The Role of CD4 and CD8 T Cells in MHV-JHM-Induced Demyelination

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1. INTRODUCTION

Demyelination following central nervous system (CNS) infection with the neurotropic coronavirus, mouse hepatitis virus, strain JHM (MHV-JHM) has been shown to be dependent on the immune response of the host (Perlman, 1998). Although controversial, both CD4 and CD8 T cells are thought to contribute to demyelination (Perlman, 1998). However, the mechanisms by which each T cell subset contributes to demyelination remains unknown. To address this issue, the following questions were raised. First, what is the magnitude of the T cell response during immune-mediated demyelination? Second, what is the timing of the immune response leading to demyelination? Third, what are the roles of CD4 and CD8 T cells - in particular virus-specific T cells - during MHV-JHM-induced demyelination? In order to address these questions, a previously described adoptive transfer system (Wu and Perlman, 1999) was utilized to measure the quantity of virus-specific T cells during the process of immune-mediated demyelination. Furthermore, the individual contributions of CD4 or CD8 T cells to MHV-JHM-induced demyelinating disease were determined by depletion studies.

The Nidoviruses (Coronaviruses and Arteriviruses).
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2. MATERIALS AND METHODS

2.1 Virus

The neuroattenuated variant of MHV-JHM, strain J2.2-v1 (MHV-J2.2-v1), was generously provided by Dr. J. Fleming (University of Wisconsin, Madison).

2.2 Adoptive Transfer

Mice with genetic disruption of the recombination activating gene (RAG1-/-), obtained from Jackson Laboratories (Bar Harbor, ME), were infected with $1x10^3$ PFU MHV-J2.2-v1 in 30 μL by intracranial injection (Wu and Perlman, 1999). Donor splenocytes were isolated from C57Bl/6 (B6) mice, obtained from the National Cancer Institute (Bethesda, MD), that were immunized intraperitoneally (i.p.) with wild-type MHV-JHM. These cells were delivered to infected RAG1-/- mice 3 days post-inoculation (p.i.) as previously described (Wu and Perlman, 1999). In some experiments, CD4 or CD8 T cells were depleted prior to transfer as previously described (Wu *et al.*, 2000). In order to grade the clinical disease observed following adoptive transfer, the following scoring system was utilized: 0 – asymptomatic, 1 – limp tail, 2 – wobbly gait with righting difficulty, 3 – hind-limb weakness, 4 – hind-limb paralysis, 5 – moribund/dead. Brains and spinal cords from adoptive transfer recipients were harvested 7 to 15 days post-transfer (p.t.).

2.3 FACS Analysis

Antigen-specific T cells were identified by staining for intracellular IFN-γ using PE-conjugated anti-IFN-γ antibody (Pharmingen), as previously described (Wu *et al.*, 2000). Peptides corresponding to the CD4 (M-133-147; I-A^b restricted) and CD8 (S-510-518, H-$2D^b$ restricted; S-598-605, H-$2K^b$ restricted) T cell epitopes were used at a final concentration of 5 μM and 1 μM, respectively.

2.4 Histology

Preparation of spinal cord sections was done as previously described (Wu and Perlman, 1999). 8 μm sections were stained with luxol fast blue (LFB) and digitized. The percentage of demyelinated white matter was quantified as previously described (Xue *et al.*, 1999).

