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Mouse hepatitis virus

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Inoculation of mice with most neurotropic strains of the coronavirus mouse hepatitis virus results in an immune response-mediated demyelinating disease that serves as an excellent animal model for the human disease multiple sclerosis. Recent work has shown that either virus-specific CD4+ or CD8+ T cells are able to mediate demyelination and also that the antibody response is crucial for clearing infectious virus. Another exciting advance is the development of recombinant coronaviruses, which, for the first time, will allow genetic manipulation of the entire viral genome.

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Current Opinion in Microbiology 2001, 4:462–466

1369-5274/01/$ – see front matter

Abbreviations

CNS	central nervous system
CTL	cytotoxic T lymphocyte
IFN-γ	interferon γ
MHV	mouse hepatitis virus
pi	post infection
S	surface

Introduction

Depending on the strain used, mouse hepatitis virus (MHV) causes a variety of diseases, such as enteritis, hepatitis and demyelinating encephalomyelitis, in susceptible rodents [1]. Infection of susceptible strains of mice with strain MHV-3 results in severe hepatitis caused, in part, by overexpression of prothrombinase [2]. This infection may serve as a useful model for acute, fulminant hepatitis in humans [3]. Infection of immunocompetent mice with the neurotropic JHM strain of MHV results in acute encephalitis, followed, variably, by the development of chronic demyelination in survivors. Virus is not cleared from the central nervous system (CNS), effectively resulting in a persistent infection, although infectious virus is only recovered from chronically infected mice in some models. Viral RNA is readily detected in the CNSs of these mice [4] and, more recently, virus-specific CD8+ T cells were also recovered from the CNSs of chronically infected mice at more than 60 days post infection (pi) [5]. Several characteristics of the CNS foster its predisposition to persistent viral infection, such as low-to-undetectable expression of MHC classes I and II molecules on oligodendrocytes, neurons and astrocytes, limited immune surveillance, and low apoptotic rate of CNS cells [6].

Although it was initially believed that JHM-induced demyelination was caused by virus destruction of oligodendrocytes, more recent studies using immunodeficient mice showed that the process of myelin destruction is largely immune response-mediated [7–11] (see also Update). However, the precise role of individual components of the immune system in this process is not known and remains an area of intense investigation.

This review provides a brief discussion of recent work with MHV that has helped define the association between viral persistence in the CNS and chronic immune stimulation, which ultimately results in neuropathology. Also discussed are recent advances that allow, for the first time, genetic manipulation of the genomes of coronaviruses, the largest known RNA viruses.

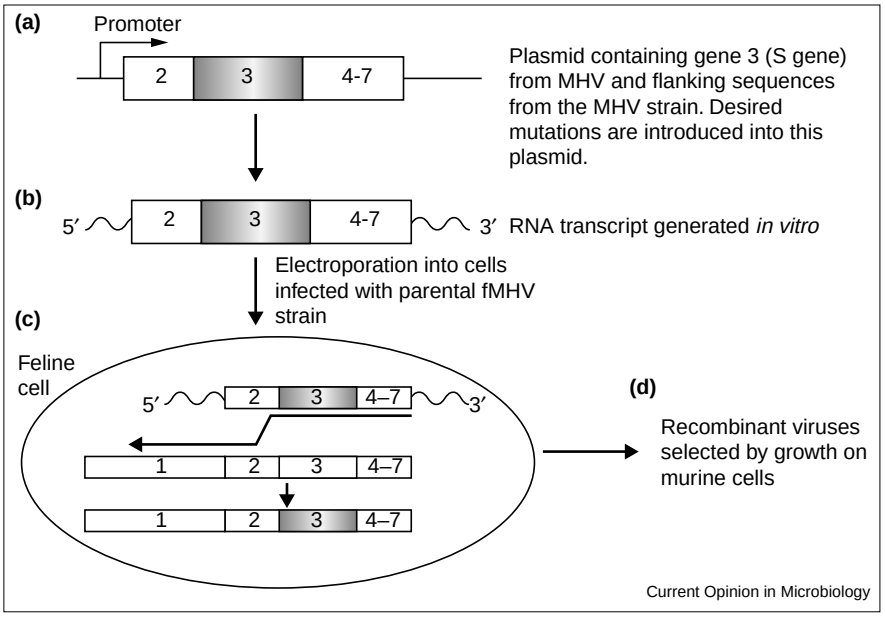
T-cell responses in the CNS following infection with strain JHM

