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## Delayed-type hypersensitivity response in the central nervous system during JHM virus infection requires viral specificity for protection

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### Summary

The JHM strain of mouse hepatitis virus (JHMV) elicits an *I-A*-restricted delayed-type hypersensitivity (DTH) response mediated by a Thy-1<sup>+</sup>, Lyt-1<sup>+</sup>, and CD4<sup>+</sup> T cell. Adoptive transfer of these polyclonal CD4<sup>+</sup> T cells from immunized mice prevents death in lethally infected recipients without significantly reducing virus titer in the central nervous system (CNS). These observations raise the possibility that the recruitment of mononuclear cells into the CNS may play a critical role in survival from a lethal CNS infection. Transient DTH response to nonviral antigens induced an accumulation of monocytes in the CNS that was maximal at 48 h post-challenge and virtually resolved by 5 days post-challenge. By contrast the induction of prolonged DTH responses resulted in the accumulation of a large number of monocytes that persisted in the CNS for at least 5 days post-challenge. Neither type of DTH reaction suppressed virus replication or prevented death from concomitant lethal JHMV infection.

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## Introduction

Many viral infections are accompanied by delayed-type hypersensitivity (DTH) responses; however, the role of the DTH response in viral pathogenesis or resistance is unclear (Liew, 1982). DTH is an antigen-specific response mediated by T cells which express the CD4 cell surface marker and respond to antigen presented in the context of major histocompatibility complex (MHC) class II molecules. Antigen-induced activation of T<sub>DTH</sub> cells results in the release of lymphokines, increased vascular permeability and the local accumulation of antigen-nonspecific monocytes (Liew, 1982; Askenase and Van Loveren, 1983; Geczy, 1984). Our laboratory has previously shown that the adoptive transfer of CD4<sup>+</sup> cell T<sub>DTH</sub> clones specific for the neurotropic JHMV strain of mouse hepatitis virus protects mice from death due to a lethal infection (Stohlman et al., 1986). Protection requires only a small number ( $5 \times 10^4$ ) of these clonal T cells, is MHC class II (*I-A*) restricted, is prevented by immunosuppression of the host and results in the accumulation of large numbers of perivascular mononuclear cells (Stohlman et al., 1986). These characteristics are consistent with the induction of a DTH response (Liew, 1982). The data suggest that the DTH-stimulated local accumulation of monocytes within the central nervous system (CNS) may play a pivotal role in providing protection to the host from a lethal infection. In addition, protection was not accompanied by a reduction of virus in the CNS, which suggests that the DTH response may play a role in the establishment of persistent JHMV infection in the CNS, leading to chronic JHMV-induced demyelination (Stohlman and Weiner, 1981).

In comparison with the well-defined roles of both antibody in the neutralization of infectious virus and cytotoxic T lymphocytes (CTL) in the lysis of infected cells, the role of DTH-inducer T cells in providing antiviral effector function is unclear; however, the recruitment of antigen-nonspecific bone marrow-derived monocytes to the site of viral infection is a primary effect of their activation (Allison, 1974; Liew, 1982). The CD4<sup>+</sup> phenotype and MHC restriction of T<sub>DTH</sub> cells are identical to T cells that provide help in the augmentation of both antiviral antibody and the CTL responses. Although adoptive transfer of JHMV-specific CD4<sup>+</sup> T cell clones prevents death from lethal JHMV infection, it does not result in a significant increase in serum antiviral antibody titer, suggesting that protection is not due to T cell-mediated help provided to antibody producing B cells (Stohlman et al., 1986). CTL responses are pivotal in protection and reduction of viral titer in several systemic infections (Sethi et al., 1983; Byrne and Oldstone, 1984; Morrison et al., 1986). Although JHMV-specific CTL have been recently described (Kyuwa et al., 1988), it is not clear if they play a significant role in protection from lethal JHMV infection.

Since the JHMV-specific DTH response in the CNS confers survival to mice undergoing an otherwise fatal infection, it is important to determine if protection is mediated via the action of the bone marrow-derived monocytes recruited into the CNS and/or other antigen-nonspecific components of the response. In this paper we demonstrate that JHMV elicits an *I-A*-restricted, CD4<sup>+</sup> T cell-mediated DTH response that provides protection from lethal infection upon adoptive transfer to

naive recipients. However, the induction of DTH responses by nonviral antigens resulting in the recruitment of monocytes into the CNS during a lethal JHMV infection is not protective. This suggests that the antigen-nonspecific components of a DTH response in the CNS are not critical to survival from lethal JHMV infection.

