine HEV marker (6); and MALA-2, the murine homologue of human intercellular adhesion molecule 1 (ICAM-1) (7, 8).

Materials and Methods

Animals and Immunization. Female SJL/J mice (The Jackson Laboratory, Bar Harbor, ME), 6–8 wks of age, were maintained in an NIH/AAALAC approved facility. Donor SJL/J mice were immunized with guinea pig myelin basic protein (MBP) for the transfer of MBP-sensitized lymphocytes into naive, syngeneic recipients (9), and were sampled during the pre-clinical (24– 48 h before onset of signs), acute, remitting, and relapsing stages. Animals first showed signs of EAE 6–8 d post-transfer (PT).

Tissue Processing and Immunocytochemistry. Frozen sections (10 μm) of CNS tissue were fixed in acetone for 10 min and stained with the avidin-biotin-peroxidase complex (ABC) technique and 3',3-diaminobenzidine as the chromagen. As controls, kidney and lymph node tissues were studied. Incubations were performed overnight with the rat monoclonals MECA-325 (6), used at a 1:10 dilution; YN1/1, which detects MALA-2 (7, 8), used as straight supernate; anti–lymphocyte function–associated antigen (LFA-1) (Boehringer-Mannheim Biochemicals, Indianapolis, IN), used at 1:20; biotinylated Ia^s (from MKS-4 hybridoma) (American Type Culture Collection, Rockville, MD), purified by affinity chromatography, used at 1:25; and anti-L3T4 (Becton Dickinson & Co., Mountain View, CA), used at 1:100. Control sections were incubated with PBS. For double labeling, rabbit anti–glial fibrillary acidic protein (GFAP) antibody, (gift of J.E. Goldman, Columbia University), was used at 1:100.

Results

Spinal cord sections reacted for MECA-325 showed that during the preclinical phase, this HEV marker was expressed at low levels on the surface of EC. It was not seen in the CNS of normal mice. MECA-325 expression was greatly upregulated on the majority of postcapillary venules in spinal cord white matter lesions during the acute phase within 24–

48 h after onset of paralysis, at which timepoint inflammatory cell invasion was prominent (Fig. 1 a). Unaffected white matter, grey matter, and rostral CNS regions showed no MECA-325 reactivity. MECA-325 decreased during clinical remissions to near preclinical levels (Fig. 1 b), and then increased markedly again during each relapse (Fig. 1 c). Differences in MECA-325 staining between preclinical and acute stages, and between relapses and remissions, were statistically significant (Fig. 2).

