Coronavirus-induced encephalomyelitis: balance between protection and immune pathology depends on the immunization schedule with spike protein S

Egbert Flory, Albert Stühler, Vesna Barac-Latas, Hans Lassmann and Helmut Wege

Institute of Virology and Immunobiology, University of Würzburg, Versbacher Straße 7, D-97078 Würzburg, Germany, Institute of Neurology, Schwarzeningerstraße 17, A-1090 Vienna, Austria and Federal Research Centre for Virus Diseases of Animals, Friedrich Loeffler Institute, D-17498 Insel Riems, Germany

The neurotropic mouse hepatitis virus MHV-JHM induces central nervous system (CNS) demyelination in Lewis rats that pathologically resembles CNS lesions in multiple sclerosis. The mechanisms of MHV-JHM-induced demyelination remain unclear and several studies have implicated the role of the immune response in this process. We have shown previously that protective immunity against MHV-JHM-induced encephalomyelitis was induced by immunization with a vaccinia virus (VV) recombinant expressing MHV-JHM S-protein (VV-S). Here, we present evidence that the time of MHV-JHM challenge after immunization with VV-S plays a critical role in protective immunity. The induction of virus-neutralizing S-protein-specific antibodies prior to the MHV-JHM challenge modulates the disease process and a subacute encephalomyelitis based on a persistent virus infection developed. Typical pathological alterations were lesions of inflammatory demyelination. In addition, the results indicate that after seroconversion, CD8+ T cells were no longer essential for virus elimination in contrast to their role in protection during acute encephalomyelitis.

Introduction

Coronavirus infection of rodents provides a useful experimental model system to study the mechanisms of virus-induced demyelination and persistence. Interest in the pathogenicity of murine coronaviruses, in particular mouse hepatitis virus (MHV-JHM), has centered on their ability to produce both acute and chronic central nervous system (CNS) diseases.

The coronaviruses are enveloped, with a single-stranded RNA genome of positive polarity (Compton et al., 1993; Lai, 1990). The virion contains four major structural proteins. The glycoproteins, spike (S), the haemagglutinin–esterase (HE) and membrane (M) proteins are expressed on the virion surface. The predominant spike protein is the S-protein on the virion surface, which interacts with the viral receptor on the surface of target cells and is the major determinant of viral pathogenicity (Collins et al., 1982; Fleming et al., 1986; Wege et al., 1988; Gallagher et al., 1990; Gombold et al., 1993).

Infection of Lewis rats with the neurotropic strain MHV-JHM results in an acute and fulminant encephalomyelitis (AE) with little evidence of demyelination. The other course of the disease, the subacute demyelinating encephalomyelitis (SDE) involves paralysis and is characterized by selective loss of myelin and inflammation in the white matter of the CNS (Nagashima et al., 1978; Sorensen et al., 1980; Watanabe et al., 1983; Koga et al., 1984; Wege et al., 1984b; Zimprich et al., 1991).

Establishment of a persistent virus infection in vivo requires that the virus must persist within cells as a nonlytic infection. In addition, infected cells must remain viable within the organism in the presence of antiviral immune responses (Oldstone, 1991). Results from several studies on MHV-JHM-induced encephalomyelitis indicate that the host immune response plays a dual role. On the one hand, an effective cellular and humoral immune response is able to protect against acute lethal viral infection (Sussman et al., 1989; Williamson & Stohlman, 1990; Körner et al., 1991; Schwender et al., 1991; Bergmann et al., 1993; Wege et al., 1993). On the other hand, there is evidence that the immune response is able to promote the establishment of a chronic demyelinating disease associated with virus persistence (Shubin et al., 1990; Wang et al., 1990). Suckling mice and rats nursed by immune mothers can develop a subacute disease after infection with an otherwise lethal dose of MHV-JHM, indicating that IgG in the milk may contribute to the course of disease (Perlman et al., 1987).
Finally, the passive transfer of antiviral antibodies can modulate the course of an acute encephalomyelitis to a subacute disease in mice (Buchmeier et al., 1984).

We have previously shown that vaccinia virus recombinants (VV) expressing S-protein (VV-S) protect against MHV-JHM-induced AE. In these experiments, challenge with an otherwise lethal dose of MHV-JHM was performed prior to the induction of virus neutralizing S-protein specific antibodies (Flory et al., 1993). In vivo depletion of the CD8+ T cell subset indicated that these VV-S primed cells are a primary mechanism of immunological defence against MHV-JHM. Virus elimination and hence protective immunity depend on the presence of CD8+ T cells in the early acute phase of the disease process.

