CD4+ T cells are important for clearance of DA strain of TMEV from the central nervous system of SJL/J mice

Xiaoqi Lin¹, Xiaoxing Ma¹, Moses Rodriguez² and Raymond P. Roos¹

¹Department of Neurology, University of Chicago, Chicago, IL 60637, USA
²Departments of Neurology and Immunology, Mayo Clinic, Rochester, MN 55905, USA

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Abstract

DA and other TO subgroup strains of Theiler’s murine encephalomyelitis virus synthesize a protein called L* which interferes with DA virus clearance from the central nervous system of susceptible SJL mice, thereby fostering virus persistence and a late demyelinating disease. By using CD4 (−/−) and CD8 (−/−) mice as well as adoptive transfer studies we demonstrate that susceptible mouse strains are capable of clearing DA virus through a CD4+ T cell-dependent activity that is inhibited by L*.

Introduction

Theiler's murine encephalomyelitis viruses (TMEV) are members of the Cardiovirus genus of Picornaviridae. TMEV strains fall into two subgroups on the basis of their differing biological activity (1). GDVII strain and other members of the GDVII subgroup cause an acute neuronal disease with no evidence of virus persistence. In contrast, inoculation of DA strain and other members of the TO subgroup of TMEV into susceptible mouse strains (e.g. SJL/J [H-2s] and B10.S [H-2s]) leads to virus persistence in the central nervous system (CNS) and the induction of an immune-mediated late demyelinating disease (1). DA virus-induced demyelination is considered to be an excellent model of multiple sclerosis (MS) because both disease processes have a similar inflammatory demyelinating disease pathology that appears to be mediated by immune factors.

TO subgroup strains synthesize an 18 kDa protein called L* both in vitro and within the CNS from an AUG 13 nucleotides downstream and from out of frame with the polyprotein (2,3). L* has been hypothesized to interfere with virus clearance and has been implicated as key to DA virus persistence (with subsequent demyelination) on the basis of the following information. Members of the GDVII subgroup, which neither persist nor demyelinate, have an ACG corresponding to the location of the DA L* initiating AUG, so that no L* protein synthesis is predicted to occur. A mutant of DA virus, called DAL*-1 virus, which has a change of the L* initiating AUG to ACG (3), as well as other DA virus mutants which synthesize a truncated L* (4), are cleared from the CNS and produce little or no demyelinating disease in susceptible mouse strains. L* is not an absolute requirement for persistence and demyelination, since mice inoculated with recombinant GDVII/DA viruses that are not predicted to synthesize L* have minimal evidence of virus persistence and demyelination. Our previous studies showed that 7 days following infection with DAL*-1 virus, SJL mice generate a prominent H-2K-restricted late cytotoxic (CTL) T cell response (5). Of interest, this H-2K restricted CTL response has not been associated with virus clearance (6). Although our initial studies suggested that L*, which is synthesized in the wt-DA virus infection, could interfere with the virus-specific CTL response in susceptible strains of mice, it remained unclear whether the inhibition of this response was key to the failure of the host to clear wt-DA virus or whether L* interfered with other aspects of the immune response. In the present study we sought to clarify these issues by making use of CD4- and CD8-deficient SJL/J mice as well as carrying out adoptive transfer experiments. We found that CD8+ T cells are unable to clear DA virus from the CNS of susceptible mouse strains, and that DA virus is cleared from susceptible mouse strains through a CD4+ T cell-dependent activity that is inhibited by L*.

The first two authors contributed equally to this work.

Correspondence to: R. P. Roos; E-mail: roos@neurology.bsd.uchicago.edu

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Methods

Viruses

wt-DA virus was prepared from a wild-type DA full-length infectious clone, DAFL3 (7). The generation of DAL*-1 virus has been described (5). The viruses were propagated in BHK-21 cells.

Animals

Four-week-old female SJL/J mice were purchased from Jackson Laboratories (Bar Harbor, ME). CD4 (--/--) or CD8 (--/--) mice that had been backcrossed for 8 to 12 generations to SJL/J mice have been previously described (8). All mice used in the study were inoculated intracerebrally (IC) with wt-DA or DAL*-1 virus at 2 × 10⁶ PFU.

Adoptive transfer studies

CNS-infiltrating lymphocytes (CNS-ILs) were harvested 7 days post-inoculation from SJL/J mice which had been IC inoculated with wt-DA virus or DAL*-1 virus; the CNS-ILs were isolated by Percoll gradient and FACSorted into CD4+ and CD8+ T cells using previously published methods (9). A second group of SJL/J mice were inoculated IC with wt-DA virus at the same time that they were intravenously (IV) inoculated with 5–10 × 10⁵ of the CD4+ T cells or CD8+ T cells that had been purified from CNS-ILs 7 days PI. This second group of mice was sacrificed 45 days post-inoculation to determine the effect of the transferred CD4+ or CD8+ T cells on wt-DA virus infection.

