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Research paper

Active immunization with proteolipid protein (190-209) induces ascending paralysing experimental autoimmune encephalomyelitis in C3H/HeJ mice

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ABSTRACT

Experimental autoimmune encephalomyelitis (EAE) is a demyelinating disease of the central nervous system (CNS) that shares clinical and pathophysiological feature with multiple sclerosis (MS) and is commonly used as an animal model for the human disease.
Upon active immunization, different myelin proteins and other neuronal antigens are known to induce EAE in susceptible mouse strains. However, there are rodent strains reputed to be resistant to actively-induced EAE and the correct combination of animal strains and their respective autoantigen is absolutely critical as some antigens are encephalitogenic in one animal strain, but not in another. Here we describe actively-induced EAE in C3H/HeJ mice with different myelin peptides. Whereas no clinical signs could be found by immunization with myelin oligodendrocyte glycoprotein 35-55, significant weight loss as well as rapidly occurring severe ascending paralysis was found in animals immunized with proteolipid protein 190-209 ($PLP_{190-209}$). Histologically, this form of EAE was characterized by predominant involvement of the spinal cord.
As PLP is one of the major lipid antigens putatively involved in the pathogenesis of MS, this model may be useful for further studies of the disease.

1. Introduction

Experimental autoimmune encephalomyelitis (EAE) is one of the most extensively studied animal models and is commonly used to model inflammatory aspects of multiple sclerosis (MS) (Denic et al., 2010; Krishnamoorthy and Wekerle, 2009). Since initial experiments in the 1930s (Rivers et al., 1933), EAE has been actively induced in different mammal species (Denic et al., 2010).

Over the years, the protocols for EAE induction have been redefined. The use of Freund's adjuvant during immunization facilitated immunization regimens, such that EAE can be induced with a single immunization (Stromnes and Goverman, 2006). Addition of pertussis toxin improved the efficiency of EAE induction (Levine and Sowinski, 1973). Specific encephalitogenic epitopes fractioned from spinal cord like myelin basic protein (MBP) (Martenson et al., 1969), proteolipid protein (PLP) (Olitsky and Tal, 1952) and myelin oligodendrocyte glycoprotein (MOG) were identified (Lebar and Vincent, 1981; Linnington et al., 1984).

Current protocols typically use purified or recombinant myelin proteins or synthetic peptides as immunogens suspended in different adjuvants initiating a peripheral activation of myelin-specific CD4 T cells that have escaped immune tolerance and circulate in the periphery (Seamons et al., 2003). This activation allows myelin-specific CD4 T cells to migrate

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across the blood brain barrier more efficiently than naïve (Hickey, 1991; Brabb et al., 2000) initiating a cascade of proinflammatory events in the central nervous system (CNS) including recruitment of macrophages. This neuroinflammatory response leads to infiltrates composed of T cells, B cells and macrophages with focal plaques of demyelination and axonal damage that are similar to the pathology seen in MS (Sospedra and Martin, 2005).

However, there are rodent strains reputed to be resistant to actively-induced EAE (Duong et al., 1994; Clark et al., 1997). Generally, responsiveness in each strain depends on the nature of antigens applied, so that some antigens are encephalitogenic in one animal strain, but not in another (Lando et al., 1979; Mendel et al., 1995; Krishnamoorthy and Wekerle, 2009).

C57Bl/6 mice immunized with MOG_{35-55} have become the animals of choice, especially for studies involving transgenic mice (Krishnamoorthy and Wekerle, 2009). The characteristic clinical feature of EAE in this strain is ascending paralysis, beginning at the distal end of the tail and moving rostrally to affect the hindlimbs and sometimes the forelimbs (Greer et al., 1992; Cross et al., 1993; Krishnamoorthy and Wekerle, 2009). Typically animals suffer from a chronic course with only incomplete recovery after the initial inflammatory peak depending on the exact immunization regime. In this form of EAE, CNS inflammation and demyelination is located predominantly in the spinal cord (Cross et al., 1993; Mendel et al., 1995).

EAE with interchanging relapses and remissions, reminiscent of early human MS, can be induced in SJL/J or Biozzi ABH mice (Tuohy et al., 1992; Amor et al., 1993; Amor et al., 1994). Other clinical signs have been described in some strains of mice with atypical signs like forelimb paralysis in the absence of hindlimb paralysis or disturbance of balance (Kerlero de Rosbo et al., 1995; Greer et al., 1996). To recapitulate the diverse clinical and pathological aspects of MS, different

