Acute CNS Infection is Insufficient to Mediate Chronic T Cell Retention

[1]NORMAN W. MARTEN, [2]MAUREEN HOHMAN, [2,3]STEPHEN A. STOHLMAN, [1]ROSCOE D. ATKINSON, [1]DAVID R. HINTON, AND [2,3]CORNELIA C. BERGMANN

Departments of [1]Pathology, [2]Molecular Microbiology and Immunology and [3]Neurology, Keck School of Medicine, University of Southern California, Los Angeles, CA 90033

1. INTRODUCTION

$CD8^+$ T cells control infection by the neurotropic JHM strain of mouse hepatitis virus (JHMV) by eliminating infectious virus and reducing CNS pathology (Stohlman et al. 1995). Virus-specific CTL in BALB/c mice ($H\text{-}2^d$) respond almost exclusively to a single immunodominant epitope in the nucleocapsid (N) protein (Bergmann et al. 1993). During acute JHMV infection, $CD8^+$ T cells account for up to 40% of CNS mononuclear cells (MNC) (Bergmann et al. 1999). Following viral clearance, virus specific $CD8^+$ T cells remain in CNS for several weeks (Bergmann et al. 1999).

This report elucidates the requirement of persisting antigen (Ag) in retaining primary effector $CD8^+$ T cells within the CNS by comparing the responses to two antibody selected variants of JHMV, 2.2-V-1 and 2.2/7.2-V-2, abbreviated V-1 and V-2, respectively (Fleming et al. 1996 & 1997). V-1 forms a persistent CNS infection accompanied by demyelination (Fleming et al. 1996). By contrast, V-2 causes little to no demyelination (Fleming et al. 1997). Herein, we demonstrate that whereas V-1 persists in the form of vRNA within the spinal cord, infection with V-2 is transient and vRNA is reduced to below detectable limits. Analysis of CNS MNC revealed similar peaks of T cell infiltration during acute infection followed by clearance of both infectious viruses. However, in V-2 infected mice

The Nidoviruses (Coronaviruses and Arteriviruses).
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which clear vRNA, CNS T cells eventually decline to levels typical of naïve mice. By contrast T cells were maintained within the spinal cords of mice persistently infected with V-1. Virus mediated retention of infiltrating cells was specific for T cells, as other cell types including NK cells, B cells and peripheral macrophages, were not retained within the CNS of mice persistently infected with V-1.

2. MATERIAL AND METHODS

Mice and viruses. Male BALB/c (H-2^d) mice were purchased from National Cancer Institute (Frederick, MD) at 6 weeks of age and infected within 1 week of arrival. Acute and persistent CNS infections were induced by intracranial injection of 1000 plaque forming units of JHMV variants 2.2-V-1 or 2.2/7.2-V-1 (Fleming et al. 1996 & 1997).

CNS MNC. MNC were derived separately from either spinal cords alone or both the brain and spinal cord of infected mice as described previously (Bergmann et al. 1999).

Determination of viral RNA. RNA isolation and RT-PCR amplification of viral N and host HPRT were performed as described (Marten et al. 2000b).

FACS analysis of CNS derived MNC. Surface markers were determined by staining with the following mAb: CD8 (53.67), CD4 (GK1.5), CD19 (1D3), CD45 (30-F11), panNK (DX5) (Pharmingen, San Diego, CA) and F4/80 (Cl:A3-1) (Serotech, Raleigh, NC) and the L^d-N318 MHC class-I tetramer has been described elsewhere (Bergmann et al. 1999).

3. RESULTS

To confirm viral persistence following V-1 infection of BALB/c mice and establish that V-2 is cleared from the CNS, mice were analysed for the presence of vRNA from the viral N gene (Table 1) in brain and spinal cord. Following a single RT-PCR amplification, vRNA was detected in all RNA preparations from infected mice sacrificed at d 7 and 11 p.i. However, levels of vRNA fell sharply by d 33 p.i. and all samples from mice sacrificed at d 33 and 63 p.i. were reamplified with a nested N primer set. Following the second amplification, vRNA was detected in all but one spinal cord sample from V-2 infected mice at d 33 p.i. However, by d 63 p.i., vRNA was undetectable within the CNS of V-2 infected mice. Although vRNA was only detected in the brains of 2 out of 4 V-1 infected mice at d 63 p.i., vRNA was still present within spinal cords of all V-1 infected mice. No differences

in product yield were observed following HPRT amplification nor was N RNA detected in naïve mice (data not shown). Although both virus strains replicate with similar efficiencies (Marten et al. 2000a), V-1 preferentially infects spinal cords during persistence, whereas V-2 vRNA was cleared from the CNS.

