Effective Clearance of Mouse Hepatitis Virus from the Central Nervous System Requires both CD4⁺ and CD8⁺ T Cells

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Both CD4⁺ and CD8⁺ T cells are required for the clearance of virus from the central nervous system following infection with the JHM strain of mouse hepatitis virus. Development of antiviral antibodies requires the presence of CD4⁺ T cells but appears to play a minimal role in the reduction of virus. The data presented are consistent with the hypothesis that clearance of JHM virus is mediated by virus-specific CD8⁺ T cells, which appear to require the presence of CD4⁺ T cells.

The JHM strain of mouse hepatitis virus (JHMV) induces an acute encephalomyelitis with both acute and chronic demyelination in its natural host, the mouse (5, 6, 16). The host immune response plays a critical role both in protection from death and in modulating the development of chronic disease and associated demyelination (8, 12). JHMV infection induces both cellular (T-cell-mediated) and humoral (B-cell-mediated) responses; however, the role(s) of the individual components of the immune system in providing protection from death and in preventing or exacerbating demyelination is not clear. For example, the passive transfer of monoclonal antibodies specific for the spike, matrix, and nucleocapsid structural proteins is able to protect mice from lethal infection (3, 7, 15). Protection is apparently due to the sparing of the neuronal population and is associated with histological evidence of demyelination (3).

A role for the cell-mediated immune response in protection has been demonstrated by observations from adoptive transfer experiments with polyclonal or clonal CD4⁺ delayed-type hypersensitivity (DTH)-inducer T cells. Transfer of these cells into JHMV-infected mice prevents neuronal infection and subsequent death, without significantly altering the replication of JHMV within glial cells in the central nervous system (CNS) (11). As with monoclonal antibodies, the transfer of DTH-effector T cells also leads to protection associated with significant demyelination in survivors (4). Interestingly, neither protection afforded by monoclonal antibodies nor that afforded by transferred CD4⁺ DTH-inducer T cells results in the complete clearance of virus from the CNS.

Recently, we have shown that another population of CD4⁺ cells distinct from the CD4⁺ DTH-inducer cells also protects mice from lethal JHMV infections (13). Mice thus protected show no evidence of demyelination, and only low levels or no virus are found in their CNS. The observation that reduction of viral titers requires class I histocompatibility between protective donor cells and the recipient suggests that the clearance of virus is not directly mediated by the donor non-DTH-inducer cells but that host CD8⁺-effector T cells may be involved (14).

The present studies were undertaken to assess more clearly the roles of different components of the immune response in the clearance of virus from the CNS. Depending

on the strain of mouse used, JHMV replication in the CNS peaks at approximately 5 to 6 days postinfection. During a fatal infection, a decline in titer is observed prior to the death of the animal. The presence of infiltrating mononuclear cells in the CNS suggests that this drop in titer may represent an abortive protective attempt on the part of the host immune response. To confirm the importance of the host immune response in reduction of JHMV in the CNS, mice were immunosuppressed by whole-body irradiation (800 R) at 24 (day 1) or 1 (day zero) h prior to intracerebral infection with 1,000 PFU of JHMV variant DS as described previously (10). C57BL/6 mice were obtained from Jackson Laboratories, Bar Harbor, Maine, at 5 weeks of age. Representative mice were bled and ascertained to be seronegative for JHMV prior to infection.

Whole-body irradiation of mice 24 h before infection resulted in their complete inability to clear virus from the CNS (Fig. 1). All infected mice in this group were dead by day 9 postinfection. In mice which were irradiated immediately prior to infection, an early reduction in viral titer was observed. However, this reduction was not sustained, and by day 5, viral titers had increased to the initial levels observed in mice irradiated 24 h prior to infection. All mice in this group also succumbed to infection by day 9. In contrast, viral titers obtained from CNS tissue of untreated mice showed a gradual decline, which reached undetectable limits by day 11.

The observation that irradiated mice could not clear virus from their CNS confirms the importance of the host immune response in reduction of viral replication. In nonirradiated hosts, the greatest reduction in viral titer was observed after day 7. Presumably, the number of host cytotoxic cells is at a maximum at this time, as has been observed in infections with other viruses (2). However, the response is apparently too late to prevent death, even though viral titers in the CNS are significantly reduced.

