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Cellular Immunology 233 (2005) 140–147
Cellular Immunology
www.elsevier.com/locate/ycimm

Neuroglial response after induction of experimental allergic encephalomyelitis in susceptible and resistant rat strains

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Received 21 April 2005; accepted 21 April 2005
Available online 15 June 2005

Abstract

Experimental allergic encephalomyelitis (EAE), the animal model for multiple sclerosis in humans, a T-cell mediated disease of the central nervous system is characterized by inflammatory infiltrates of myelin antigen(s)-specific T cells and consecutive demyelination. Spinal cord tissue emulsified in complete Freund's adjuvant clinical disease in the genetically susceptible Dark Agouti rats (DA) but not in Albino Oxford (AO) rats although similar inflammatory infiltrates in the CNS are observed in both strains 10–12 days after induction. We have shown that the resistance to clinical disease of AO rats is associated with rapid clearance of infiltrating mononuclear cells by a mechanism of apoptosis. Here, we demonstrate by immunohistochemical and FACS analyses of the expression of CD11b/c that microglial cells respond differently to disease induction in the two strains. Whereas microglial cells are activated throughout the period of day 10–28 days after EAE induction in AO rats they are only activated at the inception and resolution phases but not at the peak of clinical disease in DA rats when there is the highest level of CD4+ T cell infiltration. Our findings are compatible with the notion that microglia terminate effector T cells by apoptosis and that lack of this mechanism as evidenced by the lack of CD11b/c expression, support T cell survival and clinical expression of disease.

Keywords: Encephalomyelitis; Macrophages; Microglia activation; Mononuclear cells; OX-42

1. Introduction

Experimental allergic encephalomyelitis (EAE), the animal model for multiple sclerosis in humans, is a T-cell mediated disease of the central nervous system characterized by local inflammatory infiltrates of myelin antigen-specific T cells and demyelination. Injection of encephalitogen, spinal cord homogenate or myelin basic protein induces disease in the genetically susceptible Dark Agouti rats (DA) but not in Albino Oxford (AO) rats although inflammatory infiltrates of similar intensity are observed in both strains early after disease induction [1]. Recently, we postulated that the resistance to clinical disease of AO rats is associated with rapid clearance of lymphoid cell infiltrates in the CNS by a mechanism of apoptosis early after disease [1,2]. Studies on EAE in mice have shown that the severity of clinical disease may reflect interaction between infiltrating T cells and the resident CNS glial cells especially microglial and astrocytes [3].

It has been proposed that at the initial stages, EAE arises as a result of Th1 response [4–6] following recruitment of CD4+ T helper cells which then become restimulated by antigen presenting cells (APCs) in the target tissue [6]. Once CD4+ T cells have gained entry into the CNS, the microenvironment of the CNS becomes very important for the outcome of the disease. Inflammation as well as macrophage activation and tissue destruction leading to clinical disease results from the secretion of proinflammatory cytokines, such as interferon-γ (INF-γ), IL-23, IL-1β, and tumor necrosis

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0008-8749/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.cellimm.2005.04.023

factor (TNF-α) [7–9]. The secretion of transforming growth factor-β1 (TGF-β) [3], the Th2 cytokines IL-4, IL-10, and IL-13 [9–12], and the apoptotic elimination of T cells [13] by CNS parenchymal cells have been proposed to limit and terminate cell-mediated immune pathology in the CNS.

Both microglia and astrocytes appear to play significant roles in the clinical history of EAE. Microglial cells secrete both proinflammatory IL-1 and TNF and anti-inflammatory IL-10 and TGF-β cytokines [14–16] and hence would be important for both initial and late stages of the disease. Furthermore, microglial cells have been shown to act as APCs during immune-mediated CNS disease and therefore could play a role in T cell restimulation [17–19]. Recently, it has also been argued that activated microglia may downregulate Th1-induced pathology [20]. Further, there is evidence that suggests that blood-borne monocyte/macrophages, and not microglial cells, might be the APCs [21] and effector cells in EAE [22].

