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Consistent induction of chronic experimental autoimmune encephalomyelitis in C57BL/6 mice for the longitudinal study of pathology and repair

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HIGHLIGHTS

- We provide a detailed experimental autoimmune encephalomyelitis (EAE) protocol.
- This protocol uses two myelin oligodendrocyte glycoprotein (MOG_{35-55}) injections.
- Ascending paralysis is consistently observed in 80-100% of MOG_{35-55} immunized mice.
- Robust multiple sclerosis (MS)-like central nervous system pathology is observed.
- Providing this protocol facilitates comparability between pre-clinical studies.

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ABSTRACT

Background: While many groups use experimental autoimmune encephalomyelitis (EAE) as a model to uncover therapeutic targets and understand the pathology underlying multiple sclerosis (MS), EAE protocol variability introduces discrepancies in central nervous system (CNS) pathogenesis and clinical disease, limiting the comparability between studies and slowing much-needed translational research.
Optimized method: Here we describe a detailed, reliable protocol for chronic EAE induction in C57BL/6 mice utilizing two injections of myelin oligodendrocyte glycoprotein (35–55) peptide mixed with complete Freund's adjuvant and paired with pertussis toxin.
Results: The active MOG_{35-55}-EAE protocol presented here induces ascending paralysis in 80–100% of immunized mice. We observe: (1) consistent T cell immune activation, (2) robust CNS infiltration by peripheral immune cells, and (3) perivascular demyelinating lesions concurrent with axon damage in the spinal cord and various brain regions, including the optic nerve, cortex, hippocampus, internal capsule, and cerebellum.
Comparison with existing method(s): Lack of detailed protocols, combined with variability between laboratories, make EAE results difficult to compare and hinder the use of this model for therapeutic development. We provide the most detailed active MOG_{35-55}-EAE protocol to date. With this protocol, we observe high disease incidence and a consistent, reliable disease course. The resulting pathology is MS-like and includes optic neuritis, perivascular mononuclear infiltration, CNS axon demyelination, and axon damage in both infiltrating lesions and otherwise normal-appearing white matter.
Conclusions: By providing a detailed active MOG_{35-55}-EAE protocol that yields consistent and robust pathology, we aim to foster comparability between pre-clinical studies and facilitate the discovery of MS therapeutics.

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1. Introduction

Multiple sclerosis (MS) is an autoimmune, demyelinating, and neurodegenerative disease of the central nervous system (CNS) that presents with varied clinical and pathological manifestations. The hallmark of MS is the demyelinated lesion, which is characterized by demyelination, axon damage, mononuclear cell infiltrates, and astrocytic scar formation (Mahad et al., 2015). While the etiology of MS is currently unknown, a prevailing hypothesis involves immune cells, including macrophages, B cells, and T cells, gaining access to the CNS, where they release an array of pro-inflammatory mediators (Friese and Fugger, 2009; Hollenbach and Oksenberg, 2015). This results in regions of demyelination and axon degeneration, called "plaques." As the disease progresses, debilitating motor symptoms develop, eventually leading to complete paralysis and death (Lassmann, 2007b; Lucchinetti and Bruck, 2004; Trapp et al., 1999). The devastating and complex nature of MS, combined with the lack of an effective cure, has led to the employment of animal models to aid in elucidating the mechanisms of MS progression and the development of novel therapeutics.

1.1. EAE protocol development

Many experimental animal models have been developed to study MS pathology. Currently, these include: (1) chemically-induced demyelination models, such as cuprizone, ethidium bromide, or lysolecithin administration (Fernandes et al., 1997; Woodruff and Franklin, 1999); (2) viral models, such as infection with Theiler murine encephalomyelitis virus or murine hepatitis virus (Rodriguez, 1988; Sorensen et al., 1980; Mecha et al., 2012); and (3) targeted or transgenic mouse models to knock-out or overexpress chemokines or cytokines in specific cell types (*e.g.*, overexpression of interferon γ in astrocytes) (Kipp et al., 2012). However, the oldest and most studied animal model of MS, experimental autoimmune encephalomyelitis (EAE), has been shown to most closely recapitulate MS pathogenesis (Baxter, 2007; Mangiardi et al., 2011; Sternberger et al., 1984; Wekerle et al., 1994).

The first reported cases of EAE-like symptoms were described in human patients following rabies inoculations by Louis Pasteur. Pasteur's early rabies vaccine was generated by drying and homogenizing infected rabbit spinal cords and administering increasingly virulent spinal cord emulsions in a series of injections. Initially, the treatments were effective and had no detrimental side effects. However, the use of more virulent (*i.e.*, less dried) spinal cords resulted in cases of muscle weakness, paralysis, and, sometimes, death. Interestingly, these side effects were not directly associated with the rabies virus itself, as the pathology was histologically distinct [reviewed by Baxter (2007)].