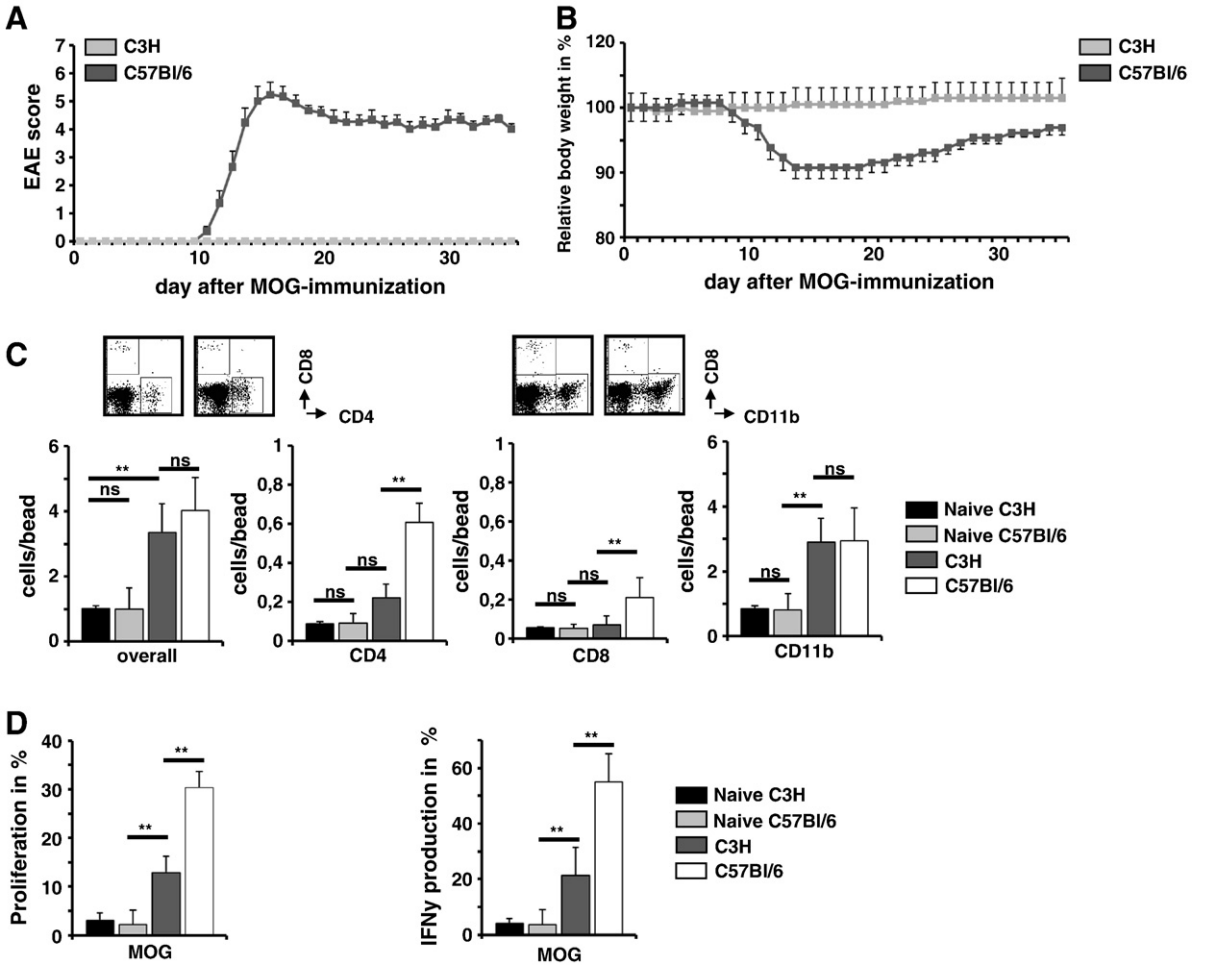

Fig. 1. MOG_{35-55} immunization in C57Bl/6 and C3H mice. (A) Disease course of C57Bl/6 (dark grey) and C3H mice (light grey) after immunization with MOG_{35-55}. (B) Body weight relative to the initial values assessed in C57Bl/6 (dark grey) and C3H mice (light grey) during the course of EAE. (C) Flow cytometry assessment of CNS invading cells: total numbers of CNS-invading cells (overall), numbers of CD4 T cells, CD8 T cells and CD11b cells were analyzed in naïve C3H (black) and C57Bl/6 mice (light grey) as well as in MOG_{35-55}-immunized C3H (dark grey) and C57Bl/6 animals (white) 35 days after immunization. Representative flow cytometry analyses are shown. (D) Proliferation and IFNy production in response to in vitro MOG_{35-55} stimulation in splenocytes from naïve C3H (black) and C57Bl/6 mice (light grey) as well as in MOG_{35-55}-immunized C3H (dark grey) and C57Bl/6 mice (white) 35 days after immunization. Results are presented as mean ± SEM. $^{**}p<0.05$, ns = not significant ($p>0.05$).