Pathologic analysis

Mice were anesthetized and perfused 45 days post-inoculation with Trump’s fixative (phosphate-buffered 4% formaldehyde, pH 7.2) and the spinal cord was processed for pathologic analysis after embedding in 2-hydroxyethyl methacrylate and staining with a modified erichrome method and cresyl violet counterstain (5). Pathological abnormalities were blindly quantitated on coronal sections. Statistical significance was determined by comparing the total number of quadrants with and without a pathological abnormality in the spinal cord of one group of mice with the total number of quadrants from the other group. In the case of the adoptive transfer studies, statistical significance was determined by comparing the total number of quadrants with a pathological abnormality in the spinal cord of mice that were inoculated with wt-DA virus under one adoptive transfer condition with the total number of quadrants from wt-DA virus infected mice that had no adoptive transfer. Statistical significance was reported as \( P < 0.05 \) by Chi square analysis.

In situ hybridization

In situ hybridization was performed on sections from the CNS using previously published methods (10).

Immunohistochemical studies

The fixed sections from the CNS of mice were immunohistochemically stained with a monoclonal antibody that reacts with VP1 of all TMEV strains (11) that had been biotinylated. The biotinylated antibody was detected with a Vectorstain ABC kit (Vector Laboratories, Burlingame, CA) following the manufacturer’s protocol.

Results

DAL*-1 virus induces virus persistence and demyelination in CD4 (--/-) mice but not wt or CD8 (--/-) mice

Inoculation of wt-DA virus into wt SJL/J mice induced substantial demyelination 45 days post-inoculation whereas, as expected from our previously reported data (5), DAL*-1 virus induced little evidence of demyelination (\( P < 0.001 \)) (Table 1). Similar results to those seen with wt mice were present following wt-DA virus and DAL*-1 virus infection of CD8 (--/-) mice, i.e. the DAL*-1 virus infected mice had little demyelination compared to the wt-DA virus infected mice. In contrast, inoculation of both DAL*-1 virus and wt-DA virus into CD4 (--/-) mice showed similar and severe demyelination, which was actually greater than that seen with wt-mice; the enhanced disease of wt-DA virus in CD4 (--/-) compared to CD8 (--/-) (\( P < 0.0001 \)) has been previously noted, and might reflect the importance of a pathogenic cytotoxic T cell response to the demyelinating disease (8). Most relevant to the present study was the significant demyelination following DAL*-1 virus infection of CD4 (--/-) mice compared to that seen with wt and CD8 (--/-) mice, suggesting that DAL*-1 virus is cleared from the CNS of wt and CD8 (--/-) mice, but not CD4 (--/-) mice (\( P < 0.001 \)); similar results were seen when the amount of white matter inflammation was compared in these groups. In situ hybridization confirmed the presence of DAL*-1 virus RNA genome in the spinal cord of CD4 (--/-) mice, but not of wt mice or CD8 (--/-) mice. In summary, these results suggested that DAL*-1 virus can be cleared from the CNS of wt and CD8 (--/-) infected mice, but not of CD4 (--/-) mice, and therefore that a CD4+ T cell-mediated response is able to clear DA virus (which has a mutated L*) from SJL/J mice.

Table 1. Demyelination in the spinal cords of mice 45 days following IC inoculation with wt-DA or DAL*-1 virus

<table>
<thead>
<tr>
<th>Strain</th>
<th>Virus</th>
<th>No. mice</th>
<th>No. quadrants with demyelination/total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt-SJL</td>
<td>wt-DA</td>
<td>10</td>
<td>157/596 (26)</td>
</tr>
<tr>
<td>wt-SJL</td>
<td>DAL*1</td>
<td>5</td>
<td>0/296* (0)</td>
</tr>
<tr>
<td>CD4--/- SJL</td>
<td>wt-DA</td>
<td>9</td>
<td>202/417*** (48)</td>
</tr>
<tr>
<td>CD4--/- SJL</td>
<td>DAL*1</td>
<td>6</td>
<td>224/338** (66)</td>
</tr>
<tr>
<td>CD8--/- SJL</td>
<td>wt-DA</td>
<td>5</td>
<td>37/288 (15)</td>
</tr>
<tr>
<td>CD8--/- SJL</td>
<td>DAL*1</td>
<td>10</td>
<td>2/508* (0)</td>
</tr>
</tbody>
</table>

\* \( P < 0.001 \) comparing the presence of demyelination following DAL*-1 virus infection for a mouse strain vs the presence of demyelination following wt-DA virus infection in the same strain of mouse.