## Materials and methods

### *Mice*

A/J, A.SW, BALB/c, C57BL/6, C57BL/10, B10.A(2R), B10.A(5R), B10.BR, B10.MBR, CBA, C3H/He, SJL and SWR mice were purchased from The Jackson Laboratory (Bar Harbor, ME) at 6 weeks of age. Mice were used as recipients in the adoptive transfer experiments within 6 days of arrival. Sera obtained from representative mice were tested for JHMV antibody by enzyme-linked immunosorbent assay (ELISA) as previously described (Fleming et al., 1986) and found to be seronegative.

### *Virus*

The DS small plaque variant of the neurotropic JHMV strain of mouse hepatitis virus (MHV) was used for intracerebral (i.c.) infection (Stohlman et al., 1982a). The DL large plaque variant of JHMV was used for intraperitoneal (i.p.) immunizations (Stohlman et al., 1982a). Both viruses elicit comparable immune responses following i.p. injection (unpublished data). Viruses were propagated in DBT cells, a continuous murine astrocytoma, as previously described (Stohlman and Weiner, 1981). Mice were inoculated i.c. with approximately  $5 \times 10^3$  plaque-forming units (pfu) of JHMV in a volume of 0.03 ml. Animals succumbing to this infection have acute encephalomyelitis with evidence of acute and chronic demyelination (Stohlman and Weiner, 1981; Stohlman et al., 1982a; Erlich et al., 1987).

### *Delayed-type hypersensitivity*

Mice were immunized by injecting approximately  $10^6$  pfu of JHMV i.p. in a volume of 1.0 ml. Five days after primary immunization with JHMV, mice were injected in the right footpad with 25  $\mu$ l of a lysate of JHMV-infected cells prepared as previously described (Woodward et al., 1984). The same protocol was used for immunizations with 150  $\mu$ g of keyhole limpet hemocyanin (KLH; Calbiochem, La Jolla, CA) or 150  $\mu$ g of ovalbumin (OVA; Sigma, St. Louis, MO) in 1.0 ml of phosphate-buffered saline (PBS), pH 7.4. Mice immunized with sheep red blood cells (SRBC; Colorado Serum, Denver, CO) received  $1 \times 10^9$  SRBC i.p. Immunized mice were challenged 5 days later with 150  $\mu$ g of KLH, 250  $\mu$ g of OVA or  $10^7$  SRBC injected into the right footpad in a volume of 25  $\mu$ l. Control antigens (25  $\mu$ l) were injected in the left footpad. The dorso-ventral thickness of the footpad was measured 24 h after challenge. Data are presented as the thickness of the right foot minus the thickness of the left foot plus or minus one standard error of the mean. Statistical significance was determined by two-tailed Student's *t*-test.

### *Adoptive transfer*

For adoptive transfer experiments, the spleens were removed aseptically from mice 5 days post-immunization, teased, and the cells washed twice with Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS; Gibco Laboratories, Grand Island, NY). The cell concentration was adjusted to  $5 \times 10^7$  per ml in DMEM containing 10% FCS. A total of  $2 \times 10^8$  cells were incubated on columns of nylon wool (Fenwall, Morton Grove, IL) for 60 min at  $37^\circ\text{C}$ . Nonadherent cells were eluted and washed twice prior to intravenous (i.v.) transfer of  $5 \times 10^7$  cells to recipient mice. Flow cytometric analysis revealed that approx. 85% of these cells were Thy-1.2<sup>+</sup>. For the DTH response, mice were challenged with antigen in the footpad on the same day and the DTH response determined as described above. To determine survival, mice were challenged with  $5 \times 10^3$  pfu of JHMV injected i.c. on the same day.

### *Antiserum depletions*

Nylon wool-purified T cells were depleted of Thy-1.2 and CD4 bearing cells using monoclonal antibodies HO-13-14 and G.K. 1.5, respectively. Both antibodies were obtained from the American Type Culture Collection (Rockville, MD). Depletions of Lyt-1.2 and Lyt-2.2 (CD8) bearing cells were performed with monoclonal antibodies purchased from Cedarlane Laboratory (Westbury, NY). Briefly,  $10^7$  cells per ml were incubated for 60 min on ice with each antibody, washed in DMEM containing 1% bovine serum albumin and incubated at  $37^\circ\text{C}$  for 60 min with prescreened rabbit serum (Cedarlane Laboratory) as a source of complement. Cells were resuspended to the original viable cell concentration and transferred to recipient mice i.v.