Low levels of ICAM-1 were noted in the spinal cord during the preclinical phase, similar to MECA-325. ICAM-1 was greatly upregulated in lesion areas during the acute phase (Fig. 1 d), tapering off during remission (Fig. 1 e). ICAM-1 expression subsequently increased with each relapse (Fig. 1 f),
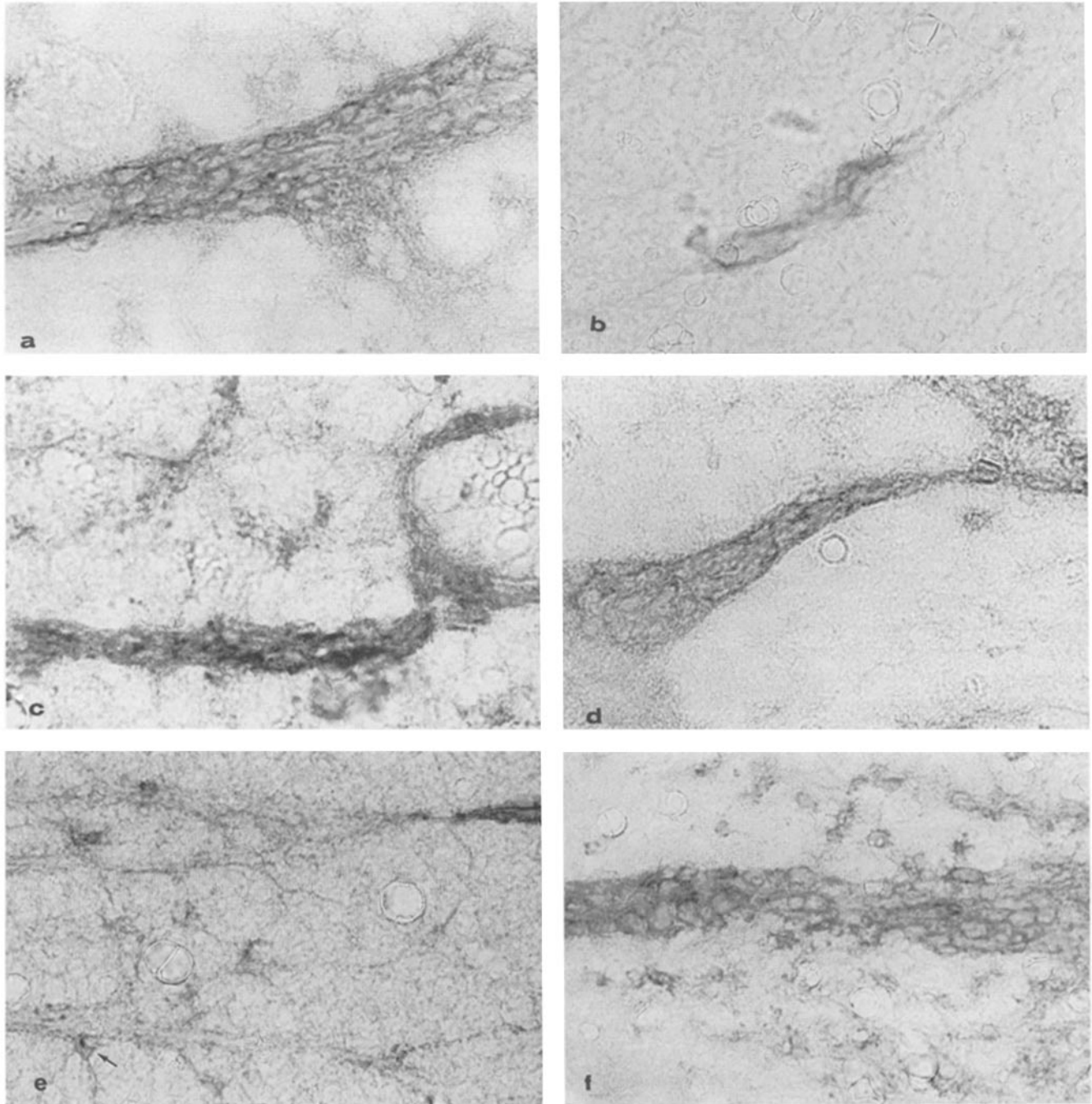
Figure 1. Sections of spinal cord tissue from mice with chronic relapsing EAE demonstrate fluctuations in expression of adhesion molecules, MECA-325 and MALA-2 (ICAM-1), coincident with clinical activity. Upregulated expression of MECA-325 is seen on the surface of EC during the acute phase of adoptively transferred EAE (a); this decreases during clinical remission (b), and increases again during relapse (c). The same fluctuating pattern is shown for MALA-2 expression during acute disease (d), remissions (e), and relapses (f). In e, note the lack of expression along the majority of the vessels, the small area to the right which is MALA-2$^+$, and the positively staining astrocytes (arrows). a–f (×550).
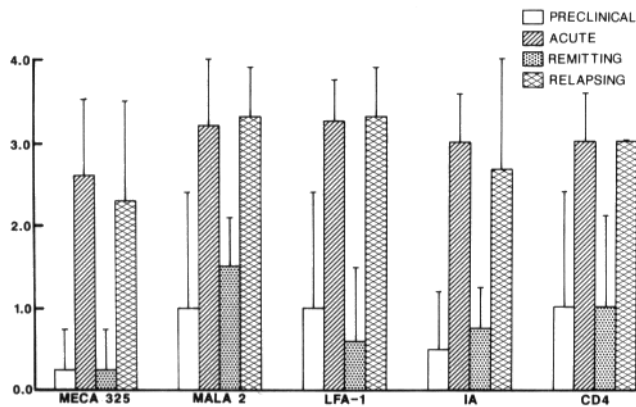
Figure 2. A histogram illustrates the up- and downregulation of adhesion molecules in the CNS of mice with adoptively transferred EAE correlating with clinical activity. Expression of MECA-325, MALA-2, and LFA-1 was significantly different when the preclinical vs. acute stage, and relapses vs. remissions were compared. MECA-325, $p < 0.002$ and $p < 0.05$; MALA-2, $p < 0.05$ and $p < 0.01$; LFA-1, $p < 0.05$ and $p < 0.01$, respectively. Differences in Ia expression were significant only for preclinical vs. acute stages ($p < 0.05$), and in CD4 expression, for relapses vs. remissions ($p < 0.05$). The score was based on the density of positive immunoperoxidase-labeled cells as determined by two blinded observers. Frozen sections from 15 animals were analyzed. Numerical values (0–4) were assigned corresponding to the percentage scale, and significance was determined by the student's t test.

and was found on EC of all vessels, perivascular cells, and scattered astrocytes (Fig. 1 *e*), which were also GFAP⁺. Unlike MECA-325, low levels were seen on EC and glial cells in the CNS of normal mice. Statistical comparison of ICAM-1 at various stages of disease revealed the differences to be significant (Fig. 2).

