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Experimental Autoimmne Encephalomyelitis : An animal model of human demyelinating disease multiple sclerosis

T. K. Shin

Department of Veterinary Medicine, Research Institute of Animal Science,
College of Agriculture, Cheju National University,
Cheju, 690-756, Republic of Korea

Tel : (82)(64)754-3363
Fax : (82)(64)756-3354
e-mail : shint@cheju.cheju.ac.kr

1. What is Experimental autoimmune encephalomyelitis?

Experimental autoimmune encephalomyelitis(EAE) is an autoimmune T cell-mediated central nervous system disease(CNS) and is used as an animal model for the human demyelinating disease multiple sclerosis(Raine, 1984). After immunization of brain tissue antigen, including myelin basic protein, susceptible animals develop paralysis followed by a spontaneous recovery.

EAE lesion is characterized by edema, perivascular cuffing of T cells, macrophages and some bystander cells. In EAE, apoptotic elimination of inflammatory cells is one of the most important phenomena in the recovery of damaged central nervous systems(Schmied et al, 1993; Pender et al, 1992).

Little is known on the neuropathogenesis of autoimmune disease in the central nervous system in human and animal models including EAE.

2. Subarachnoid space(SAS) as a site for precursor T cell proliferation and effector T cell selection in EAE.

To characterize the phenotype of inflammatory cells in the CNS in EAE, Lewis rats were immunized with MBP and frozen sections of spinal cords with EAE were examined immunohitochemically using a panel of monoclonal antibodies against T cells and adhesion molecules.

In the early stage of EAE, inflammatory cells first appeared in the SAS and

infiltrated in subpial region. The majority of inflammatory cells in SAS expressed TCR alpha/beta and either CD4 or CD8 molecules. However, only CD4-positive T cells infiltrated the parenchyma while the majoriety of CD8-positive cells remained in SAS(Table 1). A similar differential localization of T cells was observed in with regard to CD45RC molecules. Inflammatory cells in SAS consisted of both CD45RC-positive and CD45RC-negative polulation, while those in the parenchyma were largely CD45RC-negative.

With regard to adhesion molecules, the leptomeninges constitutively expressed fibronectin and intercellular adhesion molecule 1(ICAM-1). Most SAS inflammatory cells expressed very late antigen 4(VLA-4) and, to a less extent, lymphocyte function-associated antigen 1(LFA-1) in the early stage of EAE. On the other hand, parenchymal infiltrating cells expressed LFA-1 more strongly in the peak stage of EAE.

These findings suggest that VLA-4/fibronectin and LFA-1/ICAM-1 interactions between infiltrating cells and leptomeningeal cells may be involved in the early and peak stage of EAE(Shin et al, J Neuroimmunol 1995:56:171).

Table 1. Summary of immunohistochemical staining of SAS and parenchymal cells of the spinal cords with EAE.

	Early stage			Peak stage		
	SAS cells	Parenchymal cells	brain cells	SAS cells	parenchymal cells	brain cells
CD4	++[a]	+	Mic[b] ±	+++	+++	Mic +
CD8	++	±	-	+++	±	-
CD45RC	++	±	-	+++	±	-
VLA -4	++	+	-	+	+++	Mic +
Fibronectin	D+++[c]	-	EC[d] ++	D+++	-	EC++
LFA-1	+	+	Mic ±	+	+++	Mic++
ICAM-1	D+++	±	EC ++	D+++	+++	MIC+++ EC++

[a] The number of positive cells are categorized into five grades : +++, many; ++, moderate; +, several; ±, very few(one or two); -, no positive cells were found in the indicated region under medium magnification.

[b] Microglia

[c] Diffusely positive.

d endothelial cells.

