# T-Cell-Mediated Clearance of Mouse Hepatitis Virus Strain JHM from the Central Nervous System

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Clearance of the neurotropic JHM strain of mouse hepatitis virus from the central nervous system was examined by the transfer of spleen cells from immunized donors. A T cell with the surface phenotype of Thy1.2$^+$ CD4$^+$ CD8$^-$ asialo-GM$_1$$^+$ Mac-1$^-$ was found to be necessary for viral clearance. The surface phenotype and adherence to nylon wool suggest that these cells are activated helper-inducer T cells. Adoptive transfer to congenic histocompatibility strains demonstrated the necessity for compatibility at the *D* locus of the major histocompatibility complex. The expression of the CD4 surface marker and the requirement for major histocompatibility complex class I were further studied by the transfer of cells to recipients treated with anti-CD4 or anti-CD8 monoclonal antibodies. Treatment of recipients with either the anti-CD8 or the anti-CD4 antibodies inhibited virus clearance from the central nervous system. This suggests that the CD4$^+$ cell acts as a helper and that virus is cleared from the central nervous system by CD8$^+$ cells that recognize viral antigen in the context of the *H-2D$^b$* gene product.

Infection with the JHM neurotropic strain of mouse hepatitis virus (JHMV) results in an acute encephalomyelitis with demyelination in both mice and rats (6, 15, 25, 26, 33, 36, 37). Demyelination appears to result from a lytic infection of oligodendroglial cells (15, 37). Survivors show evidence of chronic demyelination with persistent viral antigen (6, 14); however, infectious virus has rarely been recovered from animals with chronic disease (14). It has recently been suggested that a myelin-specific autoimmune response can be induced during JHMV infection (34); however, the relative roles of autoimmunity and persistent viral infection in the pathogenesis of the chronic phase of JHMV-induced demyelination are not clear. Although JHMV induces both T- and B-cell responses, the mechanism of virus clearance from the central nervous system (CNS) has not been examined. Antibody is apparently ineffective in eliminating virus from the CNS, since antiviral antibody is often detected in the cerebrospinal fluid during chronic CNS infections, including infections with JHMV (5, 7). Indeed, the adoptive transfer of anti-JHMV monoclonal antibodies protects susceptible mice from lethal JHMV infection (2, 6a, 17, 21, 35); however, these antibodies have little effect on the replication of JHMV in the CNS (2, 6a, 35). Although the mechanism of protection in the absence of suppression of viral replication is not clear, it has been noted that following administration of the antiviral antibodies, infection occurred predominantly in oligodendroglial cells, with little or no infection of neurons (2, 6a). These observations are consistent with our recent report of a monoclonal antibody-selected variant of JHMV that replicates in the CNS almost as well as parental JHMV but is not fatal (8). This variant also preferentially infects glial cells but not neurons, suggesting that survival of JHMV infection is correlated with the absence of neuronal infection (8).

Cell-mediated immunity has also been shown to be important in protection from JHMV infection. The adoptive trans-

fer of JHMV-specific CD4$^+$ polyclonal splenic T cells and delayed-type hypersensitivity (DTH) inducer T-cell clones prevents death due to JHMV infection (30, 31). No decrease in CNS viral titer accompanied DTH inducer T-cell-mediated protection (30, 31), and predominant infection of glial cells in the white matter was found in protected animals (S. S. Erlich, G. K. Matsushima, and S. A. Stohlman, J. Neurol. Sci., in press).

The titer of JHMV in the CNS peaks early in infection and then declines (30). It is not known whether cytotoxic T cells participate in either protection or viral clearance during JHMV infection in vivo, although the clearance of infectious virus is generally accomplished by CD8$^+$ major histocompatibility complex (MHC) class I-restricted cytotoxic T cells. Three cell types have been described which exhibit cytotoxicity for mouse hepatitis virus-infected cells in vitro, including an NK-like cell (3), a cytolytic B cell (11), and CD8$^+$ cytotoxic T cells (40). However, their role in the antiviral immune response in the CNS is unclear. Although protection from a lethal JHMV infection can be at least partially attributed to both DTH inducer T cells and antiviral antibody, neither of these effector mechanisms suppressed viral growth in the CNS.

