

Establishment of a Model to Examine the Early Events Involved in the Development of Virus-Induced Demyelinating Lesions

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ABSTRACT: The ability to study the immediate, early events in the demyelinating process has been difficult on account of the lack of model systems that address this phase of lesion development. The vast majority of animal models used to study multiple sclerosis (MS) focuses on the later events of myelin destruction. To address this deficiency, we have modified the currently used Theiler's murine encephalomyelitis virus (TMEV)-induced model of demyelination to precisely identify the area where virus-induced demyelination first occurs. Following surgical exposure of the spinal cord, we directly injected TMEV into the spinal cord of female SJL/J mice. Characterization of the events in the spinal cord in the days following injection of virus support the use of this model to dissect the pathways triggered in the host in the early phases of demyelination. A complete understanding of the genesis of the sclerotic plaque may provide insights into enhanced treatment for patients with central nervous system (CNS) demyelination.

KEYWORDS: Theiler's murine encephalomyelitis virus; multiple sclerosis; demyelination; virus-induced demyelination

INTRODUCTION

Multiple sclerosis (MS) is an immune-mediated demyelinating disease, but neither the cause of the disease nor the initial host cell target has been conclusively identified. The well-characterized Theiler's murine encephalomyelitis

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virus (TMEV) mouse model of MS induces pathology similar to that observed in human MS.¹⁻⁶ Demyelination in this model is triggered initially by an environmental agent, TMEV,² and the host subsequently develops an immune response to self-epitopes, which exacerbates demyelination.^{7,8} This is analogous to the hypothesis that virus infection of humans triggers the initial demyelinating process, which culminates in autoimmune responses to central nervous system (CNS) antigens. One of the key mysteries in both human MS and animal models of the disease concerns the early events in the demyelinating process. The ability to study the immediate, early events in the demyelinating process has been difficult and hampered by the lack of suitable models. The most commonly studied murine models of MS, experimental autoimmune encephalomyelitis (EAE) and TMEV, provide researchers with excellent models to study demyelination initially triggered by autoimmune responses or virus infection, respectively (reviewed in Ref. 9). However, these models suffer from a significant shortcoming in that it is not possible to determine the precise age of any particular lesion in the animal. To address this deficiency, we have modified the currently used TMEV-induced model of demyelinating disease to establish a model where the processes invoked by the host response to virus can be dissociated from the damage induced by the immune response to the infection. Furthermore, in the model described herein, we can accurately identify the site of the initial insult, thereby allowing for characterization of the immediate, early processes in the myelin-damaging cascade of events.

Establishment of a Model of Direct Infection of the Spinal Cord with TMEV

To establish a model of CNS demyelination in which we could precisely determine the age of the demyelinating lesion, we exposed the spinal cord of female SL/J mice and then directly injected TMEV (2×10^4 pfu, Daniel's strain) into the spinal cord white matter of the animal. The rationale for development of this model is as follows: Normally in the TMEV model of demyelination, virus is intracerebrally (i.c.) injected into susceptible strains of mice, and by day 21 post infection (p.i.) demyelinating lesions form throughout the spinal cord and brainstem.² While the initial demyelination is virus-mediated, epitope spreading occurs and the chronic ongoing immune response is immune-mediated. Self-reactivity develops to a variety of myelin epitopes, including proteolipid protein (PLP), myelin basic protein (MBP), and myelin oligodendrocyte glycoprotein (MOG).^{7,8} Direct injection of the virus into the spinal cord has an advantage over the traditional method of i.c. injection of TMEV as precise location of the initial site of damage in the spinal cord can be identified; in the i.c. model of infection the lesions form randomly throughout the spinal cord following TMEV injection. Furthermore, the direct injection of the spinal cord

with TMEV permits us to dissociate the initial response of the CNS to virus from the responses of the immune system to viral insult.

To demonstrate that TMEV antigens could be detected in the spinal cord white matter following direct injection of virus into the murine spinal cord, immunohistochemical staining was performed using a polyclonal antisera to TMEV¹⁰; detection was performed using an Alexa-Fluor-conjugated secondary antibody. At 4 days p.i., TMEV antigens were visualized within the spinal cord white matter. To determine whether the immunohistochemical staining was detecting residual viral proteins from the inoculum or whether the spinal cord was supporting a productive infection with TMEV, we dissected tissue from the injection site at day 4 p.i., prepared a 10% (wt/vol) homogenate of the tissue, and performed plaque assays using L2 cells as an indicator cell line.¹¹ At this time point, we detected approximately a 100-fold increase in virus titer ($1-2 \times 10^6$ pfu TMEV) within the lesion site, indicating that virus was replicating at the site of injection and that residual inoculum was unlikely to account for the immunostaining results.

Changes in Myelin PLP mRNA Levels Are Observed following Virus Injection of the Spinal Cord

Several studies have demonstrated that injury to the myelin-producing oligodendrocytes of the CNS results in alterations in mRNA levels of a variety of genes involved in myelination.¹²⁻¹⁶ To determine whether direct injection of virus into the spinal cord white matter of mice affects the levels of myelin gene transcripts, we injected the spinal cord white matter of SJL/J mice with TMEV as described above, isolated RNA from the lesion site, and performed real-time polymerase chain reaction (PCR) using primers and a probe for myelin PLP.¹⁷ PLP mRNA levels were chosen as a surrogate indicator of myelin damage as PLP transcripts have been shown to be altered swiftly after injury in the TMEV model of demyelination.¹⁶ Control animals were mock-infected with virus diluent (Hanks' balanced salt solution). Within 12 h of injection, PLP transcript levels in spinal cord segments from virus-injected animals were markedly increased (six- to eightfold) compared to PLP transcript levels in spinal cord segments from mock-injected animals. PLP mRNA levels increased over the next 48 h in the spinal cords of virus-injected animals and remained elevated through at least 72 hours p.i. These data indicate that there are alterations in gene transcription within the lesion site of mice that have been directly injected with virus, which are consistent with changes that have been described in the demyelinating processes.

DISCUSSION

In these studies, we describe a novel model of virus-induced damage to the CNS with characteristics consistent with demyelination. In this model, we

have the capability of studying the early events in the demyelinating process in the TMEV model in the absence of immune-mediated demyelination, as lymphocytes are not observed in the lesion area in the first 3 days after spinal cord injection. The extent of the spinal cord region first affected by virus injection into the spinal cord can be identified via the addition of Hoechst dye to the inoculum. The presence of the DNA-binding dye in the viral inoculum permits identification of the cells exposed to virus at time zero, enabling the accurate determination of the age of the demyelinating lesion.

Ongoing studies are designed to further characterize this model and allow for a greater insight into the changes that occur in the early phases of lesion development. The model described will allow for study of lesions within minutes of the primary insult to the host, thereby finally allowing us to identify the earliest pathways triggered in this pathogenic process.

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