Intracerebral or intranasal infection with the JHM strain induces a massive influx of inflammatory cells, including CD8+ and CD4+ T cells, into the murine CNS. The development of techniques for the identification of antigen-specific T cells has facilitated measurement of virus-specific T cell responses [12,13]. Both CD4+ and CD8+ T cells are, in large part, MHV-specific in the acutely infected CNS, but the specificity of all T cells has not been ascertained in any model.

Following infection with strain JHM, approximately 50–70% of CD8+ T cells [14••,15] and 35% of CD4+ T cells [16•] are virus-specific in immunocompetent mice at seven days p.i. CD8+ T cells are critical for virus clearance and, as described below, are able to induce demyelination. During persistence, CD8+ T cells lose cytolytic activity [14••]. CD8+T cells that remain in the CNS at 35–45 days p.i. are still able to secrete interferon-γ (IFN-γ) when specifically stimulated, showing that they have not completely lost effector function [14••], as has been described in mice persistently infected with lymphocytic choriomeningitis virus [17]. IFN-γ appears to be critical for controlling viral replication, particularly in oligodendrocytes [18]. Consistent with this, more IFN-γ-secreting CD8+ T cells are detected in the CNSs of asymptomatic mice than in mice with clinical disease [19].

CD8+ T cell numbers decline as infectious virus is cleared, but a portion of recruited T cells (both MHV-specific and non-MHV-specific) are retained within the CNS. This retention is correlated with the continued presence of viral RNA [19]. These data contrast what was shown after infection with a neurotropic strain of influenza virus. In this model system, activated CD8+ T cells are maintained in the CNS in the absence of influenza virus or viral antigens [20]. The contrast in CNS T cell retention between these two viral infections may reflect either differential viral tropism, altered T cell activation status at the time of CNS entry, or both [5].

Figure 1



Targeted recombination. The development of targeted recombination has facilitated the introduction of mutations and/or different sequences into the genome of MHV. In the example shown in this figure, a recombinant virus is produced whose gene 3 (S gene) differs from that of the parental virus. Exchange of the S gene permits tissue culture selection of recombinant viruses by altering host-cell specificity. Recombinant viruses may also have unique pathogenic profiles, as described in the text. **(a)** Initially, a plasmid was constructed that contains the murine S gene (shaded gene 3). The plasmid could contain either a mutation in any of the MHV genes present in the plasmid or a new sequence to be introduced into the MHV genome, and flanking sequences from MHV. **(b)** An RNA copy of the MHV sequence within the plasmid was generated *in vitro*. **(c)** The *in vitro*-generated transcript containing the murine S gene was electroporated into feline cells. These cells were previously infected with a chimeric MHV that expressed the S protein from feline infectious peritonitis virus (unshaded gene 3, termed fMHV for feline MHV). This virus has the ability to infect feline cells and not murine cells. Recombination takes place (as indicated by the right to left arrow) between the fMHV RNA genome and the generated transcript containing the murine S gene. As a result, replication-competent viruses will be produced that have exchanged the fMHV S gene for the murine S gene (indicated by the downward arrow). **(d)** Recombinant viruses are, in turn, selected by their ability to infect mouse cells and not feline cells.

Further evidence for the critical role that $CD8^+$ T cells have in virus clearance came from studies of MHV cytotoxic T lymphocyte (CTL) escape mutants. Suckling C57BL/6 mice infected with MHV are protected from developing acute encephalitis by nursing with dams previously immunized to MHV. However, a significant fraction of these mice later develops demyelinating encephalomyelitis. All viruses recovered from these chronically infected mice are mutated in the dominant $CD8^+$ T cell epitope recognized in this strain [21]. These results also indicate that a key part of the MHV pathogenic process is infection of cells expressing MHC class I molecules.