While these studies demonstrate in detail the roles of microglial and astrocytes in EAE at its inception and termination when spontaneous resolution occurs, very little, if anything is known about these cells at the height of clinical disease. It must be assumed that microglia remain activated to perform functions that result in either the appearance or resolution of disease. We present histological, immunohistochemical, and flow cytometric evidence that while microglial cells are activated at the inception and resolution of EAE in DA rats, they appear attenuated at the height of clinical disease. Also, significantly, the level of attenuation appears to correlate with the level of clinical disease and T cell infiltration. Interestingly, microglial cells remain activated in the resistant AO rats.

2. Materials and methods

All experimental procedures were conducted using aseptic techniques and were in accordance with the protocol for projects involving the use of laboratory animals of the Animals Research Ethics Subcommittee of the Research Grants Awarding Committee of the Faculty of Medicines and Health Sciences, United Arab Emirates University. Every effort was made to minimize the number of animals used.

2.1. EAE induction and evaluation

Fifteen 8–12-week-old DA and AO rats were immunized in the left hind foot pad with 0.1 ml antigenic emulsion containing rat spinal cord tissue in complete Freund adjuvant (CFA). Animals were monitored daily for clinical signs of disease starting from day 5 after inoculation. The severity of disease was assessed by grading tail, hindlimb and forelimb weakness, each on a scale of 0 (no disease), 1 (loss of tail tonicity), 2 (hindlimb weakness), 3 (hindlimb paralysis), and 4 (moribund or death) as previously described [23,24]. Rats were anaesthesized by an overdose of ether and spinal cords were excised from the spinal canal on days 0, 10, 14, and 28 to coincide with the clinical phases of diseases namely inception, most severe clinical signs, and resolution of disease respectively.

For histological and immunohistochemistry studies animals were perfused with 4% paraformaldehyde solution followed by immersion in the same solution at 4 °C for 2 h before spinal cords were excised. For FACS analysis whole spinal cords were treated as detailed below.

2.2. Histological evaluation of disease

Pieces of the spinal cord were routinely embedded in paraffin wax and 7.0 μm sections routinely stained with haematoxylin and eosin. The level of mononuclear cellular infiltration was graded 0 (no infiltration), 1 (mild infiltration observed around pial vessels), 2 (single cell infiltration within CNS), 3 (infiltration with mild perivascular cuffing), and 4 (very intense infiltration with perivascular cuffing).

2.3. Immunohistochemistry

Seventy-five micrometer thick cryostat sections of the spinal cords were stained by the ABC streptavidin technique using mouse monoclonal OX-42 antibody, a sensitive marker for activated microglia ([25]; gift from Prof. J. Reynolds, Imperial College, London, UK) and glial fibrillary anti-protein (GFAP) for astrocytes ([26]; Sigma, St. Louis, MI, USA]. Thick sections were immunostained by the floating method. The sections were incubated in 3% hydrogen peroxide in absolute methanol for 30 min to block endogenous peroxidase after which they were incubated with monoclonal mouse OX-42 diluted 1:20 or rabbit polyclonal GFAP diluted 1:30,000 both in 0.3% Triton X in 0.1 M PBS overnight at room temperature. The sections were washed in PBS and incubated with the link antibody comprising biotinylated anti-mouse IgG for OX-42 immunostained and biotinylated anti-rabbit (Jackson ImmunoResearch laboratories, USA) for the GFAP treated sections both diluted 1:500 in 0.3% Triton X in 0.1 M PBS for 1 h and then peroxidase labelled-extravidin (Sigma, St. Louis, USA) diluted 1:1000 in 0.3% Triton X in 0.1 M PBS for 1 h. After three washes in 0.1 M PBS, peroxidase activity was demonstrated with diaminobenzidene. The sections were air-dried and dehydrated in ethanol to 100%, cleared in xylene and coverslipped using Cytoseal 60 mounting medium (Stephens Scientific, Riversdale, NJ, USA). These slides were examined on a Zeiss Axiophot photomicroscope.