In the experiments reported here, we evaluated the influence of S-protein-specific neutralizing antibodies in the presence of virus-specific CD8+ T cells on the course of the disease. For this purpose, adult Lewis rats were immunized with VV-S and then challenged with MHV-JHM, at a time when S-specific virus-neutralizing antibodies were detected at a high level. Moreover, we analysed whether the presence of CD8+ T cells after seroconversion was essential for subsequent protection in the late chronic phase of the disease process.

Methods

Viruses. The MHV-JHM wild-type virus used in this study was passaged three times in mouse brain and twice in rat brain (Nagashima et al., 1978). The recombinant vaccinia virus expressing the MHV-JHM wild-type S-protein (here designated VV-S) has been described in detail previously (Flory et al., 1993). VV-wt denotes the original vaccinia virus strain WR.

Animals. Specified pathogen-free Lewis rats (MHC-RT1) were purchased from the Zentralinstitut für Versuchstierzucht Hannover, Germany. Female Lewis rats (3 to 4 weeks) were immunized by intraperitoneal (i.p.) injections of 1 x 107 p.f.u. of recombinant vaccinia virus (VV-S).

Protection against lethal MHV-JHM challenge infection. Five to nine Lewis rats per experimental group were challenged by intracerebral infection (i.e.) with 5 x or 32 x LD50 of MHV-JHM after vaccination, which had been done either 21 or 7 days previously. The challenge virus (volume 40 ll) was inoculated into the left brain hemisphere. The incidence of disease was registered over a time period of 7 weeks.

Serological assays and virus titration. Antiviral IgG antibodies were measured by ELISA and neutralization assay (Wege et al., 1984a, b). The amount of infectious virus in brain homogenates was determined by plaque assays using cultures of DBT cells (Wege et al., 1988).

Depletion of CD8+ T cells in vivo. CD8+ T cells were depleted in animals following two injections (i.p.) of MRC Ox8 monoclonal antibody (200 ll) on two consecutive days, as described previously (Körner et al., 1991; Flory et al., 1993). This treatment resulted in complete disappearance of the CD8+ T cell population from lymph nodes for at least 20 days as determined by FACS analysis. Lymph nodes were prepared as a single cell suspension and stained with antibody Ox8 and isotype control antibody Ox21 against rat T cell surface molecules.

Results

VV-S immunization induces a MHV-JHM S-protein-specific IgG response

The induction of a S-protein-specific humoral immune response was determined in Lewis rats immunized with VV-S. At various times after vaccination rat sera were taken and analysed by ELISA and neutralization assay. The first S-protein-specific IgG antibodies were detectable 10 days after immunization and reached a maximal level at day 21 (Fig. 1a). Virus-neutralizing...
antibodies were seen first 15 days after immunization and reached highest titres 21 days after immunization (Fig. 1b).

**VV-S-induced S-protein-specific antibodies modulate the course of MHV-JHM-induced encephalomyelitis**

The protective capacity of VV-S immunization against lethal coronavirus disease was investigated. Lewis rats were protected against lethal encephalomyelitis when immunized with VV-S 7 days prior to infection (Table 1; Flory et al., 1993). At the time of challenge, no S-specific neutralizing antibodies were detected (Fig. 1b). Of the VV-S-vaccinated animals, only one out of eight rats developed AE and died within 12 days of the challenge. Histological analysis of brain tissue from protected Lewis rats was performed up to 40 days after MHV-JHM challenge. No pathological changes were seen and no virus antigen was detectable by immunohistochemistry (Table 1).

We were interested to learn whether the induction of S-protein-specific antibodies prior to infection modulates the course of disease. Therefore, Lewis rats were challenged with MHV-JHM 21 days after immunization with VV-S, when S-specific-neutralizing antibodies are detected. At this time interval between priming and challenge infection, immunization results again in a protective immune response against MHV-JHM-induced AE (Table 1). All Lewis rats in the experimental group overcame the acute phase of the disease and showed no clinical signs (mortality 0/9). Although these rats did not display clinical signs, histological analysis revealed interesting pathological changes. Groups of three rats were dissected 3, 4 and 6 weeks p.i. In 7/9 cases, extensive lesions of inflammatory demyelination were found (Fig. 2a, b, c). The lesions were mainly located in the white matter of the spinal cord, brain stem and periventricular area (Table 1). Within the lesions, virus antigen was detected in few cells (Fig. 2d, e). These changes are typical of a subacute demyelinating encephalomyelitis (SDE), which is a more chronic form of the disease. In the brain tissue of one rat no virus antigen or other pathological changes were found with the exception of rare small scars of old lesions. One animal did not show any pathological alteration in the CNS.

To demonstrate the specific effect of the immune response induced by VV-S, vaccination was also done with VV-wt, which does not express S-protein. All these VV-wt-vaccinated animals developed AE and died within 12 days of challenge (Table 1).