Anti-MHV $CD4^+$ T cells are also critical for virus clearance. $CD4^+$ T cells are necessary for $CD8^+$ T cell survival in the infected CNS. In their absence, $CD8^+$ T cells rapidly undergo apoptosis after entering the parenchyma of the CNS [22]. In addition, lymphocyte and macrophage infiltration into the CNS is delayed in the absence of $CD4^+$ T cells. This effect is probably mediated by RANTES, as $CD4^+$ T cells are an important source of this chemokine [23]. Furthermore, fewer MHV-specific $CD4^+$ T cells are present in the CNSs of chronically infected mice when assayed by IFN-γ secretion [16•]. Whether this is the result of specific T cell deletion or a loss of effector function remains to be determined.

Recently, MHV-specific antibody was shown to be necessary to prevent viral reactivation in the infected CNS [24••]. $IgM^{-/-}$ mice, which are blocked in B cell development and therefore do not make antibodies, initially clear virus normally. However, virus titers rebound and mice succumb to the infection. Passive administration of antibody after initial clearance prevented the recrudescence of infection. These results were unexpected and raise questions about the efficacy of the $CD8^+$ T cell response in the absence of B cells.

Host factors critical in the demyelinating process

Some progress has been made in identifying the immune cells and cellular functions required for demyelination. Infection of mice lacking T cells (severe combined immunodeficiency [SCID] or recombination-activating gene knockout [$RAG1^{-/-}$] mice) results in fulminant encephalitis without evidence of demyelination [10,11]. Adoptive transfer of splenocytes from syngeneic (genetically identical) immunocompetent mice into infected SCID or $RAG1^{-/-}$ mice resulted in rapid development of demyelination within seven to nine days post transfer (p.t.). Demyelination occurs with transfer of splenocytes that have been depleted of $CD4^+$ or $CD8^+$ T cells, but not of both cell types, demonstrating that T cells are necessary for demyelination to develop. Additional experiments indicated that either T cell subset ($CD4^+$ or $CD8^+$) is capable of initiating this process. Mice that receive splenocytes depleted of $CD4^+$ T cells survive longer and develop more demyelination than do mice that receive splenocytes depleted of $CD8^+$ T cells, indicating that the roles of each T cell subset in this disease process are not identical [25••].

Following T cell transfer, a massive increase in the numbers of macrophages or microglia is observed at areas of demyelination, suggesting that, in agreement with previous studies, these cells may be the final effector cells in this process [11,26]. Depletion of hematogenous macrophages using the drug dichloromethylene diphosphate does not decrease the level of demyelination, suggesting that perivascular macrophages and parenchymal microglia, neither of which is depleted by the drug, may mediate demyelination [27].

Thus far, no single lymphocyte function has been shown to be essential for demyelination to develop. Neither IFN-γ, tumor necrosis factor α (TNF-α), perforin, IL-10 nor inducible nitric oxide synthase (NOS2) is required for demyelination either in the transfer model or in the MHV-infected immunocompetent mice [18,28–30]. One can conclude from all these data that the effector mechanisms of demyelination are complex and largely redundant.

In contrast, the factors involved in the initiation of the immune response to MHV following infection may not be redundant. Chemokines, secreted by many cells in the body (such as astrocytes or microglia) serve to recruit specific cellular subsets. Several chemokines, such as IP-10, CRG-2, MIP-1β, MCP-1, MCP-3, RANTES, MIP-2 and Mig, are expressed after MHV infection [31]. Neutralization of IP-10 or Mig at the time of infection with MHV results in increased mortality, higher viral loads and markedly decreased T cell infiltration into the CNS [32•,33•]. In contrast, mice treated with RANTES antiserum have delayed viral clearance, decreased T cell infiltration, and significantly less demyelination than do untreated mice, but show no change in mortality [23].