### *T cell clones*

The isolation and characterization of the JHMV-specific T cell clones have been previously reported (Woodward et al., 1984). Briefly, popliteal lymph node cells were obtained from C57BL/6 mice immunized with a lysate of JHMV-infected DBT cells emulsified in complete Freund's adjuvant. The T cells were cloned by limiting dilution and maintained in RPMI 1640 medium supplemented with 10% FCS, 100 U/ml of penicillin, 50  $\mu\text{g}/\text{ml}$  of streptomycin, 2 mM glutamine,  $5 \times 10^{-5}$  M 2-mercaptoethanol, JHMV antigen, irradiated (2000 Rad) syngeneic spleen cells as a source of antigen-presenting cells, and partially purified mouse interleukin-2 (Woodward et al., 1984). The clone used in this report is designated 4B10 and has the cell surface phenotype Thy-1.2<sup>+</sup>, Lyt-1.2<sup>+</sup>, CD8<sup>-</sup> and CD4<sup>+</sup> (Woodward et al., 1984; Stohlman et al., 1986). One passage prior to use, the 4B10 clone was cultured in the presence of ultraviolet-inactivated JHMV antigen to prevent carry-over of infectious virus. Viable cells were isolated by centrifugation on Lympholyte M (Cedarlane Laboratories), and  $1 \times 10^6$  cells were transferred i.c. to recipients on the same day as i.c. challenge with  $5 \times 10^3$  pfu of JHM.

### *Viral titer*

Viral titers were determined 5 days post-infection as previously described (Stohlman and Weiner, 1981). Briefly brains were removed aseptically from sacrificed

mice, placed in ice-cold Dulbecco's PBS, pH 7.4, and homogenized manually using 7 ml Tenbrock tissue homogenizers. Homogenates were clarified by centrifugation at  $800 \times g$  for 7 min at 4°C. Virus titer in the supernatants was determined by adsorbing 0.2 ml of 10-fold serial dilutions onto monolayers of L2 cells in 24-well plates (Falcon Plastics, Oxnard, CA). After incubation for 60 min at 37°C, the inoculum was removed, followed by the addition of 1.0 ml DMEM supplemented with 2% FCS. Viral titers were determined 48 h later from duplicate or triplicate assays of groups of three or four mice and the results expressed as the mean titer per gram of tissue.

### *Histology*

The pathogenesis of JHMV infection and the DTH response in the CNS were monitored by histological evaluation of representative coronal sections. Brains were fixed by immersion in Clark's fixative for 3 h, embedded in paraffin and sectioned at 7  $\mu$ m. Tissue sections were stained with Ehrlich's hematoxylin and eosin (H&E), dehydrated and mounted in Permount prior to light microscopy.

## **Results**

### *JHMV-induced DTH responses in various mouse strains*

It has been suggested that JHMV induces a relatively poor DTH response in some inbred strains of mice, including the C57BL/6 strain (Kyuwa et al., 1984). To compare the JHMV-induced DTH response in the C57BL/6 mice used in our previous studies (Stohlman et al., 1986) to that in other mouse strains, groups of 6-week-old mice of various strains were immunized with JHMV and challenged 5 days later. Table 1 shows that there are indeed strain-specific differences in responsiveness to JHMV using an identical immunization and challenge protocol. However, C57BL/6, which were previously reported to be low responders (Kyuwa et al., 1984), are clearly high responders. The JHMV-induced DTH response is not linked to H-2, since CBA (H-2<sup>k</sup>) and A.SW (H-2<sup>s</sup>) responded, while C3H (H-2<sup>k</sup>) and SJL (H-2<sup>s</sup>) mice showed a minimal or no response, as previously reported (Kyuwa et al., 1984; Stohlman et al., 1985). Although the basis for the discrepancy in these results is unclear, C57BL/6 mice are clearly responders in the immunization and challenge protocol described in this report.

### *Characterization of JHMV-induced DTH effectors*

To define the characteristics of the cells mediating the DTH response to JHMV and the nonviral antigen KLH, spleen cells from immunized mice were adoptively transferred to naive recipients. Mice were immunized by i.p. injection with either KLH or JHMV. Spleens were removed 5 days later and the nylon wool-purified cell population transferred i.v. to naive recipients. Antigen was injected on the same day and the DTH response measured 24 h later. Table 2 shows that the transfer of nylon wool-purified spleen cells from mice primed with either KLH or JHMV results in an antigen-specific DTH response in naive recipients. Complement-mediated antibody

TABLE 1  
DTH RESPONSE OF VARIOUS MOUSE HAPLOTYPES TO JHMV

Strain	H-2 haplotype	DTH response <sup>a</sup>
C57BL/6	b	37.8 ± 8.3
CBA	k	35.3 ± 11.9
BALB/c	d	27.7 ± 13.6
A.SW	s	26.8 ± 4.2
C57BL/10	b	26.8 ± 7.5
A/J	k/d	24.2 ± 4.3
SWR	q	17.3 ± 5.2 <sup>b</sup>
C3H/He	k	9.5 ± 3.7
SJL	s	4.6 ± 2.8

<sup>a</sup> Results expressed as the difference of the right footpad minus left footpad ± one standard error of the mean × 10<sup>-2</sup> mm as described in Materials and Methods.