The goal of this study was to examine the ability of immune cells to reduce virus titer in the CNS during JHMV infection. We have identified a nylon-wool-adherent (NWA) CD4$^+$ T cell which may function as a helper cell for the induction of MHC class I (*D$^b$*)-restricted CD8$^+$ cells, which are apparently the primary effectors in reducing virus titer in the CNS of JHMV-infected mice.

## MATERIALS AND METHODS

**Mice.** C3Heb/Fe, C57BL/6, B10.A(2R), B10.A(5R), B10.BR, and B10.MBR mice were purchased from Jackson Laboratory, Bar Harbor, Maine, at 5 to 6 weeks of age. The mice were used within 7 days of arrival. Serum samples obtained from representative mice were tested for JHMV-specific antibody by enzyme-linked immunosorbent assay as previously described (9) and found to be negative.

**Cell lines.** DBT cells, a continuous murine astrocytoma

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cell line, were grown in minimal essential medium (GIBCO Laboratories, Grand Island, N.Y.) containing 7% newborn calf serum (Biocell Laboratories, Carson, Calif.), 100 U of penicillin per ml and 100 μg of streptomycin per ml (GIBCO) at 37°C in a humidified atmosphere containing 7.5% $CO_2$. L-2 fibroblast cells were maintained in Spinner flasks (Wheaton Industries, Millville, N.J.) containing Joklik medium (GIBCO) supplemented with 8% fetal calf serum (FCS) at 37°C.

**Virus and viral antigen.** Viruses were propagated on DBT cells in minimal essential medium containing 10% tryptose phosphate broth and 2% fetal bovine serum (Irvine Scientific, Irvine, Calif.). The viral titer was determined by a plaque assay with DBT cells as previously described (8, 29, 39). Mice were inoculated intracerebrally with approximately 5 × 10³ PFU of the DS strain of JHMV in a volume of 0.03 ml. Immunizations were performed by intraperitoneal injection of 1.0 ml containing approximately 1 × 10⁶ PFU of JHMV. Viral titers in the brains of infected mice were determined by limiting-dilution analysis with L-2 cells in 24-well plates as previously described (31). JHMV antigen used for DTH footpad tests consisted of a lysate of infected DBT cells sonicated in phosphate-buffered saline (pH 7.2), as previously described (30, 31, 39). A control lysate of uninfected DBT cells was similarly prepared.

**Adoptive transfers and use of nylon wool columns.** At 6 days after immunization, spleens were removed aseptically and teased, and the cells were washed twice with RPMI 1640 medium containing 10% FCS. The cells were adjusted to the appropriate concentration in RPMI 1640 medium supplemented with 2% FCS before being transferred intravenously (i.v.) to recipient mice. The mice were infected immediately following adoptive transfer. Nylon wool (Fenwal Labs, Deerfield, Ill.) was prepared as previously described (31). Approximately 8 × 10⁸ cells were adsorbed for 1 h onto each column, which contained 2.4 g of nylon wool in a 30-ml syringe at 37°C. The nylon-wool-nonadherent (NWNA) cells were collected by washing the column with 3 volume equivalents of RPMI 1640 medium containing 10% FCS. The NWA population was removed by allowing the column to run dry and refilling it with ice-cold Hanks balanced salt solution. The cells were expelled from the column by using the syringe plunger. Approximately equal numbers of cells were recovered in each population. Cells were washed once, suspended in RPMI 1640 medium supplemented with 2% FCS, and adjusted to the appropriate concentrations prior to transfer to recipient mice. Statistical significance was tested by using the two-tailed Student $t$ test.

