



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.

## Relapsing encephalomyelitis following transfer of partial immunity to JHM virus\*

Richard A. Shubin,<sup>1</sup> Mark A. Sussman,<sup>2†</sup> John O. Fleming<sup>1,2</sup> and Stephen A. Stohlman<sup>1,2‡</sup>

*Departments of Neurology<sup>1</sup> and Microbiology,<sup>2</sup> The University of Southern California School of Medicine, Los Angeles, CA 90033, U.S.A.*

(Received January 5, 1989; accepted in revised form September 8, 1989)

---

Shubin, R. A. (Dept of Neurology, The University of Southern California School of Medicine, Los Angeles, CA 90033, U.S.A.), M. A. Sussman, J. O. Fleming and S. A. Stohlman. Relapsing encephalomyelitis following transfer of partial immunity to JHM virus. *Microbial Pathogenesis* 1990; **8**: 305–314.

Mice infected with the JHM strain mouse hepatitis virus (JHMV) develop a fatal encephalomyelitis with evidence of demyelination. It has previously been shown that the adoptive transfer of  $5 \times 10^7$  nylon wool adherent (NWA) spleen cells from immunized donors to lethally infected recipients clears virus from the central nervous system (CNS) and prevents demyelination. Adoptive transfer of a smaller number ( $1 \times 10^7$ ) of NWA spleen cells from immunized donors also protects from death but does not significantly alter virus replication in the CNS during the acute phase of the infection. Moreover, these mice develop a transient non-fatal encephalomyelitis which occurs approximately 3 weeks post-infection. This delayed encephalomyelitis is associated with a mononuclear cell infiltration into the CNS but little or no evidence of virus replication or increased viral antigen. A virus-specific delayed-type hypersensitivity (DTH) response precedes this delayed onset of disease by 24 to 48 h. Resolution of disease correlates with a selective and permanent suppression of the JHMV-specific DTH reactivity. In addition, no virus-specific DTH is detected following adoptive transfer of viral-specific DTH effectors derived from immunized donors. In contrast, these mice respond to a heterologous antigen, KLH, suggesting that the resolution of the encephalitis is accompanied by a profound suppression in viral-specific DTH response.

*Key words:* coronaviruses; neurovirulence; delayed-type hypersensitivity; cellular immunity.

---

### Introduction

The JHM strain of mouse hepatitis virus (JHMV) is a murine coronavirus which causes acute encephalitis and both acute and chronic demyelination in mice and rats.<sup>1</sup> JHMV infection has been studied extensively as a model of multiple sclerosis, which may be caused by a viral infection encountered early in life that triggers an autoimmune response to oligodendrocytes and/or myelin components in individuals with an appropriate genetic background.<sup>2</sup> Our laboratory has been investigating the role of the

\* Supported by Public Health Service grants NS 07149 and NS 18146.

† Present address: Scripps Clinic and Research Foundation, MB-14, 10666 N. Torrey Pines Road, La Jolla, CA 92037, U.S.A.

‡ Author to whom correspondence should be addressed: S. A. Stohlman, University of California School of Medicine, MCK 142, 2025 Zonal Avenue, Los Angeles, CA 90033, U.S.A.

immune system in the pathogenesis of JHMV-induced disease and the ability of immune therapy to modify the course of disease. Mice and rats infected with JHMV have an acute neutralizing encephalomyelitis with neuronophagia.<sup>1</sup> To understand the pathogenesis of encephalitis and demyelination following JHMV infection of the CNS, we and others have examined the roles of the humoral and cellular immune response to JHMV and related mouse hepatitis viruses (MHV). Rodents can be protected from death by administration of monoclonal antibodies specific for the three virus structural proteins, E1,<sup>3</sup> E2<sup>4,5</sup> and the nucleocapsid protein.<sup>6,7</sup> Wege *et al.*,<sup>5</sup> demonstrated in rats that only anti-E2 monoclonal antibodies which neutralized virus and prevented cell fusion were protective. Protection by virus-specific monoclonal antibodies in mice is associated with sparing of neurons during JHMV infection and the subsequent development of subacute demyelination.<sup>4</sup>