**Isolation of MHV-JHM from the brain of VV-S-immunized and challenged Lewis rats**

The presence of infectious MHV-JHM in the brain of immunized and challenged Lewis rats was analysed by virus re-isolation assays. At various times post-MHV-JHM infection, re-isolation was performed by cultivation of homogenized brain tissue on DBT cells (Table 2). Brain tissue from animals vaccinated with VV-wt 21 days prior to the challenge infection contained high titres of
Fig. 2. Spinal cord from a Lewis rat inoculated i.c. with MHV-JHM after vaccination 21 days previously and examined at day 20 post-infection. (a) Large demyelinating plaque in anterior column of spinal cord. Luxol fast blue × 40. (b) Serial sections stained by immunohistochemistry for GFAP. Extensive glia reaction in the border of the lesion; reduction of GFAP⁺ cells in the centre of the lesion. Anti-GFAP × 40. (c) Serial sections stained with ED1. Massive macrophage infiltration in demyelinated plaque and some ED1⁺ cells in the surrounding grey and white matter. Anti-ED1 × 40. (d) Serial sections of the same lesion; virus-infected cells (arrows) are present in the plaque. Anti-MHV-JHM × 100 (e) Higher magnification showing several virus-infected cells (arrows) and some myelinated nerve fibres with MHV-JHM antigen in the oligodendrocyte cell processes (arrowheads). Anti-MHV-JHM × 430.

Table 2. Isolation of MHV-JHM from the brain tissue of vaccinated and challenged Lewis rats

<table>
<thead>
<tr>
<th>VV-R vaccination*</th>
<th>Time of challenge (days post-immunization)*</th>
<th>Virus titre (p.f.u./g tissue) on indicated day post challenge†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+3</td>
<td>+6</td>
</tr>
<tr>
<td>VV-S</td>
<td>7</td>
<td>0.1 × 10⁵</td>
</tr>
<tr>
<td>VV-wt</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>VV-S</td>
<td>21</td>
<td>9.7 × 10⁴</td>
</tr>
<tr>
<td>VV-wt</td>
<td>5.3 × 10⁶</td>
<td>1.8 × 10³</td>
</tr>
</tbody>
</table>

* Lewis rats were vaccinated i.p. with a dose of 1 × 10⁵ p.f.u. of VV and challenged with 5 × LD₅₀ MHV-JHM 7 days later or 21 days later with 32 × LD₅₀ MHV-JHM.
† Virus titres were determined in three to five individual rats per group.
‡ 0, Virus titre below the level of detection (< 0.1 × 10⁵ p.f.u./g tissue).
§ -, Lewis rats died with AE 7 to 9 days after challenge infection.

MHV-JHM. Rats in this experimental group died 7 to 9 days p.i. with AE. In contrast, brain tissue from Lewis rats immunized with VV-S and challenged 21 day later yielded a very low titre of infectious virus at days 3 and 6 following infection (Table 2). No infectious virus was detected 10 and 18 days after the challenge infection. This is similar to the findings in rats challenged 7 days after immunization, where infectious virus was eliminated.

Effect of CD8⁺ T cell depletion on the demyelinating disease process

To investigate the role of CD8⁺ T cells in VV-S-immunized Lewis rats in the presence of S-protein-specific IgG antibodies with virus-neutralizing activity,
the animals were depleted of CD8+ T cells by two injections of Ox8 monoclonal antibodies (anti-CD8 MAb) given 3 days and 1 day prior to challenge infection. The efficiency of the depletion was monitored by FACS analysis of cervical lymph node cells and resulted in a physical loss (reduction of more than 99%) of CD8+ T cells (Fig. 3). The depletion had no influence on the protection against acute and chronic disease mediated by VV-S immunization 21 days before MHV-JHM challenge (Table 1). Of eight animals seven survived the challenge with an otherwise lethal virus dose. In the brain of these Lewis rats there were no neuropathological changes detected 20 to 32 days following infection. Only one rat died of AE 12 days after challenge. By contrast, when immunized Lewis rats were challenged within 7 days of immunization, all anti-CD8-treated animals developed AE and died (Flory et al., 1993).

Discussion

Previous experiments have demonstrated that VV-S immunization protects against MHV-JHM-induced encephalomyelitis. This protective effect depends on the presence of S-protein-specific CD8+ T cells and is associated with virus elimination (Flory et al., 1993). By using an altered vaccination protocol, we have obtained evidence that the antiviral humoral immune response and the presence of CD8+ T cells may contribute to virus persistence and CNS demyelination. Moreover, after seroconversion, when S-protein-specific antibodies are present, the CD8+ T cells are no longer the principal mechanism in protective immunity.