**Monoclonal antibodies.** Hybridoma cell lines which secrete monoclonal antibodies Mac-1 and GK1.5 (specific for CD4) were purchased from the American Type Culture Collection, Rockville, Md. The monoclonal antibody NK1.1 was kindly provided by G. Koo, Merck, Sharp & Dohme Research Laboratories, Rahway, N.J. Anti-Thy-1.2 and anti-Lyt-2.2 monoclonal antibodies were obtained from Cedarlane Laboratories Ltd., Hornby, Ontario, Canada. Anti-asialo-$GM_1$ polyclonal serum was obtained from DAKO Laboratories, Santa Barbara, Calif. For injection into mice, the anti-L3T4 and anti-Lyt-2.2 monoclonal antibodies were concentrated by precipitation with saturated ammonium sulfate. Recipients were injected intraperitoneally with approximately 100 μg of the concentrated monoclonal antibodies 2 days before, the same day, and 3 days after the transfer of NWA spleen cells from immunized donors. The concentrated anti-Lyt-2.2 was shown to effectively inhibit the induction of anti-lymphocytic choriomeningitis virus CD8⁺ killer cells (kindly

tested by R. Ahmed, University of California at Los Angeles School of Medicine).

**Complement-mediated antibody depletions.** Depletions of NWA cells were carried out in RPMI 1640 medium containing 1% bovine serum albumin and 10 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) buffer (both from Sigma Chemical Co., St. Louis, Mo.). Cell suspensions at a final concentration of 10⁷ cells per ml were incubated with antibodies for 45 min on ice at 4°C. After incubation, the cells were washed three times with ice-cold medium and suspended to the original volume in Low-Tox M rabbit complement (Cedarlane Laboratories) at a final dilution of 1:10. The suspension was incubated for 60 min at 37°C. The cells were then washed three times and suspended to the original viable-cell concentration prior to i.v. transfer. Depletion was verified by trypan blue exclusion.

**DTH.** Footpad swelling was used as a measure of DTH as previously described (30, 31, 39). Within 2 h of the i.v. transfer of cells, recipient mice were injected in the right footpad with 20 μl of JHMV antigen. Control antigen (20 μl) was injected in the left footpad. The dorsoventral thickness of the footpad was measured 24 h later with a micrometer (Miyutoyo Instruments, Tokyo, Japan). Data are presented as the difference between the right footpad measurement and the left footpad measurement.

## RESULTS

**Transfer of CD4⁺ cells reduces viral titer in the CNS.** The maximum JHMV titer in the brain occurs at 4 to 5 days postinfection, and the titer subsequently decreases until mice succumb to the infection (30). No decrease in viral titer is found in lethally infected irradiated (850 rads) mice, suggesting that the decrease is immune system mediated (J. O. Fleming, personal communication). To determine whether cell-mediated immunity might contribute to the reduction in viral titer in the CNS, spleen cells from mice immunized 6 days previously with JHMV were adoptively transferred to recipient mice, which were immediately infected with JHMV. The titer of JHMV in the brains of these mice was determined 5 days later, corresponding to the time of peak viral titer in the CNS (30). Spleen cells from immunized donors reduced the viral titer in the brains of recipient mice approximately 500-fold compared with untreated controls (Table 1). Spleen cells from immunized mice were applied to nylon wool columns to enrich for T cells as previously described (31). Both the NWNA and NWA fractions were adoptively transferred to lethally infected mice. The NWNA population failed to significantly reduce the viral titer in recipient mice, even though it contained approximately 80% of the total T cells (Table 1). Surprisingly, the active cells were found in the NWA fraction (Table 1). The titer decrease in mice receiving pooled NWNA and NWA fractions was not significantly different from the reduction of viral titer in recipients of NWA cells alone (Table 1).

To determine the surface phenotype of the cells responsible for the reduction of the JHMV titer, antibody-plus-complement lysis was used to deplete NWA cells of cells expressing specific surface markers before the cells were transferred to JHMV-infected recipients. Depletion of the Thy-1⁺ population from the NWA fraction completely inhibited the reduction of JHMV titer in the brains of infected mice (Table 2, experiment 1). Flow-cytometric analysis showed that the NWA fraction was composed of approximately 25% Thy-1⁺ cells, which represents approximately

TABLE 1. Viral titer reduction by adoptive transfer of nylon-wool-separated spleen cells from JHMV-immunized donor mice

| Cells transferred[a] | Viral titer[b] | Decrease[c] in viral titer |
|---|---|---|
| None | 4.8 ± 0.4 | |
| Whole spleen | 2.3 ± 0.7 | 2.5 |
| NWNA | 4.5 ± 0.7 | 0.3 |
| NWA | 2.8 ± 0.9 | 2.0 |
| NWNA + NWA[d] | 2.0 ± 0.5 | 2.8 |

[a] Unseparated spleen cells ($10^8$) or nylon-wool-separated spleen cells (5 × $10^7$) were transferred i.v. on the same day as virus challenge from C57BL/6 donors to C57BL/6 recipients. Three mice were tested per group.