Other studies have focused on the protective role that cellular immunity may play in JHMV pathogenesis. Using the adoptive transfer of JHMV-immune spleen cells into JHMV-challenged recipients, two distinct protective T lymphocyte populations have been identified. First, nylon wool non-adherent (NWA) cells (Thy 1<sup>+</sup>, CD4<sup>+</sup>, Lyt-2<sup>-</sup>) will induce a delayed-type hypersensitivity (DTH) response in recipients. These cells are restricted by major histocompatibility complex (MHC) Class II (I-A) identify between donor and recipient. Protected mice show vigorous perivascular mononuclear cell responses in the CNS but viral replication is not suppressed, and surviving mice show marked demyelination in central white matter. Second, nylon wool adherent (NWA) cells (Thy 1<sup>+</sup>, CD4<sup>-</sup>, Lyt-2<sup>-</sup>) will protect mice, suppress viral replication, and prevent demyelination. The effect of these cells is dependent on MHC Class I (H-2 D) identify between donor and recipient and is blocked by administration of anti-CD8<sup>+</sup> antibody to the recipient. Protection mediated by the administration of either donor NWA CD4<sup>+</sup> or NWA CD4<sup>+</sup> populations is prevented by immunosuppression of the recipients, implying that both donor T cell populations function primarily as helpers for host cells. Thus, NWA CD4<sup>+</sup> cells induce a DTH response, prevent a lethal encephalitis, but are unable to block viral replication and subsequent demyelination; NWA CD4<sup>+</sup> cells induce CD8<sup>+</sup> effector cells which protect mice, reduce viral replication, and prevent demyelination.

In the present report we have focused on the influence of the second T helper population, CD4<sup>+</sup> NWA cells, on JHMV-induced demyelination. Interestingly, when a reduced number of these cells are administered, sufficient to protect only partially from an ordinarily lethal JHMV challenge, many recipient surviving mice show a biphasic, relapsing disease. This finding is consistent with the observation that chronic CNS pathology often occurs when there is an equivalence or balance between the ability of a virus to replicate and the ability of immune system to clear the virus. We have also found that the onset and resolution of delayed disease is correlated with the level of anti-JHMV cellular immunity in these mice. This system provides a useful model for dissecting the regulation of immunological responses to a viral infection of the CNS with regard to clinically relevant phenomena, such as the onset and resolution of an episode of demyelination.

## Results

### *Kinetics of the biphasic disease*

The adoptive transfer of NWA, but not NWA cells, from immunized donors to lethally infected mice mediates the clearance of JHMV.<sup>11</sup> Previous results from our laboratory have indicated that the active cells in this population are CD4<sup>+</sup> T cells that function by providing help for CD8<sup>+</sup> cells, because treatment of the recipients with anti-Lyt-2

**Table 1** The effect of transfer of nylon wool adherent spleen cells into JHMV-infected mice

Number of cells transferred <sup>a</sup>	Live <sup>b</sup> / total	Viral titer <sup>c</sup>	Percent with late disease	Histological appearance <sup>g</sup>	
				Cellular infiltrate <sup>e</sup>	JHMV antigen <sup>e</sup>
None	0/11	5.3 ± 0.4	–	++	++
5 × 10 <sup>7</sup>	8/8	2.1 ± 0.4	0	0 <sup>f</sup>	0
1 × 10 <sup>7</sup>	24/28	4.5 ± 1.0	83%	++	++
5 × 10 <sup>6</sup>	1/11	4.5 ± 1.0	0	++ <sup>f</sup>	++
5 × 10 <sup>5</sup>	0/5	6.0 ± 1.0	–	++	++

<sup>a</sup> Nylon wool adherent spleen cells from immunized mice.

<sup>b</sup> At 30 days post-infection.

<sup>c</sup> Log pfu per g brain at 5 days post-infection.

<sup>d</sup> At 21 days post i.c. inoculation.

<sup>e</sup> 0 = absent, + = scanty (occasional positive cells), ++ = abundant (many positive cells).

<sup>f</sup> Not applicable.

<sup>g</sup> At 5 days post-infection.

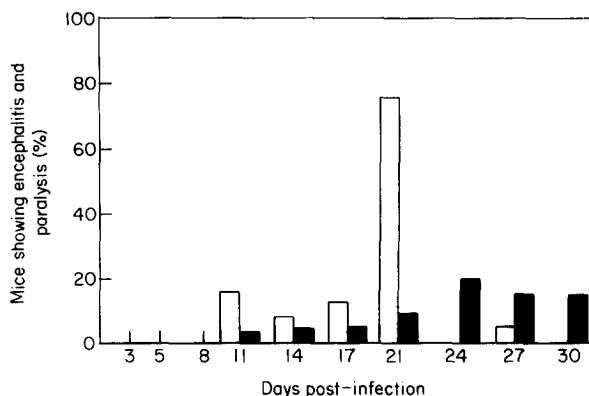
(CD8<sup>+</sup>) monoclonal antibody prevents the reduction of JHMV in the CNS.<sup>11</sup> Table 1 shows that adoptive transfer of 5 × 10<sup>7</sup> NWA cells results in the reduction of JHMV in the brain of infected mice as previously described.<sup>11</sup> To determine the number of cells required for protection, 1 × 10<sup>7</sup> and 5 × 10<sup>6</sup> NWA cells were transferred. The transfer of 1 × 10<sup>7</sup> NWA cells also protects against lethal JHMV infection; however, this cell number does not mediate reduction of the viral titer in the CNS (Table 1). Adoptive transfer of 5 × 10<sup>6</sup> or fewer cells neither protected mice nor cleared virus from the CNS (Table 1). Complement-mediated antibody depletions prior to transfer of 1 × 10<sup>7</sup> NWA cells per recipient indicated that the phenotype of the cell responsible for protection is a CD4<sup>+</sup> T cell (data not shown), similar to our previously published report.<sup>11</sup>