Targeted recombination allows detailed molecular analysis of MHV

As stated in the Introduction, pathogenic outcome is strongly dependent on the inoculating virus strain. Indeed, this is evident even among strain variants derived from strain JHM, some of which display pathogenic profiles distinct from those described in the previous section. It is therefore clear that the study of pathogenic mechanisms of MHV infection can be furthered through creation of defined strain chimeras and site-directed mutants. Recent developments in the manipulation of large coronavirus RNA genomes have now allowed for creation of these defined mutants. These viruses are being used in rodent infection models to understand the viral genetic basis of pathogenicity. The sheer size of the MHV genome (32 kb) has hindered the development of an infectious cDNA clone. A recently developed technique uses the high level of RNA recombination observed in MHV-infected cells to introduce mutations or new genetic sequences into MHV [34•] (Figure 1). A modified synthetic transcript encoding sequences from the 3′ end of the MHV genome is introduced via electroporation into cells already infected with a parent virus. These transcripts contain desired mutations as well as selectable 'marker' mutations that, when fixed into recombinant MHV genomes, will confer growth advantages under certain experimental conditions. Recombinant viruses containing desired mutations are therefore selected by virtue of heat resistance or altered cell tropism [34•,35••].

This approach, termed targeted recombination, has been used to reveal the important role of virus-receptor–host-receptor interactions in MHV pathogenesis. The cellular receptor for MHV is a member of the carcinoembryonic antigen family of cell adhesion molecules (murine CEACAM1[a]). Controversy remains as to whether this is the primary receptor in the CNS [36,37]. Viral attachment is mediated by the surface (S) glycoprotein, which is cleaved into two fragments in many strains of MHV. S1 mediates binding to infected cells and S2 is critical for virus–cell fusion. Previous results, using natural and antibody-selected variants of MHV, showed that variations in the S protein had a dramatic effect on pathogenesis. It has recently been suggested that some of the differences in pathogenesis reflect instability of S1–S2 complexes. Increased stability of the complex correlates with increased growth in tissue culture cells [38]. Viruses with increased stability of the S1–S2 complex are less neurovirulent than the parental strain, suggesting that a more labile interaction may enhance viral entry in the infected murine host.

More recently, using targeted recombination, the S genes from different strains of MHV have been swapped. Introduction of the S gene of the highly neurovirulent strain JHM into the mildly neurovirulent strain MHV A59 resulted in a substantial increase in neurovirulence, paralleling that of the JHM parental strain [39]. In another study, the S gene of strain A59 was replaced with the S gene from MHV-2, a nondemyelinating virus that causes hepatitis. The resulting virus caused severe hepatitis, but not demyelination, although, unlike the strain MHV-2 parental strain, it was able to persist in the spinal cord [40]. Targeted recombination was also used to illustrate a critical role for the S protein of another coronavirus, transmissible gastroenteritis virus (TGEV), in cellular tropism and pathogenesis [41]. These studies together confirm that the S protein has a critical role in coronavirus pathogenesis. More recently, infectious cDNA clones have been developed for several coronaviruses [42•,43•]. The development of full-length infectious clones provides obvious advantages, including the ability to introduce mutations into genes at the 5′ end of the genome, elimination of the necessary selection process and increased ability to identify lethal mutations [34•]. However, given the size of the genome, targeted recombination may remain the method of choice for manipulation of the 3′ end of the genome.

Conclusions

Recent studies have solidified the link between viral persistence, chronic T cell stimulation and demyelination. Although some of the key mediators in the initiation of the

anti-MHV immune response in the CNS have been identified, much less is known about the role of specific cellular mediators in the demyelinating process. Other areas of investigation in the future will be directed at understanding how the virus persists in the infected animal. In particular, determining the cellular site and physical state of the virus during persistence will be crucial to our understanding of this process. The ability to answer these questions and others about MHV-induced disease will be advanced by the recent development of methods to manipulate the coronavirus genome.

Update

Thiel *et al.* [44••] recently showed that recombinant human coronaviruses 229E could be generated using a vaccinia virus (VV)-based system. This method should be generally useful for the development of recombinant coronaviruses, as VV vectors are stable and can be assembled by *in vitro* ligation without passage in bacteria. In another development, Dandekar *et al.* [45] showed that MHV causes axonal damage in areas of demyelination. Strikingly, axonal damage was observed nearly concomitant with myelin damage, even before gross demyelination was detectable.

Acknowledgements

We thank T Gallagher for critical review of this manuscript. This research was supported in part by grants from the National Institutes of Health (NS36592, AI43497 and NS40438) and the National Multiple Sclerosis Society (RG2867). JH was supported by an institutional training grant (P32 AI 07533).

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