[b] Titer in the brain was determined on day 5 postinfection and is expressed as log PFU per gram ± one standard deviation of the mean.

[c] The difference between the viral titer in control mice receiving no cells and the titer in recipients of adoptively transferred spleen cells expressed as log decrease.

[d] An equivalent mixture totaling $10^8$ NWNA and NWA cells was injected.

23% of the total Thy-1[1] cells (data not shown). Since we had previously shown that CD4^{+} DTH inducer T cells were unable to reduce the JHMV titer (30, 31), cells expressing CD8 were also depleted. However, depletion of CD8-bearing T cells had no effect on the ability of the NWA cells to clear JHMW. Depletion of cells expressing asialo-GM$_1$ also resulted in the inhibition of viral clearance, suggesting the possibility that natural-killer (NK) cells participate in JHMV clearance. NK cells are generally NWNA and express asialo-GM$_1$, NK1.1, and low or undetectable levels of Thy-1 (10). Since some T cells also express asialo-GM$_1$ (28), it was important to distinguish between these two cell types. Antibodies to the NK cell marker, NK1.1, and to CD4 were used in depletion experiments (Table 2, experiment 2). No effect on viral titer was observed following depletion of NK1.1^{+} cells, suggesting that the active cell was not an NK cell. By contrast, treatment with anti-L3T4 antibody plus complement completely abolished the ability of NWA cells to reduce viral titer. Elimination of the Mac-1^{+} macrophages from the NWA fraction also had no effect on the ability of the transferred cells to reduce JHMV titer and confirmed the importance of CD4^{+} T cells (Table 2, experiment 3). Together, these data indicate that the cell responsible for reducing JHMV titer in the CNS expresses the Thy1^{+} CD4^{+} asialo-GM$_1$[+] CD8^{-} NK1.1^{-} Mac-1^{-} cell surface phenotype and therefore belongs to the helper-inducer subset of T cells.

The T cells responsible for reduction of the JHMV titer are phenotypically similar to the CD4^{+} JHMV-specific DTH

TABLE 3. Viral titer reduction in irradiated recipients

| Group | Cells transferred[a] | Viral titer[b] | Decrease in titer[c] |
|---|---|---|---|
| Untreated[d] | None | 8.3 ± 0.8 | |
| | Untreated | 4.8 ± 0.6 | 3.5 |
| | C only[e] | 5.1 ± 0.7 | 3.2 |
| | Lyt-2 + C | 5.2 ± 0.3 | 3.1 |
| Irradiated[f] | None | 9.3 ± 0.3 | |
| | Untreated | 7.0 ± 0.4 | 2.3 |
| | C only | 7.2 ± 0.6 | 2.1 |
| | Lyt-2 + C | 9.3 ± 0.4 | 0 |

[a] NWA cells (5 × $10^7$) from JHMV-immunized donors were transferred i.v. to naïve recipient mice.

[b] Expressed as log PFU per gram of brain tissue ± one standard deviation of the mean.

[c] The difference between the viral titers in control mice receiving no cells and the NWA cell recipients.

[d] Naïve 6-week-old C57BL/6 mice. Three mice were tested per group.

[e] C, Complement.