Table 1. Persistence of V-1 and V-2 vRNA within the CNS

Virus	Brain				S.C.			
strain	d 7	D 11	d 33	d 63	d 7	D 11	d 33	d 63
V-1	4/4	4/4	4/4	2/4	4/4	4/4	4/4	4/4
V-2	4/4	4/4	4/4	0/4	4/4	4/4	3/4	0/4

[a]Number of mice with persisting viral N RNA within the indicated CNS tissue as determined by RT-PCR.

Spinal cords were monitored for T cell infiltration to determine the role of potential class I Ag presentation associated with persisting vRNA in retaining $CD8^+$ T cells within the CNS. Mice infected with both V-1 and V-2 were sacrificed during and following resolution of acute infection. Peak infiltration was observed on d 10 p.i. when $CD8^+$ T cells accounted for 29 to 34 % of the MNC population in mice infected with both viruses (Table 2). 37 to 43 % of CNS $CD8^+$ T cells expressed T cell receptors specific for the JHMV-N epitope as indicated by tetramer staining. Although infectious virus has been cleared for several weeks by d 33 p.i., $CD8^+$ T cells were still present in spinal cords of mice infected with both viruses, albeit at two to three fold lower levels compared to peak frequencies. However, by d 70 p.i. at which time V-2 RNA was no longer detectable, the frequency of $CD8^+$ T cells within the CNS had dropped to levels typical of naïve mice. By contrast, both $tetramer^+CD8^+$ T cells and $tetramer^-CD8^+$ T cells remained within the spinal cords of mice persistently infected with V-1 at d 70 p.i. These data suggest that persisting vRNA within the CNS is required to maintain the continued presence of both N-specific $CD8^+$ T cells as well as $CD8^+$ T cells with yet undefined specificity.

Table 2. Frequency of MNC isolated from spinal cord[a]

Cell Types	Virus	d 7 p.i.	d 10 p.i.	d 33 p.i.	d 70 p.i.
$CD8^+$ T cells	V-1	30.0 % (35.9)[b]	35.0 % (42.9)	14.8 % (25.7)	8.5% (30.6)
	V-2	20.4 % (30.4)	29.3 % (36.9)	8.5 % (36.5)	0.3 % (33.3)
$CD4^+$ T cells	V-1	19.9 %	18.6 %	9.5 %	10.7 %
	V-2	8.3 %	21.8 %	2.6 %	1.6 %
B cells	V-1	2.3 %	1.7 %	1.4 %	0.6 %
	V-2	ND[c]	1.8 %	1.1 %	0.3 %
NK cells	V-1	11.9 %	3.3 %	1.9 %	1.1 %
	V-2	ND	1.7 %	ND	ND

[a]The percentages of MNC types isolated from spinal cord were determined by flow cytometry. [b]Numbers in parenthesis represent the percentage of $tetramer^+CD8^+$ T cells among total $CD8^+$ T cells. [c]ND: not determined.

To determine if the influence of persisting vRNA was specific for retention of $CD8^+$ T cells, MNC from spinal cords of both V-1 and V-2 infected mice were also examined for $CD4^+$ T cells by flow cytometry. Both viruses induced similar peak infiltration of $CD4^+$ T cells, comprising 19 to 22 % of spinal cord MNC, at d 10 p.i. (Table 2). In contrast to $CD8^+$ T cells, the frequency of $CD4^+$ T cells in spinal cords of V-1 infected mice was at least twice that obtained from V-2 infected mice at both early (day 7 p.i.) and later time points (d 33 and 70 p.i.). By d 70 p.i. $CD4^+$ T cells comprised only 1.6% of the total spinal cord MNC population from V-2 infected mice. By contrast, $CD4^+$ T cells still accounted for greater than 10% of MNC isolated from spinal cords of mice persistently infected with V-1 at d 70 p.i. These data suggest that $CD4^+$ as well as $CD8^+$ T cells are retained in conjunction with persisting vRNA.

