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Early blood-brain barrier permeability in cerebella of PLSJL mice immunized with myelin basic protein

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Abstract

The blood-brain barrier (BBB) is dramatically but transiently compromised in the cerebella of myelin basic protein immunized mice at least 1 week prior to the development of the paralytic phase of experimental allergic encephalomyelitis (EAE). Treatment of mice with the peroxynitrite-dependent radical scavenger uric acid (UA) during the first week after immunization blocks the early increase in cerebellar BBB permeability and the subsequent development of clinical signs of EAE. These results indicate that the early loss of BBB integrity in the cerebellum is likely to be a necessary step in the development of paralytic EAE.
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1. Introduction

Despite the identification of a variety of factors that contribute to the pathogenesis of multiple sclerosis (MS) and its animal model experimental allergic encephalomyelitis (EAE), the precise mechanisms responsible for the development of the CNS inflammatory lesions that are characteristic of these neurodegenerative diseases remain poorly understood. Lesion formation is clearly associated with enhanced expression of a number of cytokines, chemokines, adhesion molecules and matrix metalloproteinases (MMPs) as well as the accumulation of immune/inflammatory cells in CNS tissues (reviewed Brown, 2001; Fazekas and Tabira, 2000; Hemmer et al., 2002; Imitola et al., 2005). Free radicals including nitric oxide ($NO^{\cdot}$) and peroxynitrite ($ONOO^-$), the product of $NO^{\cdot}$ and superoxide, have also been implicated in lesion formation both through neurotoxicity and the induction of the changes in blood-brain barrier (BBB) integrity that are associated with immune cell invasion into CNS tissues (Cross et al., 1997; Cross et al., 1998; Hooper et al., 1997; Hooper et al., 2000; Liu et al., 2001; Scott et al., 2001; van der Veen et al., 1997). Studies in EAE have provided considerable insight into the development of immune processes that lead to CNS pathology but information about contributory events in the CNS tissues that precede lesion formation is limited. Just as it is impossible to predict the onset of MS, or the appearance of new plaques in an existing case, it is difficult to predict whether an individual animal will develop EAE following appropriate immunization. For example, while immunized PLSJL mice all develop similar immune responses to myelin basic protein (MBP) by day 10–14 after immunization (Kean et al., 2000), only 30–70% of the mice generally progress to exhibit clinical signs of EAE in a particular experiment (Hooper et al., 1998). Moreover, some animals may

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Table 1
Primer and probe sequences used for real-time quantitative RT-PCR

Gene	5′Primer	3′Primer	Probe
GAPDH	GGCAAATTCAACGGCACAG	AGATGGTGATGGGCTTCCC	AGGCCGAGAATGGGAAGCTTGTCATC
iNOS	TGGCTACCACATTGAAGAAGCTG	TCTGGCTCTTGAGCTGGAAGAAA	TGGCCACCAAGCTGAACTTGAGCGA
CD68	GTGCTCATCGCCTTCTGCATCA	GGCGCTCCTTGGTGGCTTAC	CCAGCCCCTCTGAGCATCTGCCCC

become sick as early as day 12 after immunization while others take more than 30 days for symptoms to appear (Fabis et al., 2007). This is likely a reflection of the fact that the development of CNS pathology in EAE is dependent upon contributions from a number of processes including the induction of an appropriate myelin-antigen specific immune response as well as pro-inflammatory changes in the neurovasculature and CNS tissue. The latter are required to promote the invasion of the circulating immune/inflammatory cells responsible for lesion formation across the BBB and into CNS tissues.

The development of a strong pro-inflammatory response in the CNS tissues of mice immunized with myelin antigens, identified by the enhanced expression of TNF-α, is associated with the loss of BBB integrity and the development of clinical signs of EAE (Scott et al., 2004). However, there is evidence of functional changes occurring in the neurovasculature prior to the onset of disease. This includes elevated adhesion molecule expression (Archelos and Hartung, 1999; Kieseier et al., 1999; Scott et al., 2004) and transiently enhanced BBB permeability which has been detected in the cerebella of SJL/J mice 6 days following immunization with proteolipid protein (PLP) peptide aa139–151 (Tonra et al., 2001; Tonra, 2002). While it is clear that the elevation of adhesion molecules on the neurovasculature would facilitate immune/inflammatory cell invasion into CNS tissues, the contribution of transient BBB permeability in the cerebellum to the development of clinical signs of EAE several days later is unknown. We have previously demonstrated that $ONOO^-$ makes an important contribution toward the induction of enhanced BBB permeability in EAE. For example, the administration of uric acid (UA), a natural scavenger of peroxynitrite-dependent radicals, prevents the loss of BBB integrity in mice immunized with myelin antigens as well as CNS inflammation and the development of EAE (Hooper et al., 1997, 2000). Our findings indicate that UA treatment suppresses the BBB permeability changes associated with the symptomatic phase of EAE without interfering with the induction of myelin-specific immunity (Kean et al., 2000). With a view toward establishing whether or not this early loss of BBB integrity is an essential step in lesion formation and the development of clinical signs of EAE, in this investigation we have assessed the effects of UA treatment on the transient neurovascular permeability seen in the cerebella of mice immunized 6 days previously with myelin basic protein (MBP) and the subsequent development of disease.

2. Materials and methods

2.1. Induction of EAE and treatment of mice

Female, 8–10 week old, PLSJL mice (Jackson Laboratory, Bar Harbor, ME) were immunized subcutaneously at 3 sites with 200 μl of an emulsion of 100 μg MBP in complete Freund's adjuvant containing 0.05% M. Butyricum plus an additional 4 mg/ml M. tuberculosis H37 RA (Difco). Pertussis toxin, 400 ng, was given intra-peritoneally twice, on days 0 and 2. Mice were scored twice daily for clinical signs of EAE on the basis of the presence of the following symptoms: 0, normal mouse; 1, piloerection, tail weakness; 2, tail paralysis; 3, tail paralysis plus hindlimb weakness; 4, tail paralysis plus partial hindlimb paralysis; 5, total hindlimb paralysis; 6, hind and forelimb paralysis; 7, moribund/dead. (Hooper et al., 1998). UA (Sigma Chemical Co., St. Louis, MO) was administered i.p. 4 times per day, 10 mg in 100 μl saline. Control groups of mice received saline alone.

2.2. Assessment of BBB permeability

BBB permeability was assessed using sodium-fluorescein (MW 376) as a tracer molecule. Each animal received 100 μl of 10% sodium-fluorescein (Sigma) in PBS i.p. After 10 min, mice were anesthetized by i.p. administration of sodium pentobarbital (20 mg/kg body weight), cardiac blood was collected, and the animals were transcardially perfused with PBS-heparin and PBS. Sodium-fluorescein uptake into the spinal cord was determined as detailed previously (Hooper et al., 2000). Briefly, spinal cord tissue was homogenized in 7.5% trichloroacetic acid and centrifuged for 10 min at 10,000 rpm to remove insoluble precipitates. Following the addition of 5 N NaOH, the fluorescence of the sample was determined at excitation 485 nm and emission 530 nm using a Cytofluor II fluorimeter (PerSeptive

Table 2
Primer sequences used to synthesize cDNA standards for real-time quantitative RT-PCR

Gene	5′Primer	3′Primer
GAPDH	GAACGGATTTGGCCGTATTG	GGATGCAGGGATGATGTTCT
iNOS	TCCCAGCCTTGCATCCTCATT	TACTCAGTGCCAGAAGCTGGA
CD68	ATACCCAATTCAGGGTGGAAG	GTTGAGTCAGTGGCATGGTG

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