Intrigued by Pasteur's results, Thomas Rivers investigated the cause of these complications. This led to the first comprehensive description of EAE in 1933 (Baxter, 2007; Rivers et al., 1933). Rivers' initial studies involved injecting Rhesus macaques with an emulsion of brain tissue from healthy rabbits. This induced inflammatory peripheral immune cell infiltration and demyelination, similar to Pasteur's observations and observations in MS patients, and demonstrated that injection with uninfected foreign CNS tissue was sufficient to initiate acute CNS disease. Since these methods were extremely inefficient (Baxter, 2007; Rivers et al., 1933), extensive method development continued in the following decades. This has resulted in increasingly specific reagents for efficient and controlled induction in a variety of species, with mice being most widely utilized (Denic et al., 2011).

Currently, EAE can be induced in mice by immunization with specific myelin peptides (*i.e.*, antigens), such as myelin basic protein (MBP), proteolipid protein (PLP), or myelin oligodendrocyte glycoprotein (MOG) peptides, emulsified with adjuvant (*i.e.*, immunopotentiator) to initiate a T cell response directed against specific myelin proteins. When specific myelin peptides are paired with specific mouse strains, chronic non-relapsing, monophasic, or relapsing-remitting (RR) disease courses are observed, mimicking the clinical forms of MS (Robinson et al., 2014). For example, immunization of B10.PL mice with MBP_{84-104} produces a monophasic disease course (McCarthy et al., 2012), immunization of SJL mice with $PLP_{139-151}$ produces a RR disease course (McRae et al., 1992), and immunization of C57BL/6J mice with MOG_{35-55} produces a chronic, non-relapsing disease course (Mendel et al., 1995). Along with the similarities in disease pathology, this adaptability makes murine EAE the most germane model of MS.

MOG peptides exhibit autoimmune reactivity in more than 50% of MS patients (Kerlero de Rosbo et al., 1997; Kerlero de Rosbo et al., 1995). With this is mind, the MOG peptide-induced EAE mouse model was pioneered by Mendel and colleagues in the mid-1990s (Mendel et al., 1995) when they immunized female C57BL/6J mice with multiple synthetic MOG peptides: 1–21, 35–55, and 104–117. All MOG peptide-immunized mice developed a T cell response; however, more severe neurological impairment was observed in mice immunized with MOG_{35-55}. More specifically, MOG_{35-55}-immunized mice showed persistent neuropathy paired with ascending paralysis, as well as CNS inflammation, demyelination, axonal loss, and gliosis (Mendel et al., 1995; Stromnes and Goverman, 2006a). These clinical and pathological features are similar to those observed in MS patients, supporting the use of MOG_{35-55}-EAE as a model of MS (Crawford et al., 2010b; Mangiardi et al., 2011; Tompkins et al., 2002).

1.2. EAE protocol application

The chronic MOG_{35-55}-EAE model is capable of recapitulating aspects of all three MS subtypes. RR-MS is the most common form of the disease, accounting for 85% of MS patients, and is marked by acute episodes of disability followed by recovery (Lassmann, 2007a). The onset stage of MOG_{35-55}-EAE can serve as a model of early relapses, and it allows experimenters to monitor possible key effectors in MS progression and to test therapeutic interventions prior to permanent CNS damage. Typically, RR-MS patients progress to a chronic disease stage known as secondary progressive (SP) MS, during which they develop permanent motor and cognitive impairments (Lassmann, 2009). A third subtype, primary progressive (PP) MS, affects 15% of patients (Lassmann, 2009) and presents with a chronic disease course at onset, devoid of remittances. As such, the chronic nature of MOG_{35-55}-EAE recapitulates the permanent damage observed in both SP- and PP-MS. Thus, MOG_{35-55}-EAE is an invaluable model for studying both the progression and treatment of multiple MS subtypes.

Examples of how EAE has played a critical role in elucidating MS pathology include the identification of the aryl hydrocarbon receptor as a ligand-dependent transcription factor needed for the development of Th17 and T regulatory responses, and the discovery of retinoic acid receptor-related orphan receptor γ as a critical transcription factor for Th17 cell differentiation (Veldhoen et al., 2008; Ivanov et al., 2006). Additionally, multiple approved MS therapeutics, including the amino acid copolymer glatiramer acetate (Teitelbaum et al., 1999) and the $\alpha_4\beta_1$-integrin (*i.e.*, very late antigen 4) antibody natalizumab (Miller et al., 2003; Yednock et al., 1992), demonstrated efficacy in EAE models prior to proceeding to clinical trials. It has also been reported that all currently-approved MS treatments reduce EAE symptoms to a certain extent (Robinson et al., 2014).