CD8+ T-CELL PRIMING DURING A CENTRAL NERVOUS SYSTEM INFECTION WITH MOUSE HEPATITIS VIRUS

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1. INTRODUCTION

Infection with mouse hepatitis virus (MHV) provides an animal model with which to study central nervous system (CNS) diseases including both encephalitis and demyelination. Different strains of MHV induce disease with varying degrees of severity. The A59 strain induces acute encephalitis during the first week of infection and a strong CD8+ T-cell response is observed in the brain coinciding with virus clearance. Despite efficient clearance of infectious virus, demyelination is evident four weeks postinfection (p.i.). The JHM strain (also referred to as MHV-4 or JHM.SD in the literature) induces lethal encephalomyelitis within the first week of infection and virus is typically not cleared. In this study, we investigate the CD8+ T-cell responses induced during infections with A59 and JHM.

Virus specific CD8+ T cells play a protective role against MHV strain A59 and are essential for clearance of infectious virus from the central nervous system (CNS). We have previously found that only early transfer, prior to 3 days postinfection (p.i.) with RA59-gfp/gp33, of gp33-specific CD8+ T cells (obtained from P14 transgenic mice) resulted in accumulation of activated epitope-specific CD8+ T cells within the brain.2 We observed that P14 splenocytes did not accumulate in the brains of RA59-gfp/gp33 infected mice when the transfers were performed on day 3 or 5 p.i. In order to determine if this was due to a defect in trafficking or priming during the infection, we examined the expansion of transferred CFSE-labeled gp33-specific CD8+ T cells in the draining cervical lymph nodes following infection with RA59-gfp/gp33. In addition, we sought to determine why activated, virus-specific CD8+ T cells are detected at very low levels in the spleen and brain after infection with RJHM.

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2. MATERIALS AND METHODS

2.1. Mice and Viruses

Four-week-old male mice were used in all experiments; B6 or B6-LY5.2/Cr (CD45.1) mice were obtained from the National Cancer Institute. P14 mice were bred at the University of Pennsylvania. Recombinant MHV strain A59 expressing enhanced green fluorescent protein (EGFP) or expressing the gp33 epitope as fused to EGFP are described elsewhere. Recombinant A59 (RA59), recombinant JHM (RJHM) and the recombinant chimeric virus expressing the JHM spike with A59 background genes (SJHM/RA59) have been described elsewhere. Recombinant A59 (RA59), recombinant JHM (RJHM) and the recombinant chimeric virus expressing the JHM spike with A59 background genes (SJHM/RA59) have been described elsewhere.

2.2. Isolation of Mononuclear Cells for Adoptive Transfer

Spleens were removed from P14 mice and suspensions were prepared by homogenizing in a nylon bag (64 µm diameter) in RPMI 1640 medium supplemented with 1% fetal calf serum. Red blood cells were lysed with 0.83% ammonium chloride and the lymphocyte suspension was washed twice in 1 x PBS and resuspended in 1 x PBS for transfer. Cells (at a concentration of 5 x 10⁷ cells/ml) were labeled with 1 µl of 5 mM CFSE/ml. The total number of cells transferred was 2 x 10⁷ cells in 0.5 ml.

2.3. Isolation of Mononuclear Cells from Brains, Spleens, or Lymph Nodes

Mice were perfused with 10 ml 1 x PBS and organs removed. Brain lymphocytes were isolated as previously described. Cells were harvested from spleens and lymph nodes as described above. Intracellular IFN-γ was assayed as previously described.

2.4. Demyelination

Demyelination was analyzed in at least 10 sections of spinal cord from each animal and five to eight mice were examined in each of two separate experiments. Percent demyelination was calculated by counting quadrants of cross-sectioned spinal cord that was stained with the myelin specific dye, luxol fast blue. A neuropathologist examined the spinal cords to determine the severity score which was from 0 to 5 with 5 being the most severe demyelination.

3. RESULTS

3.1. Early Transfer Required for Protection and Accumulation of gp33-Specific CD8+ T cells in the Brain

Transfer of naïve, gp33-specific CD8+ T cells one day prior to infection with RA59-gfp/gp33 protected against acute encephalitis and, importantly, virus spread to the spinal cord was markedly reduced. This correlated with a dramatic reduction in the quantity and severity of demyelination seen 28 days p.i. However, mice that received adoptive transfers of gp33-specific CD8+ T cells on days 3 or 5 p.i. were not protected from acute
Table 1. Percent and severity of demyelination on day 28 p.i. is reduced by early transfer.

| Day of transfer | Percent demyelination | Severity of demyelination |
|-----------------|-----------------------|---------------------------|
| No transfer     | 30.53 +/- 3.5         | 2.35                      |
| Transfer (-1)   | 10.34 +/- 2.63        | 0.3                       |
| Transfer (+3)   | 24.2 +/- 5.7          | 2.0                       |
| Transfer (+5)   | 19.88 +/- 3.5         | 1.4                       |

\^ Percent demyelination was calculated by counting quadrants of cross-sectioned spinal cord that was stained with the myelin specific dye, luxol fast blue (see “Materials and Methods”).

\^ The severity of demyelination was observed and assessed by a neuropathologist. The scale was from 0 to 5 (see “Materials and Methods”). Data was presented in a different format in Ref. x.