<sup>b</sup> Differences observed between SWR and C3H/He or SJL were significant by Student's *t*-test (*P* < 0.025).

TABLE 2  
CHARACTERIZATION OF C57BL/6 DTH EFFECTOR CELLS

Antigen	Recipient				Treatment	DTH response <sup>a</sup>
	Strain	Haplotype				
		K	I	D		
KLH <sup>b</sup>	C57BL/6	b	b	b	None	28.2 ± 4.3
					C only	25.9 ± 5.4
					Thy-1.2 + C	5.3 ± 1.2
					CD4 + C	7.2 ± 3.8
JHMV <sup>b</sup>	C57BL/6	b	b	b	None	22.6 ± 4.7
					C only <sup>c</sup>	22.2 ± 5.5
					CD8 + C	22.0 ± 6.2
					Thy-1 + C	6.8 ± 2.8
	Lyt-1 + C	9.8 ± 1.7 <sup>d</sup>				
	CD4 + C	7.5 ± 4.4				
	B10.A(5R)	b	b	b		20.5 ± 6.2
	B10.A(2R)	k	k	b		3.3 ± 2.1
B10.MBR	b	k	q		4.5 ± 0.6	
B10.BR	k	k	k		9.5 ± 2.1	

<sup>a</sup> Results expressed as the difference of the right footpad minus the left footpad ± one standard error of the mean × 10<sup>-2</sup> mm as described in Materials and Methods.

<sup>b</sup> Six days after immunization, spleens were removed and 1 × 10<sup>6</sup> nylon wool-purified spleen cells were transferred i.v. to naive recipients.

<sup>c</sup> C = complement.

<sup>d</sup> The difference observed between positive DTH responders (C57BL/6 untreated) and low DTH responders (anti-Lyt-1 treated C57BL/6) was significant (*P* = 0.005).

depletions identify the phenotype of the DTH-inducer T cells elicited in response to both antigens as Thy-1<sup>+</sup>, CD4<sup>+</sup>. In addition, the JHMV-specific DTH-inducer T cells were Lyt-1<sup>+</sup> and CD8<sup>-</sup>. This is identical to the phenotype of the previously characterized JHMV-specific T<sub>DTH</sub> cell clones which mediate both the DTH response and the protection from lethal JHMV infection (Woodward et al., 1984; Stohlman et al., 1986). To ensure that the active cells derived from the JHMV-immunized donors had the appropriate pattern of genetic restriction, nylon wool-purified T cells from JHMV-immunized C57BL/6 (H-2<sup>b</sup>) donors were adoptively transferred to congenic strains and DTH responses were measured 24 h later. Table 2 also shows that C57BL/6 and B10.A(5R) mice, both of which are *I-A*<sup>b</sup>, responded, while B10.A(2R), B10.BR and B10.MBR mice, which are not histocompatible at *I-A* with the donor cells, did not respond. These data show that histocompatibility at *I-A* is necessary for responsiveness and thus confirm that these cells have both the surface phenotype and genetic restriction typical not only of a T<sub>DTH</sub> cell (Liew, 1982) but also of the JHMV-specific T<sub>DTH</sub> cell clones previously described (Woodward et al., 1984; Stohlman et al., 1986).

#### *Virus-induced responses*

The adoptive transfer of the JHMV-specific T cell clone 4B10 elicits a DTH response in naive mice and provides them with protection from lethal infection (Stohlman et al., 1986). JHMV titer in the CNS was not reduced in the protected mice, and there was no difference between mice receiving 4B10 cells and untreated controls in JHMV titer or kinetics during the entire course of the infection (Stohlman et al., 1986). Mice were either injected in the footpad with T cell clone 4B10 ( $1 \times 10^5$  per recipient) mixed with antigen for the DTH response or injected i.c. ( $1 \times 10^6$  cells per recipient) and challenged i.c. with JHMV to determine survival and virus replication. The data in Table 3 (experiment No. 1) confirm our initial observations. We have previously shown that injection of hen egg lysozyme-specific T cell clones plus antigen i.c. has no effect on a lethal JHMV infection (Stohlman et al., 1986). Table 3 also shows that the i.v. adoptive transfer of nylon wool-purified cells from JHMV-immunized donors ( $5 \times 10^7$  cells per recipient) was similarly capable of inducing a DTH response, as well as providing protection from death due to lethal infection without reducing JHMV titer in the CNS. Protection was accompanied by an increase in mononuclear cell infiltration and perivascular cuffing in the brain parenchyma (Fig. 1A), compared to JHMV-infected mice not receiving cells from immunized donors (Fig. 1B). These data suggest that the adoptive transfer of polyclonal DTH effector T cells from JHMV-immunized mice provides protection similar to the adoptive transfer of DTH-effector T cell clones.