#### *Clinical course of the biphasic disease*

The quantity of NWA cells transferred during infection not only determines survival and the clearance of virus from the CNS but also the clinical course of the disease (Table 1). Control mice which received JHMV and no NWA cells showed clinical evidence of acute encephalomyelitis (ruffled fur, hyperexcitability, and myoclonus) on day 11 post-infection and died by day 14.<sup>11</sup> Mice which received 5 × 10<sup>7</sup> NWA cells did not manifest any clinical signs of encephalomyelitis following infection.<sup>11</sup> In contrast, most mice which received the intermediate number of NW cells (1 × 10<sup>7</sup>) developed a non-fatal encephalitis. Figure 1 outlines the incidence of clinical disease in this group. All mice appeared normal until day 11. At that time, a subgroup (5 out of 24 or 22%) developed encephalomyelitis of variable severity from which a minority (1 out of 5 or 25%) died. The remainder of this subgroup recovered clinically by day 14. On approximately day 21 post-infection (17 out of 20 or 85%) develop a second episode of encephalomyelitis of variable severity. This second phase was lethal for one out of the 17 or 5% of the mice; however, the majority (15 of 16) exhibit signs of transient encephalomyelitis which resolves by day 25 post-infection.

#### *Viral titer and histopathology*

During lethal infection under conditions noted in the Materials and methods section with JHMV, maximum viral titer in the CNS occurs at 5 day post-infection.<sup>8,11</sup> There is abundant viral antigen demonstrated immunohistochemically as well as marked infiltration of mononuclear cells seen throughout the CNS (Table 1). Figure 2(a)



**Fig. 1.** Clinical course of JHMV infected recipients of  $1 \times 10^7$  NWA spleen cells from immunized donors. Encephalitis and paralysis was assessed using the criteria given in the Materials and methods section. Note that the majority of mice show signs of encephalomyelitis at day 21. Mice which do not receive NWA cells become sick at day 10 and die by day 14 post infection.

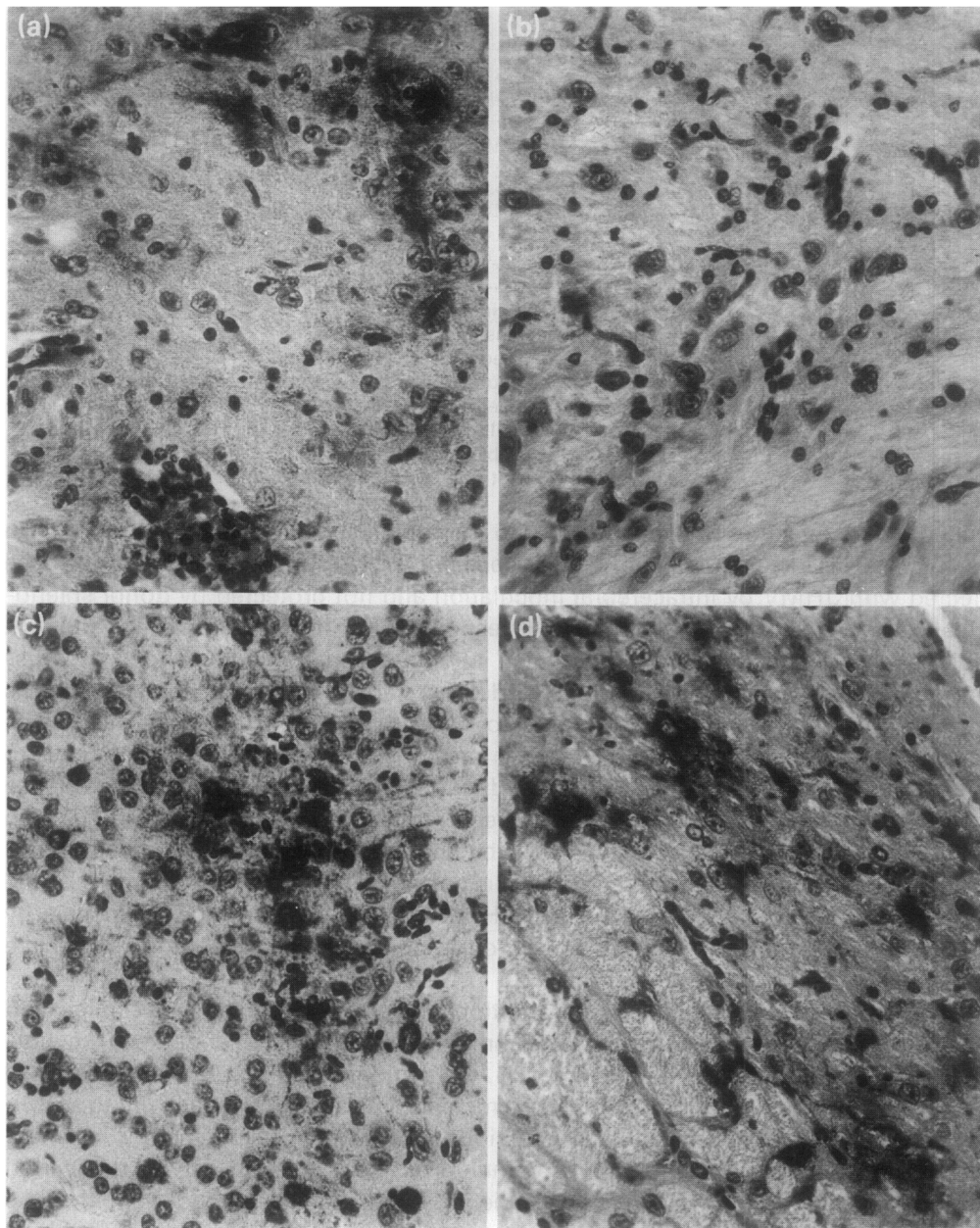
shows a representative section of cerebrum exhibiting abundant viral antigen in both neurons and glia. The degree of cellular infiltration in the CNS reaches a plateau at 7 days post-infection and remains constant until death at day 14 post-infection. In contrast, the transfer of  $5 \times 10^7$  NWA cells at the time of intercerebral (i.c.) inoculation dramatically reduces viral titer in the CNS<sup>11</sup> and results in minimal histological evidence of disease. A photomicrograph of the same region of brain as above [Fig. 2(b)] reveals an absence of viral antigen in both neurons and glia following the transfer of  $5 \times 10^7$  NWA cells. By contrast, Table 1 shows that the protection mediated by adoptive transfer of  $1 \times 10^7$  NWA cells only reduces viral titer 0.8  $\log_{10}$  pfu/g relative to the untreated controls at day 5, although the transfer of this number of NWA cells protects mice from death. Although it results in earlier clearance of virus (Table 2), histological examination shows that there is abundant viral antigen in both neurons and glial cells and a marked infiltration into the brain parenchyma, primarily in the white matter [Fig. 2(c)], similar to the controls [Fig. 2(a)].