To assess retention or ongoing recruitment of other infiltrating cell types, spinal cord MNC were examined for B cells, NK cells and macrophages/microglia. $CD19^+$ B cells comprised a minor population of no more than 2.3% of spinal cord MNC at any time point examined (Table 2). NK cells accounted for approximately 12 % of MNC from V-1 infected mice at the peak of their infiltration at d 7 p.i. (Table 2). However, at all later points NK cells comprised < 3 % of the MNC population. Macrophages/ microglia make up approximately 30 to 35 % of the MNC population at d 7 p.i. but become the dominant population at later time points as the frequencies of other MNC types decline (data not shown). To distinguish parenchymal microglia and macrophages during infection, CNS MNC were analysed for expression of CD45, which marks bone marrow derived cells and F4/80. Parenchymal microglia are characterized by a $CD45^{lo}/F4/80^+$ phenotype, whereas blood borne macrophages are $CD45^{hi}/F4/80^+$ (Carson et al. 1998). At d 8 p.i., infiltrating macrophages represent approximately 30% of $F4/80^+$ (phagocytic) CNS cells (Table 3). However, by d 62 p.i., this population had diminished to only 2%; the remainder being represented by $CD45^{lo}/F4/80^+$ microglia. These data suggest that unlike T cells, other MNC which infiltrate the CNS during acute V-1 infection do not remain within the CNS of persistently infected mice.

Table 3. Infiltrating macrophages are present during acute but not chronic stages of infection[a].

Cell Types	Naïve	d 8 p.i.	d 62 p.i
Microglia	99 %	70 %	98%
Macrophage	1 %	30%	2%

[a]MNC were isolated from CNS of naïve and V-1 infected mice sacrificed at d 8 and 62 p.i. Cells were stained with CD45 and F4/80 directly *ex vivo* to differentiate between resident microglia ($CD45^{lo}/F480^{hi}$) and infiltrating macrophages ($CD45^{hi}/F4/80^{hi}$).

4. DISCUSSION

CNS infection with JHMV induces a vigorous $CD8^+$ CTL response, which rapidly clears infectious virus (Stohlman et al. 1995). However, $CD8^+$ T cells remain in the CNS for several months following resolution of acute JHMV infection (Bergmann et al. 1999). To identify mechanisms involved in T cell retention, particularly the role of persisting virus, the immune response to two distinct JHMV variants were analysed. V-1 initiates a persistent infection within the spinal cord whereas V-2 is cleared below detection following transient infection of the spinal cord. Infection with either virus induced similar levels of peak $CD4^+$ and $CD8^+$ T cell responses. However, during persistence both $CD8^+$ and $CD4^+$ T cells were retained in the spinal cords of V-1 infected mice in conjunction with persisting vRNA. By contrast, spinal cords of V-2 infected mice were essentially free of peripheral MNC infiltrates following clearance of vRNA. These data indicate that persisting vRNA is required to maintain T cells within the CNS, possibly acting as a source of protein for ongoing Ag presentation.

The ratio of tetramer$^-$CD8$^+$ T cells to tetramer$^+$CD8$^+$ T cells remained roughly constant throughout the course of infection with either strain of virus. Furthermore, tetramer$^-$CD8$^+$ T cells were retained along with virus specific $CD8^+$ T cells within the spinal cords of V-1 infected mice during persistence. This result might be explained by the inability of the pN-L^d tetramer to distinguish a large fraction of N-specific T cell pool due to low expression of TcR or the presence of $CD8^+$ T cells with specificity to as yet undefined virus epitopes. Since both tetramer$^+$CD8$^+$ and tetramer$^-$CD8$^+$ T cells remain in spinal cords of V-1 infected but not in V-2 infected mice, it is unlikely that tetramer$^-$CD8$^+$ T cells are specific for host epitope(s).

The mechanism for retention of peripheral MNC appears to be Ag specific as neither NK cells nor peripheral macrophages were retained within the CNS of V-1 infected mice. Determination of infiltration by B cells based on CD19 expression may provide an under-estimation due to the loss of distinctive surface markers during differentiation into plasma cells. Analysis of an influenza model of CNS infection has suggested that retention of $CD8^+$ T cells in the CNS is independent of viral Ag (Hawke et al. 1998). However, the CTL responding to the influenza infection were derived from a memory population previously activated and expanded by a peripheral immunization. Thus, whereas memory T cells may be retained within the CNS independent of chronic Ag, the data presented here suggests that retention of T cells responding to a primary infection within the CNS requires the presence of persisting virus.

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