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Lipoic acid inhibits expression of ICAM-1 and VCAM-1 by CNS endothelial cells and T cell migration into the spinal cord in experimental autoimmune encephalomyelitis

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Abstract

Lipoic acid (LA) suppresses and treats murine experimental autoimmune encephalomyelitis (EAE), which models multiple sclerosis. However, the mechanisms by which LA mediates its effects in EAE are only partially known. In the present study, LA (25, 50 and 100 μg/ml) inhibited upregulation of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) in tumor necrosis factor-α (TNF-α) stimulated cultured brain endothelial cells. Immunohistochemical analysis of spinal cords from SJL mice that had received LA (100 mg/kg/day) following immunization to induce EAE exhibited markedly reduced expression of ICAM-1 and VCAM-1 compared with that of EAE mice receiving saline. Co-localization analysis showed that ICAM-1 and VCAM-1 expression increased over endothelial cells (staining positive for von Willebrand factor, vWF) in EAE and that LA decreased the expression levels to that observed in naïve mice. Spinal cords from mice receiving LA had significantly reduced inflammation (decreased CD4 and CD11b staining) as compared to EAE mice that received saline. Overall, our data suggest that the anti-inflammatory effects of LA in EAE may be partly due to inhibition of ICAM-1 and VCAM-1 expression by central nervous system (CNS) endothelial cells.

Keywords: Lipoic acid; EAE; VCAM-1; ICAM-1; T cells; Multiple sclerosis

1. Introduction

Multiple sclerosis (MS) is a common and disabling disease of the central nervous system (CNS). The loss in integrity of the blood–brain barrier (BBB), recruitment of blood borne monocytes, activation of resident microglia and perivascular macrophages, together with the infiltration of T cells result in multifocal demyelination and axonal injury that characterize the pathology of MS (Raivich and Banati, 2004). Experimental autoimmune encephalomyelitis (EAE) is an experimentally induced inflammatory demyelinating disease of the CNS that has many clinical and pathological features in common with MS.

The BBB is an anatomic and functional barrier to the entry of leukocytes from the blood into the CNS. For myelin reactive T cells to initiate disease in EAE and MS, they must first migrate through the BBB (Ransohoff et al., 2003; Sellebjerg and Sorensen, 2003). The cascade of events involved in the trafficking of T cells into the CNS are generally understood and involve interactions between the T cells and endothelial cells within post-capillary venules (reviewed in Hartung et al., 1995; Hickey, 1991; Wekerle et al., 1991; Wisniewski and Lossinsky, 1991). Activated T cells initially bind to the endothelial cells via selectins and their ligands. This reversible binding allows the T cells to roll along the endothelial cells. Pro-inflammatory T cell cytokines, such as tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ), increase the expression of cellular adhesion molecules on the endothelial cells, particularly intercellular adhesion molecule-1 (ICAM-1) and vascular

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cell adhesion molecule-1 (VCAM-1), permitting a tight binding of the T cells to the endothelial cells via their ligands on the surface of T cells, lymphocyte function-associated antigen (LFA)-1 and very late antigen (VLA)-4, respectively (Alter et al., 2003; Biernacki et al., 2004; Campbell et al., 1998; Cross et al., 1990; Grabovsky et al., 2000; Laschinger and Engelhardt, 2000; Prat et al., 2002; Raine et al., 1990). Locally produced chemokines, such as macrophage inflammatory protein (MIP)-1α, and cytokines, such as IFN-γ, increase the avidity of T cell binding to the endothelial cells and direct transendothelial migration of the T cells (Sellebjerg and Sorensen, 2003). The activated T cells produce matrix metalloproteinases (MMPs), particularly MMP-9, which enzymatically disrupt the subendothelial membrane and extracellular matrix components allowing entry of T cells into the CNS parenchyma (Madri and Graesser, 2000). Treatments that block migration of T cells and monocytes into the CNS may be beneficial in EAE and MS.