3. RESULTS

3.1 MHV-specific cells rapidly infiltrate the CNS following adoptive transfer

Infection of RAG1-/- mice, lacking B and T lymphocytes, with MHV-J2.2-v1 does not result in CNS demyelination. However, adoptive transfer of splenocytes from syngeneic B6 mice, immunized to MHV-JHM, into MHV-J2.2-v1-infected RAG1-/- mice consistently results in demyelinating disease (Wu and Perlman, 1999). In order to investigate the magnitude and timing of the immune response to MHV-JHM following adoptive transfer, intracellular cytokine staining was performed. The number of virus-specific CD4 and CD8 T cells was determined by quantifying the number of cells producing IFN-γ in response to peptides representing the known CD4 and CD8 T cell epitopes (Perlman, 1998), as described in Section 2.3. Analysis of the donor cell population, isolated from B6 mice 6 days after immunization with MHV-JHM, revealed a small fraction of CD4 T cells specific for M-133-147, the immunodominant CD4 T cell epitope (Figure 1). Similarly, a small fraction of CD8 T cells were found to produce IFN-γ in response to both the immunodominant CD8 T cell epitope, S-510-518, and the subdominant CD8 T cell epitope, S-598-605 (Figure 1). In contrast, analysis of lymphocytes harvested from the CNS of asymptomatic adoptive transfer recipients at day 4.5 p.t. demonstrated that a large fraction of both CD4 and CD8 T cells responded to the respective immunodominant T cell epitopes. Approximately 20% of CD4 T cells were specific for M-133-147, while approximately 30% of CD8 T cells responded to S-510-518 (Figure 1). However, the fraction of CD8 T cells that responded to S-598-605 remained low (less than 5%). At seven days p.t., a time at which mice have developed demyelination, the fraction of M-133-147-specific CD4 T cells remained around 20%. On the other hand, only 20% of the CD8 T cells isolated from adoptive transfer recipients on day 7 p.t. were specific for S-510-518. Nonetheless, due to the overall increase in CD8 T cells on day 7 p.t., the absolute number of CD8 T cells increased approximately 10-fold (data not shown). These data demonstrate that MHV-specific CD4 and CD8 T cells rapidly infiltrate the CNS following adoptive transfer.

3.2 CD4 and CD8 T cells make distinct contributions to MHV-JHM-induced demyelinating disease

In order to determine the specific contributions made by CD4 and CD8 T cells to the pathogenesis of demyelination following infection with MHV,

MHV-J2.2-v1-infected RAG1-/- mice were given donor cells depleted of each subset. A clinical difference was observed between recipients of CD4 T cell and CD8 T cell enriched donors. CD4 T cell enriched recipients developed a more rapid and severe course of disease, frequently becoming moribund by day 7 p.t. In contrast, CD8 T cell enriched recipients exhibited a less severe clinical phenotype, often developing clinical disease as late as 11 days p.t. (Figure 2). When adoptive transfer recipients were analysed according to the clinical scale described in Section 2.2, there was significantly more severe clinical disease observed in CD4 T cell enriched adoptive transfer recipients relative to undepleted recipients ($p < 0.5$). Spinal cord sections from each group were analysed for demyelination as described in Section 2.5. The percentage of demyelinated white matter was found to be less in CD4 T cell enriched recipients in comparison to recipients of undepleted donor cells. No difference was observed in the level of infectious virus isolated from each adoptive transfer population, indicating that the more severe clinical disease observed in CD4 T cell enriched recipients was not due to inefficient viral clearance (data not shown).

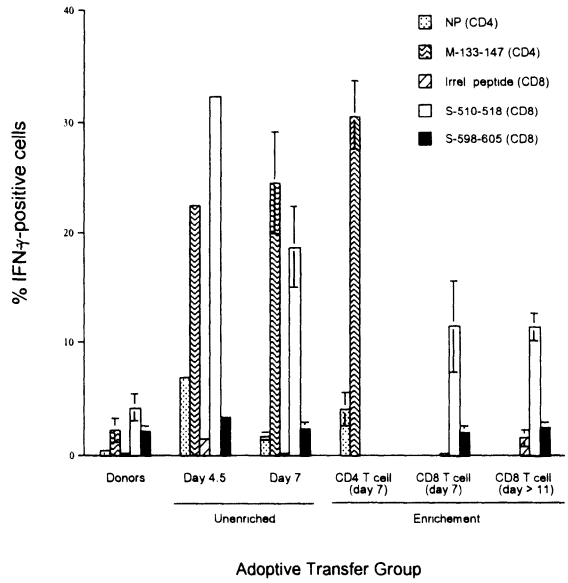

Figure 1. Quantity of MHV-specific CD4 and CD8 T cells before and after adoptive transfer. Donor splenocytes were isolated from B6 mice inoculated i.p. with MHV-JHM six days prior to adoptive transfer. The fraction of MHV-specific CD4 and CD8 T cells was determined by measuring the fraction of cells producing IFN-γ in response to peptides representing the respective epitopes (see section 2.3). Day 4.5 represents an average of 2 experiments, while all other values represent the average of at least 3 experiments.