Infiltration of the CNS correlated with clinical onset, as has been demonstrated previously (9). The majority of the infiltrating cells were LFA-1⁺. LFA-1 expression also closely paralleled that of its ligand, ICAM-1, and of MECA-325 (Fig. 2).

Class II MHC molecules, also implicated in adhesion (10, 11) were expressed during the acute and relapsing phases, similar to MECA-325 and ICAM-1 (Fig. 2). MHC class II expression occurred on perivascular cells; CNS EC appeared to be MHC class II negative. T cells expressing CD4, the ligand for MHC class II, were first demonstrated in the CNS during the preclinical stage and increased during the acute and relapsing phases. During remissions, CD4⁺ cells decreased in number. To test whether the fluctuation in adhesion molecule expression was target organ specific, kidney sections from each group (preclinical, acute, remission, and relapse) were tested for MECA-325, ICAM-1, and MHC class II, and were uniformly negative. As a positive control, lymph node tissue was routinely included and was always positive for MECA-325, ICAM-1, LFA-1, MHC class II, and CD4.

Discussion

The coexpression of two distinct adhesion-associated molecules not normally seen in CNS tissue has been shown within the CNS of mice with relapsing autoimmune demyelination. One of these, MECA-325, is normally present only in lymph node tissue, and the other, ICAM-1, while most common in hematopoietic tissue, is seen at low levels in several organs, including the CNS (12). Our study has shown a close correlation of disease activity with adhesion molecule expression. Since understanding of the mechanisms in the trafficking of immune cells to CNS white matter might be fundamental to the inflammatory demyelinating diseases, we compared the expression of a number of adhesion-related molecules within the CNS at different stages of chronic relapsing EAE.

The appearance of MECA-325 occurred at the BBB and paralleled lymphocytic infiltration. To our knowledge, this represents the first demonstration of an HEV determinant at CNS inflammatory sites. Whether the selective induction of this HEV molecule preceded or occurred during lymphocyte extravasation is not yet clear. The data suggest that the differentiation of HEV-like vessels was a gradual process occurring simultaneously with increasing lymphocytic influx.

The present results support the concept of cell traffic being associated with the modulation of expression of HEV molecules on target organ vessels. The reasons for these fluctuations remain speculative but may relate to cytokines elaborated locally. For example, interferon γ, a product of activated T cells, is known to induce MECA-325, ICAM-1, and MHC class II on a variety of cells, including EC (6, 13, 14). Previous studies using ¹⁴C-labeled T cell blasts to induce adoptively transferred EAE showed that some MBP-immune cells arrived at the CNS vasculature 24–48 h before clinical onset (4). The arrival of radiolabeled cells appeared to precede unlabeled cells, suggesting that the original CNS-immune cells induced local changes at the BBB, which then precipitated the influx of CNS antigen-nonspecific cells (4, 5). These events may have morphologic correlates in that by ultrastructure, HEV-like vessels with unique membrane specializations occurred in the CNS during early EAE (5).

MECA-325 is a member of the addressin family, a group of molecules whose apparent role is to signal and direct cell-to-cell interactions. Its ligand is unknown. With regard to function, MECA-325 antibodies do not inhibit lymphocyte binding, thus not supporting its direct role in cell adhesion (15). MECA-325 may define a distinct epitope on HEV involved in some other aspect of lymphocyte/endothelial cell interactions (15). The inappropriate presence of HEV-like vessels in the CNS, coupled with the fluctuations of MECA-325 with disease activity, appear to implicate this molecule in EAE. Parallel studies using an antibody against a constitutive antigen, von Willebrandt Factor (vWF), confirmed that the MECA-325 reactivity was specific.

Cell adhesion is an essential component of the response of T lymphocytes to antigens. Our demonstration of increased ICAM-1 in the CNS, particularly on EC, is the first to support a role for ICAM-1 during inflammation and demyelination in EAE. ICAM-1 is a member of the Ig gene superfamily and has been associated with activated lymphocytes and sites of inflammation (13). The present fluctuations in ICAM-1 expression and its receptor LFA-1 (16, 17), on inflammatory cells, suggest this interaction may play a role in the

mediation of lymphocytic traffic into CNS inflammatory sites. This speculation is supported by a recent study showing ICAM-1 in human MS and control CNS lesions (12).

The expression of MECA-325, ICAM-1, and MHC class II appears to be important in leukocyte homing and adhesion to the BBB during active stages of autoimmune demyelination. Their fluctuating patterns of expression corresponding to periods of clinical worsening support a functional role.

Non-CNS tissue from the same animals demonstrated no expression of any of the above molecules, indicating their expression to be target organ related. Since several adhesion molecules appear to be coexpressed and function cooperatively in lymphocyte homing to the CNS, the molecular events regulating cellular migration to the target organ during chronic relapsing EAE appear to be synchronized and multifactorial.

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