To address the potential roles of the CD4⁺ and CD8⁺ populations of T cells in the clearance of JHMV, mice were preferentially depleted of one or the other T-cell subpopulation prior to infection. Hybridoma cell lines secreting monoclonal antibodies GK1.5 (anti-CD4) and 2.43 (anti-CD8) were obtained from the American Type Culture Collection, Rockville, Md. The monoclonal antibodies were precipitated from serum-free culture supernatants with ammonium sulfate, dialyzed extensively against phosphate-buffered saline, and injected i.p. at 100 µg per mouse 2 days prior to, on the same

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FIG. 1. Impaired clearance of virus from the CNS of irradiated mice. Mice were immunosuppressed by whole-body irradiation with 800 R at 24 (day 1) or 1 (day zero) h prior to intracerebral infection with JHMV. Brains were obtained on the days indicated, and virus titers were estimated on monolayers of L2 cells. Results represent mean titers obtained from a representative experiment with three mice in each group at each time point. ■, Controls; ○, irradiated day zero; ●, irradiated day 1.

day as, and 2 days after infection. Initially, the efficacy of the depletion after two antibody treatments was monitored by flow cytometry. Spleen cells from untreated mice contained 24% CD4⁺ and 10% CD8⁺ cells. Following treatment with the GK1.5 anti-CD4 monoclonal antibody, no CD4⁺ cells were detected in the spleens whereas the CD8⁺ population was largely unaffected (14% CD8⁺ cells). Conversely, treatment with the 2.43 anti-CD8 monoclonal antibody eliminated the CD8⁺ population without affecting the CD4⁺ population (19% CD4⁺ cells remained). Further analysis (Fig. 2) revealed that the relevant populations remained depleted during the duration of the experiment.

Figure 3 shows the titers of JHMV recovered from the CNS of antibody-treated and untreated mice following infection with JHMV. Initially, a reduction in virus titers was observed in all three groups. After day 7, viral titers in the untreated group continued to decline whereas the clearance of JHMV from infected mice treated with anti-CD8 monoclonal antibody was significantly inhibited. By day 11, the titers of virus recovered had increased to the levels observed on day 5 and were significantly higher (>100 times higher) than those recovered from infected, untreated mice. These data are consistent with our previous suggestion that the clearance of JHMV from the CNS is mediated by CD8⁺ cells (14). The inability to sustain clearance of virus in the

FIG. 2. Fluorescence-activated cell sorter analysis of spleen cells from JHMV-infected mice at 9 days postinfection. (a and b) Profiles of CD8⁺ and CD4⁺ cells, respectively, from an infected mouse which was not pretreated with antibody; (c and d) profiles from a CD4-depleted mouse; (e and f) profiles from a mouse treated with anti-CD8 monoclonal antibody. Panels a, c, and e show staining for CD8⁺ cells; panels b, d, and f show staining for CD4⁺ cells.

FIG. 3. Reduced ability of anti-CD4- or anti-CD8-depleted mice to clear virus from the CNS following JHMV infection. Mice were depleted *in vivo* of CD4⁺ or CD8⁺ cells by treatment with the appropriate monoclonal antibody prior to intracerebral infection with JHMV. Brains were obtained on the days indicated, and virus titers were estimated on monolayers of L2 cells. Results represent mean titers obtained from a representative experiment with three mice in each group at each time point. ■, Controls; ◇, anti-L3T4 treated (CD4); ○, anti-LYT-2 treated (CD8).

anti-CD8-treated mice is coincident with the time of peak cytotoxic activity observed during infections with other viruses (2). Thus, the lack of clearance of JHMV in anti-CD8-treated mice may be attributed to a lack of CD8⁺ cytotoxic T cells, suggesting that CD8⁺ T cells play a major role in clearing JHMV.