We observed that the early transfer, performed one day prior to infection, resulted in protection from acute and chronic disease. In addition, we observed that the transferred (CD45.2 positive) cells were activated and secreted IFN-\(\gamma\) in response to gp33 peptide and accumulated to high percentages within the brains by day 7 p.i. However, the transferred cells did not accumulate in the brain on day 7 p.i. when the transfers were performed on days 3 or 5 p.i. Thus, we examined the brain-derived mononuclear cells from transfer recipients at later time points, days 10 and 12 p.i. As is evident from the data shown in Table 2 when transfers were performed on days 3 or 5 p.i. significantly fewer transferred cells accumulated at the site of infection as compared to the transfer recipients that received the transfer prior to infection. Whereas nearly half of the CD8\(^+\) T cells were the transferred cells in the early transfer recipients on day 10 p.i., only about 10.0% and less than 1.0% of the CD8\(^+\) T cells were the transferred cells in the day 3 and day 5 transfer recipients, respectively. Furthermore, on day 12 p.i. the total numbers of both CD8\(^+\) T cells as well as the transferred CD45.2-positive cells decreased as compared to day 10 p.i. We concluded that the cells transferred on days 3 or 5 p.i. were defective in their activation and/or ability to traffic into the CNS. However, when transfers were performed in RAG\(^{-/-}\), we observed the accumulation of gp33-specific IFN-\(\gamma\)-secreting cells in the brain and the later the transfer was performed the higher the percentage of gp33-specific cells observed in the brain. RAG\(^{-/-}\) do not contain endogenous T cells capable of lytic activity, thus, it is assumed that antigen presentation is prolonged. Thus, we predicted that when we transferred P14 splenocytes into B6 mice on days 3 or 5 the cells were not activated or recruited into the brain due to a lack of antigen presentation at that time point.
Table 2. Total brain-derived CD8+ T cells and transferred, CD45.2 positive, cells 7 days post transfer.

| Day of transfer | C57Bl/6 (CD8+ (% of total)) | RAG-/- (CD45.2+) gp33-specific IFN-γ+ |
|-----------------|-----------------------------|--------------------------------------|
| No transfer     | 1 x 10^5 (14%)              | --                                   |
| Transfer (-1)   | 1 x 10^5 (18%)              | ++++                                 |
| Transfer (+3)   | 6 x 10^4 (11%)              | +                                     |
| Transfer (+5)   | 5.5 x 10^4 (8%)             | +/-                                  |

*Cells harvested 7 days post transfer. Some of these data were presented in a different format in Ref.2.

3.2. Duration of Antigen Presentation During Infection of the CNS

In order to determine whether there was a block in the ability for the gp33-specific CD8+ T cells to traffic into the brain or if there was a defect in priming of the transferred cells, we developed an adoptive transfer model to trace the expansion of transferred cells. P14 splenocytes were labeled with CFSE and transferred prior to infection or on day 3 post infection.

![Figure 1.](image)
3.3. RJHM Elicits a Weak CD8+ T-Cell Response

As previously been reported in the literature, we observed that RJHM elicits a surprisingly weak CD8+ T-cell response and a poor epitope specific IFN-γ response. In order to rule out the possibility that RJHM causes destruction of the brain parenchyma that prevents recruitment of CD8+ T cells, animals were inoculated intranasally (i.n.) with their LD₅₀ dose of 100 or 1000 pfu of RJHM or SJHM/RA59 (recombinant A59 expressing the JHM spike in place of the A59 spike), respectively. Intranasal infection results in a slower course of disease with most animals dying after the first week of infection allowed the analysis of recruitment of T cells into the brain. Mice were sacrificed at 7 days p.i. in order to analyze the epitope specific CD8+ T-cell response at the site of infection. Whereas 21.9% of the cells isolated from the brains of SJHM/RA59 infected animals were CD8+, only 0.8% of the cells isolated from the brains of RJHM infected animals were CD8+. Furthermore, the specific IFN-γ response to the two epitopes within the spike protein was much higher in the SJHM/RA59 infected animals (Fig. 2). This also indicates that the low CD8+ T-cell response is not due to the RJHM spike.

In order to determine if RJHM was capable of suppressing the immune response we coinfeected mice with RJHM and RA59. Interestingly, the coinfected animals had a strong CD8+ T cell response to the subdominant S598 epitope that is present in both viruses, however, there was still no response to S510, the immunodominant epitope that is only present in RJHM.

4. CONCLUSIONS

In this study we define the window of antigen presentation during a CNS infection with RA59-egfp/gp33 to be within the first 72 hours of infection. When splenocytes were transferred on day 3 p.i., they were not activated to proliferate and, thus, did not accumulate within the brain. This provides more evidence that in order for CD8+ T cells to traffic to the site of infection they must undergo several rounds of division in the lymphoid organs. Furthermore, consistent with the idea that antigen presenting cells are destroyed by cytotoxic T lymphocytes as limiting determinant of immune activation we observed that transfers performed on days 3 and 5 p.i. in RA59-egfp/gp33 infected RAG-/- did result in the accumulation of virus-specific CD8+ T cells within the brain 7 days post transfer (Table 2).

| Virus          | Total CD8+ T cells | S510-specific | S598 |
|---------------|--------------------|---------------|------|
| RA59          | ~1 x 10⁶           | -             | ++   |
| RJHM          | ~1 x 10⁶           | +/-           | +/-  |
| SJHM/RA59     | ~2 x 10⁶           | +++           | +++  |
| RA59 and RJHM | ~2 x 10⁶           | -             | ++   |

*a Cells per brain.*
The neurotropic strains of MHV, A59, and JHM induce different courses of disease with JHM resulting in lethal encephalitis within the first week of infection. In addition, JHM induces a strong innate immune response but almost no CD8+ T-cells are found in the brains of infected animals. Following coinfection with JHM and A59, we observed that JHM is not capable of suppressing the CD8+ T-cell response but fails to elicit a CD8+ T-cell response. The weak CD8+ T-cell response induced during infections with JHM is not spike-determined as a chimeric virus expressing the JHM spike with the background genes derived from A59 results in a strong CD8+ T-cell response to both the S510 and S598 epitopes.

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6. REFERENCES

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