\** \( P < 0.001 \) comparing the presence of demyelination seen following DAL*-1 virus infection in CD4--/- mice vs the presence of demyelination following DAL*-1 virus infection in wt and CD8--/- mice.

\*** \( P < 0.0001 \) comparing the presence of demyelination seen following wt-DA virus infection in CD4--/- mice vs the presence of demyelination seen following wt-DA virus infection in CD8--/- mice.
Adoptive transfer of CD4+, but not CD8+ T cells from DAL*-1 virus infected mice clear a wt-DA virus infection in SJL/J mice

In order to confirm that an anti-virus CD4+ T cell activity is required for clearance of DA virus in SJL/J mice, we performed adoptive transfer experiments. For these studies, CNS-ILs (that had been harvested 7 days following IC inoculation of SJL/J mice with wt-DA virus or DAL*-1 virus) were sorted separately into CD4+ and CD8+ T cells and then intravenously inoculated into mice at the same time as the mice received an IC inoculation with wt-DA virus. The adoptive transfer studies (Table 2) showed abundant and similar evidence of white matter demyelination and inflammation at 45 days following wt-DA virus IC inoculation of mice which had also received: (i) no adoptively transferred cells or (ii) adoptively transferred CD8+ T cells from wt-DA virus infected or DAL*-1 virus infected mice or (iii) adoptively transferred CD4+ T cells from wt-DA virus infected mice. In contrast, mice IC inoculated with wt-DA virus at the same time as receiving an IV inoculation of CD4+ T cells from DAL*-1 virus infected mice had significantly less demyelination (P < 0.001). The effect of transferring CD4+ T cells from DAL*-1-infected mice compared to CD4+ T cells from wt-DA virus infected mice was statistically significantly different (P < 0.05), supporting the thesis that L* acts on CD4+ T cells.

In order to test whether CD4+ T cells from the CNS of DAL*-1-infected mice helped clear virus from the CNS of wt-DA virus infected mice, we stained paraffin-embedded sections for virus antigen from a random selection of half of the infected mice using a biotinylated anti-TMEV monoclonal antibody. We chose to compare virus antigen rather than actual titers of the virus since the levels of DA virus at 6 weeks post-inoculation are very low and sometimes not detectable, even in the case of wt-DA virus infection. Staining for virus antigen in situ also allowed comparison of the extent of demyelination with the level of virus protein expression. We found a similar amount of virus antigen in the wt-DA virus-infected animals in all of the treatment groups, except for a statistically significant decrease (P < 0.01) in the group of mice that had been infected with wt-DA virus and had also received CD4+ T cells from DAL*-1 infected mice (Table 2). In summary, these results suggested that CD4+ T cells in the CNS of DAL*-1 infected SJL/J mice are able to clear DA virus antigen and that the presence of L* leads to a failure in generating a CD4+ T cell response capable of clearing wt-DA virus.

Conclusions

TMEV-induced disease is an attractive model system to study virus pathogenesis because: infectious clones are available, easing preparation of recombinant or mutated viruses; the virion is small with only four structural proteins; the three dimensional crystal structure of TMEV virions has been solved; there is a great deal of information regarding the anti-virus B and T cell response; the experimental and natural host for TMEV, the mouse, is easily manipulated and well-studied immunologically and genetically; the phenotype of TO subgroup strains is unusual compared to other picornaviruses in terms of virus persistence, restricted virus expression and immune-mediated demyelination. In addition, the demyelinating disease induced by TO subgroup strains serves as an excellent experimental model for MS.

Our previous studies identified an alternatively initiated protein, L*, which is important in TO subgroup strain persistence and demyelination in susceptible mouse strains. Mutant DA viruses that lack an initiating AUG codon for L* (DAL*-1 virus) (3), or have a stop codon that truncates L* (4) fail to persist or induce significant demyelination. Mice infected with DAL*-1 virus generated more prominent H-2K-restricted anti-virus cytotoxicity in the CNS than seen with wt-DA virus (5), suggesting that L* could interfere with virus clearance, allowing the virus to persist in and demyelinate the CNS. Findings from the present study using CD4- and CD8-deficient SJL/J mice and adoptive transfer experiments suggest that susceptible mouse strains are capable of clearing DA virus through a CD4+ T cell-dependent activity that is inhibited by L*. A role for class II-restricted CD4+ T cells in clearing DA (mutant) viruses from the CNS of SJL/J mice was found in a previous investigation of DA viruses with point mutations in the VP1 capsid protein (12).