The second phase of clinically apparent disease begins at day 21 post-infection and is associated with a marked cellular infiltration into both white and gray matter [Fig. 2(d)]. A corresponding increase in infectious virus or viral antigen was not observed at this time, although occasional mice had low levels of infectious virus or occasional foci of viral antigen in areas not associated with cellular infiltration or demyelination (data not shown). These findings are consistent with previous studies<sup>9,12</sup> showing that viral antigen is generally not present in lesions at the time of demyelination and suggesting that disease may develop in areas in which active clearance of virus occurs.

#### *Immune response in protected mice*

JHMV-specific antibody levels were examined to determine the role of humoral immunity in protection afforded by adoptive transfer of  $1 \times 10^7$  NWA cells. The anti-JHMV IgG response<sup>12</sup> in both recipients and controls not given NWA cells was similar in both in kinetics and magnitude (data not shown). Similar antibody titers were obtained following the adoptive transfer of CD4<sup>+</sup> DTH inducer T cells, which also protected without altering virus replication in the CNS.<sup>8</sup>

The JHMV-specific DTH response was assessed as a measure of cell-mediated immunity in mice protected by adoptive transfer of  $1 \times 10^7$  NWA cells. Six days after intraperitoneal (i.p.) immunization with JHMV, mice exhibit a vigorous DTH response



**Fig. 2.** Viral antigen in the CNS of mice infected with JHMV (immunoperoxidase;  $\times 300$ ). (a) Cerebrum at 5 days post-infection. Note large amounts of viral antigen in neurons and glia. (b) Cerebrum of a recipient of  $5 \times 10^7$  NWA cells at 5 days post-infection. Note absence of antigen in either neurons or glia. (c) Cerebrum of a recipient of  $1 \times 10^7$  NWA cells at 5 days post-infection. Note large amounts of viral antigen in neurons and glia comparable to (a). (d) Spinal cord of a recipient of  $1 \times 10^7$  NWA cells at 24 days post-infection. Note the lack of correlation between the presence of mononuclear infiltrate in the white and gray matter and viral antigen in the white matter.

**Table 2** Replication of JHMV in NWA cell recipients

Day post-infection	Virus titer <sup>a</sup>	
	NWA cell recipients <sup>b</sup>	Untreated controls
5	5.7±0.5	6.0±0.3
7	2.7±1.0	4.3±0.5
10	2.8±1.0	2.5±0.8
12	— <sup>c</sup>	2.3±0.5
15	— <sup>c</sup>	NA <sup>d</sup>
30	— <sup>c</sup>	NA <sup>d</sup>

<sup>a</sup> Log pfu per g brain tissue.