Interestingly, the data in Fig. 3 also show that anti-CD4 treatment can inhibit clearance of JHMV. The kinetics of viral replication and viral titers obtained from anti-CD4-treated mice were very similar to those obtained from anti-CD8-treated mice. These results suggest an interdependence of the CD4⁺ and CD8⁺ T-cell subsets, with both cell types playing critical roles in the clearance of JHMV from the CNS. The observation that adoptive transfer of CD4⁺, non-DTH-inducer T cells can result in viral clearance (14), together with these data showing that virus clearance is abolished by depletion of either the CD4⁺ or the CD8⁺ population, is consistent with the hypothesis that clearance of JHMV is mediated by CD8⁺ T cells in a CD4⁺ T-cell-dependent fashion. The requirement for CD4⁺ cells for efficient clearance of JHMV is in contrast to infection with other viruses such as lymphocytic choriomeningitis virus, in which the activation of CD8⁺ effectors does not appear to depend on the concurrent presence of the CD4⁺ population (1).

Another interesting observation from the data shown in Fig. 3 is the similarity in the initial rates of viral clearance in all three groups of mice (days 5 to 7). This early reduction in viral titer was also observed in mice which received whole-body irradiation 1 h prior to infection but not in mice irradiated 24 h earlier (Fig. 1). These observations suggest that a radiosensitive cell that does not express either the CD4 or CD8 cell surface marker may mediate clearance early in infection. Since JHMV infection has previously been shown to induce NK cells through an interferon-independent mechanism (9), one possibility for the early reduction of virus in the CNS by a radiosensitive, non-T cell is the activation of NK cells. This concept is reinforced by the observation that NK cells exhibit antiviral activity early in infection (2). An alternative possibility is the B-cell-mediated

FIG. 4. Serum anti-JHMV antibody levels of untreated and monoclonal antibody-treated mice following JHMV infection. Sera were diluted 1:100, and specific antibodies were measured by enzyme-linked immunosorbent assay. Absorbances observed with sera from anti-CD4-treated mice were not significantly different from levels obtained with pooled sera from uninfected mice (data not shown). Results represent mean titers obtained from a representative experiment with sera from three mice in each group at each time point. ■, Control; ◇, anti-LYT-2 treated (CD8); ●, anti-L3T4 treated (CD4).

lysis of infected cells, which has been described for other strains of mouse hepatitis virus (17), although the *in vivo* relevance of these cells is unclear.

These results establish significant roles for both CD4⁺ and CD8⁺ cells in the reduction of virus in the CNS of infected mice. Since antiviral antibodies are produced during JHMV infection, it is possible that antibodies may also contribute to viral clearance. Furthermore, passively transferred monoclonal antibodies specific for the three viral structural proteins have been shown to be protective (3, 7). The present study, however, suggests that the presence of antibodies may not play a major role in preventing viral replication in the CNS. Serum JHMV-specific antibodies in untreated control and anti-CD4- and anti-CD8-depleted mice were measured by an enzyme-linked immunosorbent assay, as described previously (11). Figure 4 shows the kinetics of the development of antibody responses following JHMV infection. The results show that the anti-JHMV antibodies in CD8-depleted mice were equivalent to the levels observed in untreated control mice. In spite of the presence of similar levels of antibody, CD8-depleted mice were unable to clear virus, unlike untreated mice. This suggests that clearance of virus in untreated mice is effected by CD8⁺ cells and that antibodies, while present, may have a minimal or no role in clearance of virus from the CNS. The results shown in Fig. 4 also show a clear dependence of antibody production on the presence of CD4⁺ T cells. Anti-CD4-treated mice failed to generate an antibody response to JHMV, whereas anti-CD8-treated mice, whose CD4⁺ population was unaffected, had titers equivalent to those of untreated mice.

In conclusion, these data confirm a role for both CD4⁺ and CD8⁺ T cells in the antiviral immune response during JHMV infection. These observations are consistent with the hypothesis that clearance of JHMV from the CNS is mediated by CD8⁺ cells, whose induction may in turn be dependent on the presence of CD4⁺ helper-inducer cells.