[f] Naïve 6-week-old C57BL/6 mice were given 2,000 rads 24 h before infection. Two mice were tested per group.

inducer T cells. We have previously shown that these CD4^{+} DTH inducer T cells confer protection from lethal JHMV challenge without reducing the JHMV titer (30, 31). Therefore, NWA and NWNA cells were compared for the ability to induce a JHMV-specific DTH response in naïve recipients as previously described (30, 31, 39). Spleen cells from JHMV-immunized donors transferred a significant DTH response ([29.3 ± 4.7] × $10^{-2}$ mm) as previously shown (31). However, the DTH inducer activity partitioned into the NWNA fraction ([42.0 ± 5.0] × $10^{-2}$ mm) when compared with the NWA fraction ([10.0 ± 7.2] × $10^{-2}$ mm). These data indicate that the NWA T cells are functionally distinct from previously described DTH inducer T cells (30, 31), although both express the Thy-1 and CD4 cell surface markers.

**Role of CD8^{+} cells.** The data in Table 2 suggest that CD4^{+} T cells play a major role in the clearance of JHMV from the CNS. CD4^{+} cells usually function as helper cells, although CD4^{+} class II-restricted cytotoxic effector cells have been described (18). To distinguish between helper and primary effector cell function, NWA cells were initially transferred into lethally irradiated (800 rads) recipients in a preliminary experiment (Table 3). The transfer of NWA cells resulted in a ca. 100-fold reduction in the viral titer. However, removal of CD8^{+} cells from this population completely abolished any antiviral activity in the irradiated recipients. This suggests

TABLE 2. Surface phenotype of the NWA cell responsible for reduction of JHMV titer

| Antibody treatment[a] | Cells depleted | Expt 1 | | Expt 2 | | Expt 3 | |
|---|---|---|---|---|---|---|---|
| | | Viral titer[b] | Decrease in titer[c] | Viral titer | Decrease in titer | Viral titer | Decrease in titer |
| No cells transferred | | 9.0 ± 0.4 | | 6.7 ± 0.7 | | 5.3 ± 0.7 | |
| C only[d] | | 5.1 ± 0.8 | 3.9 | 2.8 ± 0.5 | 3.9 | 3.1 ± 0.5 | 2.2 |
| Thy-1 + C | T cells | 9.3 ± 0.4 | 0.0 | | | | |
| Lyt-2 + C | Cytotoxic T cells | 5.8 ± 1.5 | 3.2 | | | 2.7 ± 0.4 | 2.6 |
| Asialo-GM$_1$ + C | NK cells, some T cells | 8.8 ± 0.5 | 0.2 | | | | |
| NK1.1 + C | NK cells | | | 3.5 ± 0.3 | 3.2 | | |
| L3T4 + C | Helper T cells | | | 6.3 ± 0.4 | 0.4 | 4.8 ± 0.4 | 0.5 |
| Mac-1 + C | Macrophages | | | | | 2.7 ± 0.4 | 2.6 |

[a] NWA cells (5 × $10^7$) from JHMV-immunized donors were transferred i.v. to naïve recipient mice.

[b] Expressed as log PFU per gram of brain tissue ± one standard deviation of the mean. Three or four mice were tested per group.

[c] The difference between the viral titer in control mice receiving no cells and the titer in recipients of adoptively transferred spleen cells expressed as log decrease.

[d] C, Complement.

TABLE 4. In vivo antibody-mediated depletion

| Group[a] | Antibody | Expt 1 | | Expt 2 | |
|---|---|---|---|---|---|
| | | Viral titer[b] | Decrease in titer[c] | Viral titer | Decrease in titer |
| JHMV only | None | 8.3 ± 0.5 | | 7.0 ± 0.5 | |
| JHMV + NWA cells[d] | None | 2.9 ± 0.5 | 5.4 | 3.5 ± 0.6 | 3.5 |
| JHMV + NWA cells | Anti-L3T4 | 7.0 ± 1.2 | 1.3 | ND[e] | |
| JHMV + NWA cells | Anti-Lyt-2 | 7.1 ± 1.2 | 1.2 | 6.3 ± 1.0 | 0.7 |

[a] All mice received $5 \times 10^3$ PFU of JHMV intracutaneously.
[b] Expressed as log PFU per gram of brain tissue 5 days postinfection ± one standard deviation of the mean. Three or four mice were tested per group. ·
[c] Log difference between titers in control and recipient mice.
[d] NWA cells ($5 \times 10^7$) from JHMV-immunized donor mice were transferred i.v. on day of infection.
[e] ND, Not determined.

that some activated antiviral CD8$^+$ cells were present in the NWA fraction, even though this could not be demonstrated by adoptive transfer into immunocompetent recipients (Table 2).