<sup>b</sup> The adoptive transfer of  $1 \times 10^7$  NWA cells from immunized donors immediately prior to infection. Untreated mice were infected but received no NWA cells.

<sup>c</sup> Undetectable ( $\leq 10^2$  pfu/g of brain).

<sup>d</sup> NA = not applicable (untreated mice all die by day 14 post-infection).

elicited by the injection of viral antigen into the footpad.<sup>10</sup> We have previously shown that NWA cells do not mediate a virus-specific DTH response;<sup>11</sup> in addition, mice infected with JHMV by intracerebral inoculation and not protected by donor splenocytes do not develop a measurable DTH response prior to death (data not shown). Table 3 (experiment 1) confirms that mice immunized i.p. exhibit an excellent virus-specific DTH response. Furthermore, the adoptive transfer of unseparated spleen cells or NWA cells from immunized donors to naive recipients also results in a virus-specific DTH response. However, as shown previously,<sup>11</sup> the transfer of NWA cells to naive recipients does not result in the induction of a virus-specific DTH response (Table 3, experiment 2). Table 3 (experiment 3) shows that mice infected i.c. with JHMV and subsequently given NWA cells by adoptive transfer do not exhibit a DTH response until 18 days post-infection. This is coincident with the development of the late phase of the disease (Fig. 1). The DTH response is transient and disappears by day 24. The loss of DTH responsiveness corresponds to the resolution of the late disease by day 24.

Table 4 shows the results of two additional experiments. First, naive mice and JHMV-infected NWA cell recipients were immunized and then challenged with keyhole limpet hemocyanin (KLH). In experiment 1, we demonstrated that the DTH response to KLH was similar in both groups. To determine if the loss of DTH reactivity to JHMV was due to active JHMV-specific suppression, we performed experiment 2 in which NWA DTH effector T cells from JHMV immunized mice were adoptively transferred into naive recipients and NWA cell recipients which had recovered from the late encephalomyelitis. All mice were challenged immediately with JHMV antigen and the DTH response measured 24 h later. The average DTH response in naive mice was  $27.2 \times 10^{-2}$  mm. In contrast, an average DTH response of only  $5.1 \times 10^{-2}$  mm was found in NWA cell protected mice. This suggests that the lack of a DTH response to JHMV in NWA cell protected mice is the result of viral-specific suppression, since both groups responded equally to KLH.

## Discussion

JHMV infection has been extensively studied as a model of acute and chronic viral infection of the CNS.<sup>1</sup> The analysis of antigenic variants isolated by selection

**Table 3** Delayed-type hypersensitivity response to JHMV

	Immune status	Cells transferred	Day tested	DTH response <sup>a</sup> ( $\times 10^{-2}$ mm)
Experiment 1 <sup>b</sup>	Immunized i.p.	None	6	67.5 $\pm$ 21.9
	Infected i.c.		6	9.4 $\pm$ 5.2
Experiment 2 <sup>c</sup>	Naive	None	1	9.7 $\pm$ 6.9
		Whole spleen <sup>e</sup>	1	29.3 $\pm$ 5.2
		NWNA <sup>f</sup>	1	40.2 $\pm$ 5.3
		NWA <sup>g</sup>	1	9.9 $\pm$ 6.8
Experiment 3 <sup>d</sup>	JHMV infected i.c.	NWA	10	9.7 $\pm$ 8.9
			15	0.0 $\pm$ 5.0
			18	62.0 $\pm$ 14.1
			21	24.2 $\pm$ 8.6
			24	7.6 $\pm$ 8.1
			30	15.3 $\pm$ 13.3
			45	6.3 $\pm$ 7.1
			60	14.5 $\pm$ 7.8
			75	9.2 $\pm$ 11.9
100	0.5 $\pm$ 5.0			

<sup>a</sup> Results expressed as the difference of the right footpad minus the left footpad  $\pm$  one SD 24 h after the injection of JHMV Ag 20  $\mu$ l into the right footpad and PBS 20  $\mu$ l into the left footpad.

<sup>b</sup> Immunized (i.p.) and infected (i.c.) C57BL/6 mice were challenged at day 5 and the DTH response was measured on day 6.

<sup>c</sup>  $1 \times 10^7$  spleen cells, NWNA cells or NWA cells from immunized donors were transferred to naive C57BL/6. Recipients were challenged immediately and the DTH response was measured 24 h later.

<sup>d</sup> C57BL/6 mice which received  $1 \times 10^7$  NWA cells from immunized donors were infected (i.c.) with JHMV on day 0. Recipients were challenged with antigen and the DTH response measured 24 h later.

<sup>e</sup>  $1 \times 10^8$  spleen cells from C57BL/6 donors immunized 6 days previously by intraperitoneal injection of  $1 \times 10^6$  pfu of JHMV.