No CD8 or CD4 T cells were detected in CD4 or CD8 T cell enriched recipients, respectively (Figure 1). Interestingly, the fraction of S-510-518-specific CD8 T cells was lower in the absence of CD4 T cells (Figure 1). Furthermore, CD8 T cell enriched recipients demonstrated an increase in the amount of demyelination and clinical disease at late timepoints (Figure 2), without a corresponding increase in the percentage of S-510-518 CD8 T cells (Figure 1).

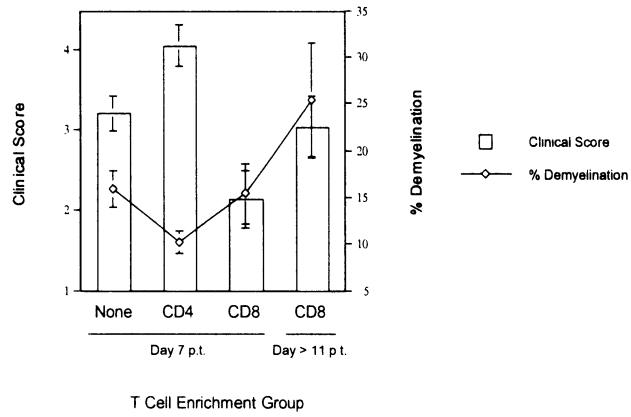

Figure 2. Clinical disease and demyelination in CD4 and CD8 T cell enriched recipients. Clinical scoring was performed using the scale described in Section 2.2. Percentage of demyelination within the spinal cord was determined as described in Section 2.5. A significantly lower percentage of demyelination was observed in CD4 T cell enriched recipients ($p < 0.05$) along with significantly more severe clinical disease ($p < 0.05$) than undepleted recipients.

4. CONCLUSIONS

1. A large percentage of MHV-specific T cells rapidly infiltrates the CNS following adoptive transfer. This rapid infiltration may be due to both the high levels of virus antigen present in the CNS of MHV-J2.2-v1-infected RAG1-/- mice prior to transfer and the activated state of donor cells. In addition, the expression of specific chemokines is likely to be important in the recruitment of T cells to the CNS. Previous reports have shown that a large fraction of CD8 T cells are specific for S-510-518 during acute and chronic MHV-induced CNS disease (Bergmann *et al.*, 1999; Pewe *et al.*, 1999).

2. In the absence of CD4 T cells, fewer MHV-specific T cells are detected within the CNS of adoptive transfer recipients. CD4 T cell help may be required for effective trafficking and/or survival of CD8 T cells

(Stohlman *et al.*, 1998). That these animals still developed demyelination, and that greater demyelination was observed without a corresponding increase in S-510-518-specific CD8 T cells at later timepoints, suggests that demyelination is induced after a certain threshold of cells is reached.

3. Neither CD4 nor CD8 T cells are essential for the development of MHV-induced demyelination. The process by which demyelination develops following adoptive transfer is therefore redundant to a degree. In contrast, CD4 T cells are critical to the development of clinical disease, inflammation and demyelination (Lane *et al.*, 2000). The studies reported herein specifically target the effector phase - rather than the induction phase - of the immune response, and therefore may be less susceptible to the depletion of CD4 T cells. The mechanisms by which CD4 and CD8 T cells induce demyelination may be unique or identical. Nonetheless, no single effector molecule has been shown to be essential. However, macrophages are most likely an important downstream mediator of demyelination (Wu and Perlman, 1999).

4. Greater clinical disease, accompanied by less demyelination, is observed in CD4 T cell-enriched adoptive transfer recipients. Macrophages may play a key role in the development of clinical disease, since a greater degree of F4/80-positive cell infiltration into the gray matter was observed in CD4 T cell enriched recipients (Wu *et al.*, 2000). Overall, these studies demonstrate the unique properties of CD4 and CD8 T cells in their contribution to MVH-JHM-induced demyelination and provide a foundation for further investigation into the mechanisms of virus-induced demyelination.

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