Lipoic acid (LA), a neuroprotective metabolic antioxidant (reviewed in Biewenga et al., 1997), has been used to improve glycemic control, treat polyneuropathies associated with diabetes mellitus, and mitigate toxicities associated with heavy metal poisoning (Biewenga et al., 1997; Smith et al., 2004). We were the first to demonstrate that LA is highly effective at suppressing and treating EAE (Marracci et al., 2002), a finding confirmed by others in another strain of mice (Morini et al., 2004). In the present study we provide evidence that the immunomodulatory effects of LA involve reduction of ICAM-1 and VCAM-1 on CNS endothelial cells. We further demonstrate that CD4 (T cells) and CD11b (microglia/macrophages) are significantly reduced in the spinal cord of mice receiving LA compared to control EAE mice that received saline. The data suggest that the reduction of ICAM-1 and VCAM-1 on CNS endothelial cells may be important to the therapeutic effects of LA in EAE.

2. Materials and methods

2.1. Murine brain endothelial cell line culture

The murine brain endothelial cell line (bEnd.3) was obtained from American Type Culture Collection. Montesano et al. (1990) confirmed the endothelial nature of the bEnd.3 cells by the expression of von Willebrand factor (FVIIIR:Ag) and uptake of fluorescently labeled low density lipoprotein (LDL). Sikorski et al. (1993) reported that the bEnd.3 cells express specific endothelial cell markers. Cells were maintained at 37 °C in 10% FBS, 1% sodium–pyruvate, 1% Penicillin–Streptomycin, 2% L-Glutamine in DMEM with 25 mM HEPES buffer (GIBCO, CA). Cells were passaged every week at a ratio of 1:5. Media was changed twice every week. bEnd.3 cells were not used beyond pass 30. For flow cytometry the cells were allowed to adhere for 24 h in 6-well plates at 0.5×10^6 cells/well in 2 ml media. Cells were treated with saline (50 μl) and LA at 25, 50, or 100 μg/ml and simultaneously stimulated with TNF-α (10 ng/ml; Sigma, MO; TNF-α from mouse, recombinant expressed in *E. coli*) at 37 °C for 18 h. We used one well per experimental condition. The cells were harvested and prepared for flow cytometry based on the methods of Sikorski et al. (1993). This experiment was repeated three times.

2.2. Flow cytometry

Techniques previously published from our laboratory were used for FACS analyses (Marracci et al., 2002, 2004). Briefly, $1-5 \times 10^5$ cells were incubated on ice for 5 min with Fc block in staining media (SM). Cells were then stained with rat anti-mouse VCAM-1 FITC/hamster anti-mouse ICAM-1 PE (1:10 each; BD Biosciences, CA) or rat IgG2a FITC/hamster IgG1 PE (isotype controls; 1:10 each; BD Biosciences, CA). Following addition of fluorescent-labeled antibodies or their isotype controls, cells were incubated for 20 min on ice, washed in ice-cold SM, and resuspended in SM for analysis on a FACScan using Cell Quest software (Becton-Dickinson). The percentage of isotype positive cells was subtracted from the percentage gated indicated in Table 1.

2.3. Induction of EAE

All procedures used in the study were approved by the Portland VA Medical Center's Institutional Animal Care Utilization Committee and were in accordance with provisions of the Animal Welfare Act, the Public Health Service's Guide for the Care and Use of Laboratory Animals, and Department of Veterans Affairs' policy. Female SJL mice were obtained from Jackson Laboratories and housed in our animal care facility. EAE was induced following previously published procedures (Marracci et al., 2002). Briefly, mice were immunized by subcutaneous (s.c.) injection with an emulsion containing proteolipid protein (PLP) 139-151 peptide (Multiple Peptide Systems, CA) and complete Freund's adjuvant (CFA) containing 150 μg of peptide and 200 μg of *Mycobacterium tuberculosis* in a total volume of 0.2 ml.

Table 1
Total percentage and mean fluorescent intensity of VCAM-1 and ICAM-1 positive cells

Treatment	Total VCAM-1 (%[a])	MFI[b] VCAM-1	Total ICAM-1 (%)	MFI ICAM-1
Saline	1.68	5	1.42	9
Saline+TNF-α	76.97	49	41.47	51
LA 25+TNF-α	41.17	19	15.24	20
LA 50+TNF-α	21.3	11	10.4	15
LA 100+TNF-α	6.83	8	9.03	16

[a] Percentage positive cells reflect single and double positive populations for each marker.
[b] MFI — mean fluorescent intensity.