<sup>f</sup>  $5 \times 10^7$  NWNA cells from C57BL/6 donors immunized 6 days previously by intraperitoneal injection with  $1 \times 10^6$  pfu of JHMV.

<sup>g</sup>  $5 \times 10^7$  NWA cells from C57BL/6 donors immunized 6 days previously by intraperitoneal injection of  $1 \times 10^6$  pfu of JHMV.

**Table 4** Delayed-type hypersensitivity response of NWA cell recipients

	Immune status	Antigen	NWNA DTH effector cells	DTH response ( $\times 10^{-2}$ mm)
Experiment 1 <sup>a</sup>	Naive	KLH	-	30.9 $\pm$ 12.2
	NWA cell recipient	KLH	-	36.3 $\pm$ 11.9
Experiment 2 <sup>b</sup>	Naive	JHMV	+	27.2 $\pm$ 5.2
	NWA cell recipient	JHMV	+	5.1 $\pm$ 3.8

<sup>a</sup> Groups of naive and NWA cell recipients were immunized with KLH, challenged in the footpad 5 days later and the DTH response to KLH determined 24 h later.

<sup>b</sup>  $1 \times 10^6$  NWNA cells from JHMV immune donors were transferred to groups of naive and NWA cell recipients. Mice were challenged in the left footpad with JHMV and in the right with PBS. The DTH response was measured 24 h later.



with neutralizing monoclonal antibodies have implicated the virus major envelope glycoprotein, designated E2, as a determinant of tropism for cell types within the CNS.<sup>12,13</sup> However, it is also apparent that the immune competence of the host plays a major role in determining the outcome of infection. For example, the passive transfer of both neutralizing and non-neutralizing monoclonal antibodies specific for both the E2 glycoprotein, which contains the neutralization determinant, and for the E1 matrix protein, can protect mice from a lethal JHMV infection.<sup>3-5</sup> The mechanism of protection is not well understood. However, it has been suggested, based on histological evidence, that these antibodies alter the apparent cell tropism for the virus within the CNS.<sup>3,4</sup> The role of the cellular components of the immune system in providing protection and suppressing virus replication within the CNS has been less well studied. The transfer of the CD4<sup>+</sup> T cell population contained in the NWA population is also able to confer protection from a lethal JHMV infection.<sup>11</sup> However, these cells could be distinguished from the CD4<sup>+</sup> DTH-inducer effector T cells previously described<sup>8,10</sup> in that they were unable to adoptively transfer a viral-specific DTH response. Further, protection was mediated via a suppression of virus replication within the CNS.<sup>11</sup> This CD4<sup>+</sup> NWA population functions as a helper cell facilitating the induction of a CD8<sup>+</sup> effector which requires Class I histocompatibility with the donor population.<sup>11</sup>

In the present study, we have confirmed that the adoptive transfer of non-DTH inducer T cells can protect mice from a lethal JHMV infection.<sup>11</sup> When high numbers ( $5 \times 10^7$ ) are transferred, the recipients show no evidence of either acute or persistent disease. Alternatively, when the number of cells transferred is reduced to  $1 \times 10^7$ , there is only a minimal effect on virus replication within the CNS. The transfer of even fewer CD4<sup>+</sup> NWA cells ( $5 \times 10^6$ ) was unable to alter virus replication within the CNS or to protect mice from a lethal infection. Surprisingly, recipients of  $1 \times 10^7$  NWA cells undergo a biphasic clinical disease wherein the second delayed phase of encephalitis is not caused by an increase in virus replication within the CNS. In addition, the data suggest that the second phase of disease corresponds to the development of a virus-specific DTH response. Similar to our previous findings,<sup>9,10</sup> the development of the DTH response does not result in the reduction of viral antigen within the CNS. The sublethal demyelinating process in these NWA cell recipients occurs in the white matter of both the brain and spinal cord and is accompanied by mononuclear cell infiltrations. The clinical and histological disease in these NWA cell recipients is similar to the description of subacute demyelinating encephalomyelitis that occurs after the infection of neonatal rats.<sup>14</sup> Although the immunological basis for the recurrent demyelination in the rats is not known, it has been suggested that T cells specific for antigens within the CNS may play at least a partial role in the induction of this disease.<sup>15</sup> The role of autoimmune cells in the murine model has yet to be examined; however, it is clear from both these models that the immune system may play a critical role in both the establishment and maintenance of JHMV-induced chronic demyelination of the CNS.