To directly address the ability of the NWA CD4$^+$ cells to provide help for antiviral CD8$^+$ cells, we treated recipient mice with either anti-L3T4 or anti-Lyt-2 antibody. The JHMV titer in the brain was determined 5 days following the transfer of the NWA cells. As expected, treatment with anti-L3T4 antibody completely inhibited the ability of the NWA cells to alter the JHMV titer in the brain (Table 4). Similarly, treatment with anti-Lyt-2 was clearly able to inhibit the ability of the NWA cells to reduce the viral titer in the brain. This suggests that the CD4$^+$ cell in the NWA population functions as a helper to induce a CD8$^+$ primary effector cell.

**Histocompatibility at MHC class I is required for JHMV titer reduction.** To determine the requirement for histocompatibility, we first demonstrated that the adoptive transfer of spleen cells from immunized C57BL/6 ($H\text{-}2^b$) or C3Heb/Fe ($H\text{-}2^k$) donors to mice of the same haplotype reduced the viral titer over 5,000-fold. Transfer of spleen cells from immunized donors to $H\text{-}2$ mismatched strains, for example, C57BL/6 into C3Heb/Fe, resulted in no decrease in the JHMV titer (data not shown). The necessity for histocompatibility at MHC class I and/or class II loci was examined by adoptive transfers of cells between various congenic donor strains. Table 5 shows the ability of cells from congenic donors to reduce the viral titer in the brains of C57BL/6 recipients. Total histocompatibility between donor and recipient resulted in a ca. 10,000-fold reduction in the viral titer. C57BL/6 recipients of cells from immunized donors matched at the $K$ locus (B10.MBR; $K^b I^k D^q$) or the $K$ and $I$ loci [B10.A(5R); $K^b I^b D^d$] showed insignificant decreases in the JHMV titer (approximately five- to eightfold). A significant reduction in the JHMV titer in C57BL/6 recipients (20,000-fold) was observed after transfer of cells from

the B10.A(2R) strain ($K^k I^k D^b$), which shares identity with C57BL/6 only at the $D$ locus.

Restriction of the $D$ locus was further investigated by the adoptive transfer of cells from immunized C57BL/6 mice into congenic recipients. The requirement for $D$-region compatibility was confirmed by using B10.A(2R) ($K^k I^k D^b$) recipient mice (Table 6). Cells from C57BL/6 donors ($H\text{-}2^b$) which matched at the $D$ locus of the B10.A(2R) strain resulted in an approximately 300-fold decrease in the titer. Cells donated to B10.A(5R) or B10.MBR strains which matched C57BL/6 at the $K$ and $I$ loci had little effect on the JHMV titer (10- to 20-fold), similar to the effect observed in totally histoincompatible B10.BR mice ($H\text{-}2^k$; approximately 10-fold). These experiments demonstrate that histocompatibility at the MHC class I $D$ locus is necessary between the donated NWA T cells and the recipient mouse for reduction of the JHMV titer.

## DISCUSSION

The ability of JHMV to infect oligodendroglial cells in the CNS of mice and rats and induce an acute encephalomyelitis accompanied by demyelination has been studied extensively as a model of human multiple sclerosis (6). It is generally accepted that the immune response plays an active role in the JHMV-induced establishment of chronic infection and ongoing demyelination. The role of antibody in the pathogenesis of JHMV infection has been studied by the adoptive transfer of JHMV-specific monoclonal antibodies and also by infection of newborn mice born of immunized mothers (23, 24). Anti-JHMV antibody prevents death from JHMV infection; however, protection does not result from direct virus neutralization, since none of the monoclonal antibodies tested to date are potent inhibitors of virus replication in the CNS (2, 6a, 35). In addition, we have recently shown that, at least in the case of nonneutralizing antibodies specific for the viral E1 matrix protein, clearance of JHMV does not occur