3. A role of nitric oxide in autoimmune CNS inflammation

NO is a free radical synthesized by NOS which includes nNOS, endothelial NOS and iNOS. The former two isoforms, called constitutive NOS(cNOS), generate a physiological level(picogram) of NO which is involved in the neurotransmission and vasorelaxation, while the latter, iNOS, produces a large amount (nanogram) of NO which plays an important role in bacteriocidal, tumorocidal and cytotoxic effects(Moncada et al, 1991: Nathan and Xie, 1994).

In a pathologic condition such as EAE, NO is detected in the spinal cords with EAE(Lin et al, 1993). Its key enzyme of iNOS has been extensively studied at the transcriptional level(Koprowski et al, 1993: Tran et al, 1997) and it has functional significance through the inhibition of iNOS activity in EAE (Zhao et al, 1996). Moreover, NO via nNOS has been found in the pathogenesis of secondary injuries after a spinal cord injury(Sharma et al, 1996). Little is known of the functional role of nNOS in autoimmune CNS injuries.

To elucidate the role of neuronal nitric oxide synthase(nNOS) and inducible NOS(iNOS) in the central nervous system with experimental autoimmune encephalomyelitis(EAE), we examined the distribution of apoptotic cells and analysed nNOS-as well as iNOS-positive cells in the spinal cords with EAE.

In EAE lesions, apoptotic cells were evenly distributed in the spinal cord parenchyma as well as in perivascular inflammatory lesions. Some apoptotic cells have intimately structural relationships with neurons and glial cells.

Immunohistochemistry shows that nNOS-positive cells including neurons increased in number at the peak stage of EAE(Table 2). The increased immunoreactivity of nNOS in neurons and astrocytes remained at the recovery stage of EAE. iNOS-positive cells, as well, increased in number mainly in perivascular lesions. Some astrocytes distant from EAE lesions also showed iNOS immunoreactivity(Table 3).

These findings suggest that nitric oxide via either nNOS or iNOS is generated in the spinal cords with EAE, and the structural relationship between apoptosis and NOS-positive cells in EAE imply that NO may play an important role in the elimination of inflammatory cells via apoptosis which results in a spontaneous recovery from paralysis in EAE(Shin et al, Korean J Gerontol 1998 : in submission).

Conclusion : When all is taken into consideration, we suppose that NO produc-

tion, mainly in macrophages via iNOS, plays a detrimental role on host tissues at the early stage of EAE. On the other hand, NO via constitutive NOS in the brain cells plays beneficial roles in the recovery of host tissues as far as autoimmune disease is concerned.

Table 2. Immunohistochemical localization of neuronal nitric oxide synthase in experimental autoimmune encephalomyelitis in Lewis rats.

Cell type of spinal cords	Normal	EAE[a]	
		Peak (grade 3)	Recovery (R.0)
Neuron	±[b]	++	+
astrocytes	±	++	+
macrophages	ND	-	-
vessels	-	+	+

[a] The spinal cords were examined at the peak stage(grade 3 : day 13 post-immunization) and the recovery stage(day 21 PI) of EAE.

[b] The number of nNOS immunoreactive cells is expressed by a small(± : under 10 cells per one field of X100), a moderate(+ : under 10 cells) and a large number(++ : over 15 cells per one field of X100) of nNOS-positive cells in the affected spinal cords.

Table 3. Immunohistochemical localization of inducible nitric oxide synthase in experimental autoimmune encephalomyelitis in Lewis rats.

Cell type of spinal cords	Normal	EAE[a]	
		Peak (grade 3)	Recovery (R.0)
Neuron	-[b]	-	-
astrocytes	-	+	+
macrophages	ND	++	+
vessels	±	+	±

[a] The spinal cords were examined at the peak stage(grade 3 : day 13 post-immunization) and the recovery stage(day 21 PI) of EAE.

[b] The number of iNOS immunoreactive cells is expressed by a small(± : under 10 cells per one field of X100), a moderate(+ : under 10 cells) and a large number(++ : over 15 cells per one field of X100) of iNOS-positive cells in the affected spinal cords.

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