After MHV-JHM infection most of the animals died of AE 7 to 10 days p.i. Depending on the MHV-JHM virus passage history, Lewis rats developed a subacute disease, at a time when the antiviral humoral immune response reached detectable levels (Nagashima et al., 1978; Koga et al., 1984). To investigate the influence of S-protein-specific immunity on the course of an MHV-JHM infection the Lewis rats were challenged with MHV-JHM after the induction of S-protein-specific virus-neutralizing antibodies by a specific immunization schedule with VV-S. The immunization schedule employed here simulates to some extent a situation in the late chronic phase of disease. Protective immunity induced with this immunization procedure is obviously not sufficient for complete virus elimination and the disease is modulated to a clinically silent, chronic course. Histological examination of rat brains indicated centres of both active and inactive inflammatory lesions and virus-infected cells throughout the brain and spinal cord, typical of SDE.

The following observations suggest that immune reactions against viral antigen are involved in the establishment of a persistent virus infection resulting in inflammatory demyelination. Mice or rats infected in the presence of maternal immunity specific for MHV-JHM developed demyelinating disease several weeks later (Wege et al., 1981; Perlman et al., 1987). Experiments in which lymphocyte subpopulations were transferred into rats immunosuppressed by irradiation have been done recently (Schwender et al., 1994). The results indicated that secreted antibodies help to restrict virus replication to small foci within the white matter. Antiviral antibodies
seem to play a pivotal role in modulating the disease. Treatment of MHV-JHM-infected mice with a MAb against S-protein modulated the disease from an acute encephalitis to a disease with demyelination and ongoing virus replication in the brain of mice (Buchmeier et al., 1984). The presence of antiviral antibodies may act predominantly to shield neurons against injury caused by virus replication and change the tropism to prevent a lethal disease. Primarily, the antibodies could directly prevent the spread of extracellular virus, but are not sufficient to eliminate the virus. Moreover, results obtained by study of Semliki Forest virus infections support the conclusion that antibodies can enter neurons and interact with replicating virus or impair viral gene expression (Levine et al., 1991).

To evade the immune system during persistent infection, the expression of glycoproteins on the cell surface can be reduced compared with that seen during acute infection (Fujinami et al., 1984; Barrett et al., 1985). This process is generally known as antigenic modulation and may also occur in vivo. The mechanism is explained by masking, shedding or internalization of viral glycoproteins from the surface of infected cells in the presence of antiviral antibodies. Such a mechanism could help the virus to escape the host's immune surveillance in the late phase of infection, or, as in the present study, the S-protein-specific neutralizing antibodies induced by immunization. In favour of this idea is the finding that in MHV-JHM-infected glial cell cultures, S-protein-specific antibodies promote the establishment of a persistent infection and reduce the relative number of cells displaying MHV-JHM S-protein on the cell surface (Wege et al., 1990). Moreover, results obtained by quantitative immunohistological analysis of demyelinating plaques in MHV-JHM-infected Lewis rats with SDE support this concept (Zimprich et al., 1991).

The results of the CD8+ T cell depletion experiment after the induction of S-protein-specific antibodies demonstrate that these cells are essential for protection only, when a humoral immune response is not yet established. After CD8 depletion the Lewis rats were protected against acute disease and even the pathological changes of SDE were no longer detected in the brain. It is possible that in the early acute phase of the disease a high precursor CD8+ T cell (CTL) frequency is induced by VV-S immunization, which mediates protective immunity against an otherwise lethal infection for only a short time after immunization. In the lymphocytic choriomeningitis virus (LCMV) system protection against lethal infection by immunization with a recombinant VV expressing LCMV glycoprotein depends on a high level of CTL precursors and/or on an activated state of memory CTL (Oehen et al., 1992; Battegay et al., 1993). The kinetics of immunological memory in the case of a time interval of 21 days between VV-S-induced priming and MHV-JHM challenge could lead to a state of low-level CTL memory and the protective CD8+ T cells are no longer indispensable for protective immunity. A functional test to demonstrate this hypothesis is not yet available for the coronavirus–rat system. However, in the late phase of the disease virus elimination could be augmented by cooperation of S-specific CD4+ T lymphocytes with other immune cells. In cytomegalovirus infection the cooperation of CD4+ T cells with other undefined cells after a long-term CD8+ T cell depletion leads to efficacious control of infection (Jonjic et al., 1990).

In natural MHV-JHM infection, the combined activation of virus-specific CD4+ T cells and CD8+ T cells plus antiviral antibodies are a prerequisite for efficient elimination of virus-infected cells. Our observations show that immunization with a recombinant VV expressing a MHV-JHM glycoprotein is able to induce protective immunity and may contribute to demyelination, depending on the time schedule of immunization. An imbalance in the immune system may promote the establishment of persistent virus infections.

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References