The resolution of the clinical disease in the NWA cell recipients corresponds with the suppression of the virus-specific DTH response. This suppression is virus-specific because we have demonstrated that these mice are still able to respond to heterologous antigen. These data are consistent with our previous data demonstrating that the DTH response plays a major role in the induction of acute demyelination following JHMV infection.<sup>8,10</sup> The DTH response is a complex of cell-cell interactions that are initiated by the recognition of antigen in the context of MHC Class II molecules expressed on antigen-presenting cells by CD4<sup>+</sup> effector cells. The breakdown of vascular permeability and the recruitment of mononuclear cells into the site are both mediated by lymphokines, including gamma interferon;<sup>16</sup> however, a variety of other lymphokine and

soluble products participate in the induction of this response.<sup>17</sup> The suppression of the DTH response may reflect an attempt of the host to reduce the adverse effects of increased vascular permeability and the infiltration of activated monocytes. Consistent with our previous results, protection from death was not due to the suppression of viral replication in the CNS;<sup>8,10</sup> however, in contrast to our previous data, protection was not correlated with a virus-specific DTH response.<sup>8,10</sup> The data presented in this report have demonstrated that the adoptive transfer of partial immunity to mice lethally infected with JHMV results in protection from death and the induction of a relapse of disease that is correlated with the virus-specific DTH response.

## Materials and methods

*Mice.* C57BL/6 male 5-week-old mice were purchased from The Jackson Laboratory (Bar Harbor, Maine). Mice were used within 8 days of arrival. Sera obtained from representative mice were tested for JHMV antibody by enzyme-linked immunosorbent assay (ELISA) as previously described<sup>12</sup> and were found to be seronegative.

*Virus.* JHMV was propagated in DBT cells, a continuous murine astrocytoma, as previously described.<sup>8</sup> Mice were inoculated i.c. with  $5 \times 10^3$  plaque forming units (pfu) of the DS strain in a volume of 0.03 ml. Animals succumb to this infection with acute encephalomyelitis with evidence of demyelination within 14 days of infection.<sup>8,9</sup> Donor mice were immunized i.p. with  $1 \times 10^6$  pfu of JHMV as previously described.<sup>10,11</sup>

*Adoptive transfer.* Six days after immunization, donor spleens were removed aseptically, teased and the cells were washed twice in RPMI 1640 medium (Gibco Laboratories, Grand Island, New York). Cells were resuspended in RPMI medium supplemented with 10% fetal calf serum (FCS; Irvine Scientific, Irvine, California). Approximately  $8 \times 10^8$  cells were adsorbed for 1 h onto a nylon wool column containing 2.4 g of nylon wool (Fenwall, Morton Grove, Illinois) prepared in a 35-ml syringe as previously described.<sup>10,11</sup> The NWNA were collected by washing the column with three volume equivalents of RPMI medium containing 10% FCS. NWA cells were dislodged by allowing the column to run dry and then refilling it with ice cold Earle's balanced salt solution (Gibco Laboratories). The cells were expelled from the column using the syringe plunger. NWA cells were washed twice and resuspended in RPMI medium at the appropriate concentrations. Cells were administered by i.v. injection immediately prior to i.c. injection with JHMV.

*Virus titration.* The virus titer in brain was determined by limiting dilution of clarified homogenates on monolayers of L-2 cells as previously described.<sup>8,11</sup> Titer was calculated from duplicate assays derived from three–four mice per group and the results expressed as the mean titer per g of brain.

*Clinical assessment.* Mice were examined for signs of encephalitis and paralysis at least every other day for the first month and at least three times per week for all subsequent points. Clinical changes were scored using the system described by Fleming *et al.*<sup>12</sup> Encephalitis was scored as present if mice were either: easily excitable, slightly or severely hunched, had ruffled fur or were lethargic. Paralysis was rated present if mice were ataxic, or exhibited hind limb paresis or paralysis.

*Histology.* Brains and spinal cords were fixed by immersion in Clarke's fixative for 3 h prior to paraffin embedding. Sections ( $7 \mu\text{m}$ ) were stained with Erlich's hematoxylin and eosin or luxol fast blue, dehydrated and mounted in permount prior to light microscopy. Adjacent tissue sections for immunohistochemistry were stained by an avidin-biotin immunoperoxidase procedure (Vectastain, Vector Laboratories, Burlingame, California) using monoclonal antibody J.3.3, specific for the JHMV nucleocapsid protein,<sup>18</sup> as the primary antibody. Horizontal sections through the basal ganglia and hippocampus and longitudinal sections of spinal cord were examined. Perivascular cuffing, parenchymal infiltrate in gray and white matter, the quantity of viral antigen in gray and white matter, and the distribution of viral antigen in neurons and glia were scored as follows: n, normal; +, slight; ++, moderate; +++, severe.

**Delayed type hypersensitivity.** Footpad swelling was used as a measure of the DTH response as previously described.<sup>8,10,19</sup> A lysate of infected DBT cells sonicated in phosphate buffered saline (PBS), pH 7.2, prepared as previously described, was used as antigen.<sup>8,10,19</sup> Mice were immunized with 150  $\mu$ g of KLH (Calbiochem, La Jolla, California) by i.p. injection. Five days later mice were injected with 20  $\mu$ l containing 150  $\mu$ g of KLH in PBS in the right footpad. Injection of 20  $\mu$ l of PBS in the left footpad served as control. The dorso-ventral thickness was measured 24 h later using a Mitutoyo micrometer (Tokyo, Japan). Data are presented as the difference of the right foot minus the left foot plus or minus one SEM.