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LITERATURE CITED

1. Ahmed, R., L. D. Butler, and L. Bhatti. 1988. T4⁺ T helper cell function *in vivo*: differential requirement for induction of anti-viral cytotoxic T-cell and antibody responses. *J. Virol.* **62**:2102–2106.
2. Biron, C. A., R. J. Natuk, and R. M. Welsh. 1986. Generation of large granular T lymphocytes *in vivo* during viral infection. *J. Immunol.* **136**:2280–2286.
3. Buchmeier, M. J., H. A. Lewicki, P. J. Talbot, and R. L. Knobler. 1984. Murine hepatitis virus-4 (strain JHM)-induced neurologic disease is modulated *in vivo* by monoclonal antibody. *Virology* **132**:261–270.
4. Erlich, S. S., G. K. Matsushima, and S. A. Stohlman. 1989. Studies on the mechanism of protection from acute viral encephalomyelitis by delayed-type hypersensitivity inducer T cell clones. *J. Neurol. Sci.* **90**:203–216.
5. Knobler, R. L., M. Dubois-Dalcq, M. V. Haspel, A. P. Clay-smith, P. W. Lampert, and M. B. A. Oldstone. 1981. Selective localization of wild type and mutant mouse hepatitis virus (JHM strain) antigens in CNS tissue by fluorescence, light and electron microscopy. *J. Neuroimmunol.* **1**:81–92.
6. Lampert, P. W., J. K. Sims, and A. J. Kniazeff. 1973. Mechanism of demyelination in JHM virus encephalomyelitis. Electron microscopic studies. *Acta Neuropathol.* **24**:76–85.
7. Nakanaga, K., K. Yamanouchi, and K. Fujiwara. 1986. Protective effect of monoclonal antibodies on lethal mouse hepatitis virus infection in mice. *J. Virol.* **59**:168–171.
8. Sorenson, O., R. Dugre, D. Percy, and S. Dales. 1982. In vivo and in vitro models of demyelinating disease: endogenous factors influencing demyelinating disease caused by mouse hepatitis virus in rats and mice. *Infect. Immun.* **37**:1248–1260.
9. Stohlman, S. A., P. R. Brayton, R. C. Harmon, D. Stevenson, R. G. Ganges, and G. K. Matsushima. 1983. Natural killer cell activity during mouse hepatitis virus infection: response in the absence of interferon. *Int. J. Cancer* **31**:309–314.
10. Stohlman, S. A., P. R. Brayton, L. P. Weiner, and M. M. C. Lai. 1982. Murine coronaviruses: isolation and characterization of two plaque morphology variants of the JHM neurotropic strain. *J. Gen. Virol.* **63**:265–275.
11. Stohlman, S. A., G. K. Matsushima, N. Casteel, and L. P. Weiner. 1986. *In vivo* effects of coronavirus-specific T cell clones: DTH inducer cells prevent a lethal infection but do not inhibit virus replication. *J. Immunol.* **136**:3052–3056.
12. Stohlman, S. A., and L. P. Weiner. 1981. Chronic central nervous system demyelination in mice after JHM virus infection. *Neurology* **31**:38–44.
13. Sussman, M. A., J. O. Fleming, H. Allen, and S. A. Stohlman. 1987. Immune mediated clearance of JHM virus from the central nervous system. *Adv. Exp. Med. Biol.* **218**:399–410.
14. Sussman, M. A., R. A. Shubin, S. Kyuwa, and S. A. Stohlman. 1989. T-cell mediated clearance of mouse hepatitis virus strain JHM from the central nervous system. *J. Virol.* **63**:3051–3056.
15. Wege, H., R. Dories, and H. Wege. 1984. Hybridoma antibodies to the murine coronavirus JHM: characterization of epitopes on the peplomer protein (E2). *J. Gen. Virol.* **65**:1931–1942.
16. Weiner, L. P. 1973. Pathogenesis of demyelination induced by a mouse hepatitis virus (JHM virus). *Acta Neurol.* **18**:298–303.
17. Welsh, R. M., Jr., M. V. Haspel, D. C. Parker, and K. V. Holmes. 1986. Natural cytotoxicity against mouse hepatitis virus-infected cells. II. A cytotoxic effector cell with a B lymphocyte phenotype. *J. Immunol.* **136**:1454–1460.