It is important to note that there are complex actions of immune cells following infection of TMEV, which vary depending on the time post-inoculation, the stage of disease and the strain of the mouse. For example, our finding of a role for CD4+ T cells in DA virus clearance of SJL/J mice is distinct from findings of studies demonstrating the role of the immune system in mediating and fostering DA-induced late demyelination in SJL/J mice; the latter studies noted a contribution of both the CD4+ and CD8+ T cells to this late immune-mediated disease (8). The present study’s results also differ from those found in investigations of DA virus clearance in resistant mouse strains (H-2b*). In the latter case, there is evidence that a class I-restricted CD8+ T cell CTL response plays a critical role in DA virus clearance. For example, wt-DA virus infection of a normally resistant mouse strain that has been made β2-microglobulin deficient leads to a low level of expression of class I antigen, a small number of CD8+ T cells, and the development of a severe demyelinating disease (13–16). Findings from a recent study of transgenic mice that express DA virus genes in an H-2b* mouse also supported the importance of class I-restricted CD8+ T-cell immune response.

Table 2. Demyelination and virus antigen in the spinal cords of SJL/J mice 45 days following IC inoculation with wt-DA virus and T cell adoptive transfer

<table>
<thead>
<tr>
<th>Virus inoculum used to infect donor of transferred cells</th>
<th>T cell type transferred from donor</th>
<th>Number of quadrants positive for abnormality/total quadrants (no. mice)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NA</td>
<td>None</td>
<td>155/846 [18]</td>
</tr>
<tr>
<td>wt-DA</td>
<td>CD4+</td>
<td>65/445 [9]</td>
</tr>
<tr>
<td>wt-DA</td>
<td>CD8+</td>
<td>22/174 [6]</td>
</tr>
<tr>
<td>DAL*-1 virus</td>
<td>CD4+</td>
<td>31/327 [6]**</td>
</tr>
<tr>
<td>DAL*-1 virus</td>
<td>CD8+</td>
<td>44/165 [5]</td>
</tr>
</tbody>
</table>

NA: not applicable; mice were inoculated with wt-DA, but received no transferred cells.

*P < 0.01; **P < 0.001 compared to animals inoculated with wt-DA virus but receiving no adoptively transferred cells.

DA virus clearance in SJL/J mice
responses in virus clearance and protection from the demyelinating disease in a resistant mouse strain (17).

There are multiple ways by which a CD4+ T cell response can assist in the clearance of DA virus, including the induction and maintenance of anti-virus-specific CTLs within the CNS, the secretion of lymphokines that activate macrophages and NK cells, and the production of anti-virus antibodies. The present study suggests that the importance of CD4+ T cells in virus clearance is not wholly related to its effect on CD8+ T cells, an effect that we had previously noted (8). Although DAL*−1 virus infection triggered a more robust H-2K restricted anti-virus CTL response in SJL/J mice than that seen following wt-DA virus infection, the CTL response did not seem critical to virus clearance since: CD8 (−/−) mice inoculated with DAL*−1 virus were still able to clear virus from the CNS; CD8+ T cells from DAL*−1 virus-infected mice were unable to clear wt-DA virus in infected mice. It also appears unlikely that the importance of CD4+ T cells on virus clearance is wholly related to the anti-virus B cell response since our previous studies showed that there was no difference in the level of anti-DA virus antibody in wt-DA virus infected mice from that seen in DAL*−1 virus infected mice (5). It may be that the secretion of lymphokines by CD4+ T cells is important in the clearance of DA virus. For example, studies have demonstrated a role for TGF-β in protection of SJL/J mice from DA virus-induced disease (18). However, it is likely that CD4+ T cells have multiple ways and pathways by which efficient virus clearance is carried out and facilitated.

Acknowledgements

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Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>CNS-ILs</td>
<td>central nervous system-infiltrating lymphocytes</td>
</tr>
<tr>
<td>CTC</td>
<td>cytolytic</td>
</tr>
<tr>
<td>IC</td>
<td>intracerebral</td>
</tr>
<tr>
<td>IV</td>
<td>intravenous</td>
</tr>
<tr>
<td>MS</td>
<td>multiple sclerosis</td>
</tr>
<tr>
<td>TMEV</td>
<td>Theiler’s murine encephalomyelitis virus</td>
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References