TABLE 5. Genetic restriction of the antiviral effects mediated by spleen cells from congenic donors

| Donor strain[a] | H-2 K I D | Compatible loci | Expt 1 | | Expt 2 | |
|---|---|---|---|---|---|---|
| | | | Viral titer[b] | Decrease in titer[c] | Viral titer | Decrease in titer |
| None | | | 8.5 ± 0.5 | | 7.5 ± 0.5 | |
| C57BL/6 | b b b | K I D | 3.8 ± 0.4 | 4.7 | 2.0 ± 0.5 | 5.5 |
| B10.MBR | b k g | K | 7.8 ± 0.4 | 0.7 | 7.0 ± 0.6 | 0.5 |
| B10.A(5R) | b b d | K I | 7.7 ± 0.7 | 0.8 | 7.0 ± 0.6 | 0.5 |
| B10.A(2R) | k k b | D | 6.3 ± 0.7 | 2.2 | 3.3 ± 0.9 | 4.2 |

[a] NWA cells ($5 \times 10^7$) from JHMV-immunized donor mice were transferred i.v. to C57BL/6 recipients.
[b] Expressed as log PFU per gram of brain tissue ± one standard deviation of the mean. Three mice were tested per group.
[c] Log difference between the titer in control and recipient mice. Reductions in titers in recipients of cells from B10.MBR and B10.A(5R) were not statistically significant ($P \geq 0.1$). Reductions in titers in recipients of B10.A(2R) cells were statistically significant ($P \leq 0.05$ [experiment 1]; $P \leq 0.01$ [experiment 2]).

TABLE 6. Genetic restriction of the antiviral effects mediated
by spleen cells from C57BL/6 donor mice

| Recipient strain[a] | H-2 | | | Compatible loci | Viral titer[b] | | Decrease in titer[c] |
|---|---|---|---|---|---|---|---|
| | K | I | D | | Control[d] | Recipient | |
| C57BL/6 | b | b | b | K I D | 7.0 ± 0.5 | 3.3 ± 0.4 | 3.7 |
| B10.A(2R) | k | k | k | D | 6.8 ± 0.5 | 4.5 ± 0.6 | 2.3 |
| B10.MBR | b | k | g | K | 7.2 ± 0.5 | 6.1 ± 0.8 | 1.1 |
| B10.A(5R) | b | b | d | K I | 7.2 ± 0.5 | 6.0 ± 0.5 | 1.2 |
| B10.BR | k | k | k | | 7.2 ± 0.5 | 6.1 ± 0.8 | 1.1 |

[a] NWA cells (5 × 10⁷) from JHMV-immunized C57BL/6 donor mice were transferred i.v. 6 days after immunization.

[b] Expressed as log PFU per gram of brain tissue ± one standard deviation of the mean. Three mice were tested per group.

[c] Log difference between viral titer in control and recipient mice. Reductions in the B10.A(2R) recipients were statistically significant ($P \leq 0.05$), whereas reductions in B10.MBR, B10.A(5R), and B10.BR were not significant ($P \geq 0.1$).

[d] Titer from mice of the same strain and age as recipients which did not receive transferred spleen cells.

via antibody-mediated complement-dependent lysis of infected cells (6a). The inability of antibody to reduce the viral titer in the CNS of protected mice, as well as results of studies of both nude mice and rats and animals immunosuppressed by lethal irradiation, suggests that cell-mediated immunity plays a critical role in the pathogenesis of JHMV infection (2, 6a, 13, 27). The adoptive transfer of either NWNA polyclonal JHMV-induced DTH effector T cells or JHMV-specific DTH inducer T-cell clones also protects mice from lethal JHMV infection. However, since neither of these cell populations was able to reduce the viral titer in the CNS (30, 31), another cell type must be responsible for this essential activity.