**Anti-virus antibody.** Blood was obtained from anesthetized mice by cardiac puncture. Anti-JHMV antibodies in serum samples from individual mice were measured by ELISA.<sup>12</sup> The concentration of antibody in sera was determined by probit analyses in comparison with IgG standards obtained from mice hyperimmunized with JHMV.

We are grateful to Cindy Fabricius-Segal and Ligaya Pen for excellent technical assistance and to Carol Flores for excellent editorial assistance.

## References

1. Wege H, Siddell S, ter Meulen V. The biology and pathogenesis of coronaviruses. *Curr Top Microbiol Immunol* 1982; 99: 165–200.
2. Waksman BH, Reingold SC. Viral etiology of multiple sclerosis; where does the truth lie. *Trends Neurosci* 1986; 9: 388–91.
3. Fleming JO, Shubin RA, Sussman MA, Casteel N, Stohlman SA. Monoclonal antibodies to the matrix (E1) glycoprotein of mouse hepatitis virus protect mice from encephalitis. *Virology* 1989; 168: 162–7.
4. Buchmeier MJ, Lewicki HA, Talbot PK, Knobler RL. Murine hepatitis virus-4 (strain JHM)-induced neurologic disease is modulated *in vivo* by monoclonal antibody. *Virology* 1984; 132: 261–70.
5. Wege H, Dorries R, Wege H. Hybridoma antibodies to the murine coronavirus JHM: characterization of epitopes on the peplomer protein (E2). *J Gen Virol* 1984; 65: 1931–42.
6. Nakanaga K, Yamanouchi K, Fujiwara K. Protective effect of monoclonal antibodies on lethal mouse hepatitis virus infection in mice. *J Virol* 1986; 59: 168–71.
7. Zecomte J, Cainelli-Gebara V, Mercier G *et al.* Protection from mouse hepatitis virus type 3-induced acute disease by an anti-nucleoprotein monoclonal antibody. *Arch Virology* 1987; 97: 123–30.
8. Stohlman SA, Matsushima G, Casteel N, Weiner LP. *In vivo* effects of coronavirus-specific T cell clones. DTH inducer cells prevent a lethal infection, but do not inhibit virus replication. *J Immunol* 1986; 136: 3052–6.
9. Stohlman SA, Sussman MA, Matsushima GK, Shubin RA, Erlich SS. Delayed-type hypersensitivity response in the central nervous system during JHM virus infection requires viral specificity for protection. *J Neuroimmunol* 1988; 19: 255–68.
10. Erlich SS, Matsushima GK, Stohlman SA. Studies on the mechanism of protection from acute encephalomyelitis by delayed-type hypersensitivity T cell clones. *J Neurol Sci* 1990; 90: 203–16.
11. Sussman MA, Shubin RA, Kyuwa S, Stohlman S. T cell mediated clearance of mouse hepatitis virus, strain JHM, from the central nervous system. *J Virol* 1989; 63: 3051–6.
12. Fleming JO, Trousdale MD, El-Zaatari FAK, Stohlman SA, Weiner LP. Pathogenicity of antigenic variants of murine coronavirus JHM selected with monoclonal antibodies. *J Virol* 1986; 58: 869–75.
13. Dalziel RG, Lampert PW, Talbot PJ, Buchmeier MJ. Site-specific alteration of murine hepatitis virus type 4 peplomer glycoprotein E2 results in reduced neurovirulence. *J Virol* 1986; 59: 463–71.
14. Nagashima K, Wege W, Meyerman R, ter Meulen V. Coronavirus induced subacute demyelinating encephalomyelitis in rats: a morphological analysis. *Acta neuropathol (Berl)* 1978; 44: 63–70.
15. Watanabe R, Wege H, ter Meulen V. Adoptive transfer of EAE-like lesions from rats with coronavirus-induced demyelinating encephalomyelitis. *Nature* 1983; 305: 150–3.
16. Issekutz TB, Stoltz JM, Meide P. Lymphocyte recruitment in delayed-type hypersensitivity. The role of IFN-g. *J Immunol* 1988; 140: 2989–93.
17. Geczy CL. The role of lymphokines in delayed-type hypersensitivity reactions. *Springer Semin Immunopathol* 1984; 7: 321–46.
18. Fleming JO, Stohlman SA, Harmon RC, Lai MMC, Frelinger JA, Weiner LP. Antigenic relationships of murine coronaviruses: analysis using monoclonal antibodies to JHM (MHV-4) virus. *Virology* 1983; 131: 296–307.
19. Woodward JG, Matsushima GK, Frelinger JA, Stohlman SA. Production and characterization of T cell clones specific for mouse hepatitis virus, strain JHM: *in vivo* and *in vitro* analyses. *J Immunol* 1984; 133: 1016–21.