The present study demonstrates that an NWA Thy-1⁺ CD4⁺ subset of T cells is able to mediate the clearance of JHMV from the CNS of infected mice. Although cytotoxic CD4⁺ T cells recognizing influenza virus antigens in the context of MHC class II molecules have previously been described (18), the decreased ability of adoptively transferred NWA CD4⁺ cells to inhibit JHMV replication in mice immunocompromised by irradiation initially suggested the possibility that these CD4⁺ cells function as helper cells to induce CD8⁺ effector cells in the recipient. This possibility was further supported by data showing that depletion of the CD8⁺ cells from the NWA cells prior to their adoptive transfer into irradiated recipients completely eliminated the reductions in viral titer in the CNS when untreated NWA cells were transferred. A role for these NWA cells as helper cells was supported by (i) the depletion of CD8⁺ cells in the immunocompetent NWA cell recipients by the passive transfer of anti-Lyt-2 antibody; (ii) the inability of the NWA CD4⁺ cells to function as DTH inducers, which have previously been suggested to be a unique subset of CD4⁺ T cells (4); and (iii) the inability of the NWA cells to lyse targets expressing H-2ᵇ histocompatibility gene products (data not shown). Furthermore, the requirement for histocompatibility at the D locus and not the K or I locus for expression of the effector function is consistent with NWA CD4⁺ cell helper function. These data suggest that a CD8⁺ effector may be derived either from the host when the NWA cells are transferred into class II histocompatible recipients or from the small number of CD8⁺ cells contained in the NWA population transferred into class II histoincompatible recipients.

The restriction of the effector mechanism to Dᵇ is intriguing, since MHV infection results in the induction of MHC

class I genes on oligodendroglial cells and astrocytes and in the induction of class II MHC antigens on astrocytes (12, 16, 19, 20, 32). In addition, the expression of both MHC D antigen and viral proteins is doubled in brain endothelial cell cultures after JHMV infection, whereas that of MHC K gene products is selectively decreased (12). The expression of MHC D on cells of the CNS in response to JHMV infection, along with the necessity for Dᵇ region compatibility in the clearance of JHMV, indicates that these MHC gene products are important in directing anti-JHMV immune responses in the CNS.

The data presented in this report suggest that the NWA CD4⁺ T cell functions as a helper T cell for the induction of a CD8⁺ MHC class I Dᵇ-restricted effector cell. Although we have not directly identified the CD8⁺ cell as a cytotoxic T cell, CD8⁺ cytotoxic MHC class I-restricted T-cell clones specific for JHMV have recently been reported (40). Adoptive transfer of these clones into JHMV-infected mice does protect them from a lethal infection; however, no dramatic decrease in the JHMV titer in the CNS was detected (K. Yamaguchi, personal communication). This finding is similar to the results obtained following transfer of antiviral monoclonal antibody and DTH-induced T cells (2, 6a, 30, 31, 35). The clearance of lymphocytic choriomeningitis virus from the CNS is also mediated by CD8⁺ cells and occurs in the absence of the tissue destruction seen in other tissues infected with lymphocytic choriomeningitis virus (1, 22). This has led to the suggestion that clearance is mediated by lymphokines, rather than by direct lysis of infected neurons. JHMV infects predominantly oligodendroglial cells, although astrocytes and neurons are also infected during the course of a fatal disease. Our inability to detect viral antigen at day 5 postinfection suggests that clearance prevents the spread of infection to astrocytes and neurons. The absence of histologically apparent encephalomyelitis following transfer of the NWA CD4⁺ cells to JHMV-infected mice (M. A. Sussman, unpublished observation) also suggests that the clearance of JHMV by the CD8⁺ cells may be due to a soluble factor rather than direct lysis of infected cells. The low frequency of both the CD4⁺ and the CD8⁺ cells in the NWA fraction, the data demonstrating that pretreatment of recipients with monoclonal antibodies to both cell surface markers inhibited viral clearance, and the lack of demonstrable cytolytic activity in the NWA population cannot exclude the possibility that the CD4⁺ cell population also carries out a critical function independent of providing help for the CD8⁺ population. However, our current understanding of the respective roles of CD4⁺ and CD8⁺ cells is consistent with the hypothesis that the CD4⁺ NWA cells function as CD8⁺ helper-inducer T cells. The understanding of the role(s) of the CD8⁺ cells in suppression of JHMV replication requires analysis of the role of CD8⁺ cytotoxic T cells in protection, which is currently under way.

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