#### *Transient nonviral-induced responses*

To determine if the DTH reaction itself provides protection against lethal JHMV infection, DTH responses to the nonviral antigens OVA, KLH or SRBC were tested for their ability to prevent death due to a concurrent JHMV infection. Table 3 (experiment No. 2) shows that all three antigens induced 24 h DTH responses in C57BL/6 mice, with KLH showing the most potent response. On the fifth day



TABLE 3  
EFFECT OF DTH RESPONSE UPON JHMV INFECTION

Treatment	DTH (mm $\times 10^{-2}$ )	No. alive/ total <sup>a</sup>	Viral titer (log pfu/g) <sup>b</sup>
<i>Experiment No. 1</i>			
None	4.3 $\pm$ 2.5 <sup>c</sup>	0/15	8.1 $\pm$ 0.6
T cell clone 4B10	29.5 $\pm$ 3.9 <sup>d</sup>	7/7	8.1 $\pm$ 0.2 <sup>c</sup>
JHMV splenic T cells	37.8 $\pm$ 5.0 <sup>f</sup>	5/5	7.8 $\pm$ 0.7 <sup>g</sup>
<i>Experiment No. 2</i> <sup>h</sup>			
KLH	43.8 $\pm$ 10.2	0/7	8.1 $\pm$ 0.7
OVA	13.2 $\pm$ 8.8	0/9	NT <sup>i</sup>
SRBC	23.2 $\pm$ 4.8	0/9	NT <sup>i</sup>
<i>Experiment No. 3</i> <sup>j</sup>			
KFCA	70.0 $\pm$ 11.0	0/10	8.1 $\pm$ 0.7

<sup>a</sup> Survival was monitored for 21 days post-infection. All mice died between days 10 and 12 post-infection.

<sup>b</sup> Viral titers were determined 5 days after challenge.

<sup>c</sup> Average response of 22 naive mice challenged with JHMV, KLH, OVA or SRBC.

<sup>d</sup> T cell clone 4B10 ( $1 \times 10^5$ ) was mixed with JHMV or control antigen and injected into the left or right footpads respectively. DTH was measured 24 h later.

<sup>e</sup> T cell clone 4B10 ( $1 \times 10^6$ ) was injected i.c. followed by i.c. injection with  $5 \times 10^3$  pfu of JHMV as previously described (Stohlman et al., 1986).

<sup>f</sup> Nylon wool-purified splenic T cells ( $5 \times 10^7$ ) from donors immunized 5 days earlier were transferred i.v. to naive recipients. Recipients were challenged immediately in the footpads with JHMV or control antigens. DTH was measured 24 h later.

<sup>g</sup> Recipients were prepared as described in footnote <sup>f</sup> and challenged i.c. on the same day with  $5 \times 10^3$  pfu of JHMV.

<sup>h</sup> Mice were immunized i.p. with antigen (KLH, OVA or SRBC) 5 days prior to challenge either in the footpads to determine DTH response or i.c. with antigen and JHMV to determine survival.

<sup>i</sup> Not tested.

<sup>j</sup> Mice were immunized i.p. with KLH 5 days prior to challenge with KFCA as described in footnote <sup>h</sup>.

post-immunization, mice were infected with JHMV and challenged i.c. with the appropriate nonviral antigen. Following 5 additional days, half of the mice in each group were sacrificed for histological examination and, in the case of KLH, to determine the virus titer in the CNS. The other half of each group was monitored for survival. None of the immunized mice challenged with these nonviral antigens survived the concurrent JHMV infection, nor was there a reduction in CNS viral titer following challenge with KLH (Table 3). All mice died between 10 and 12 days post-infection, suggesting that the concurrent DTH responses had essentially no effect upon survival. Histological examination of both infected and uninfected mice immunized with these antigens and challenged i.c. revealed a peak of mononuclear infiltration within the CNS at 48 h following secondary challenge. However, the responses in uninfected mice had diminished to almost undetectable levels by the fifth day post-infection, indicating that the response to these nonviral antigens was transient. In comparison, JHMV infection results in a progressive increase in

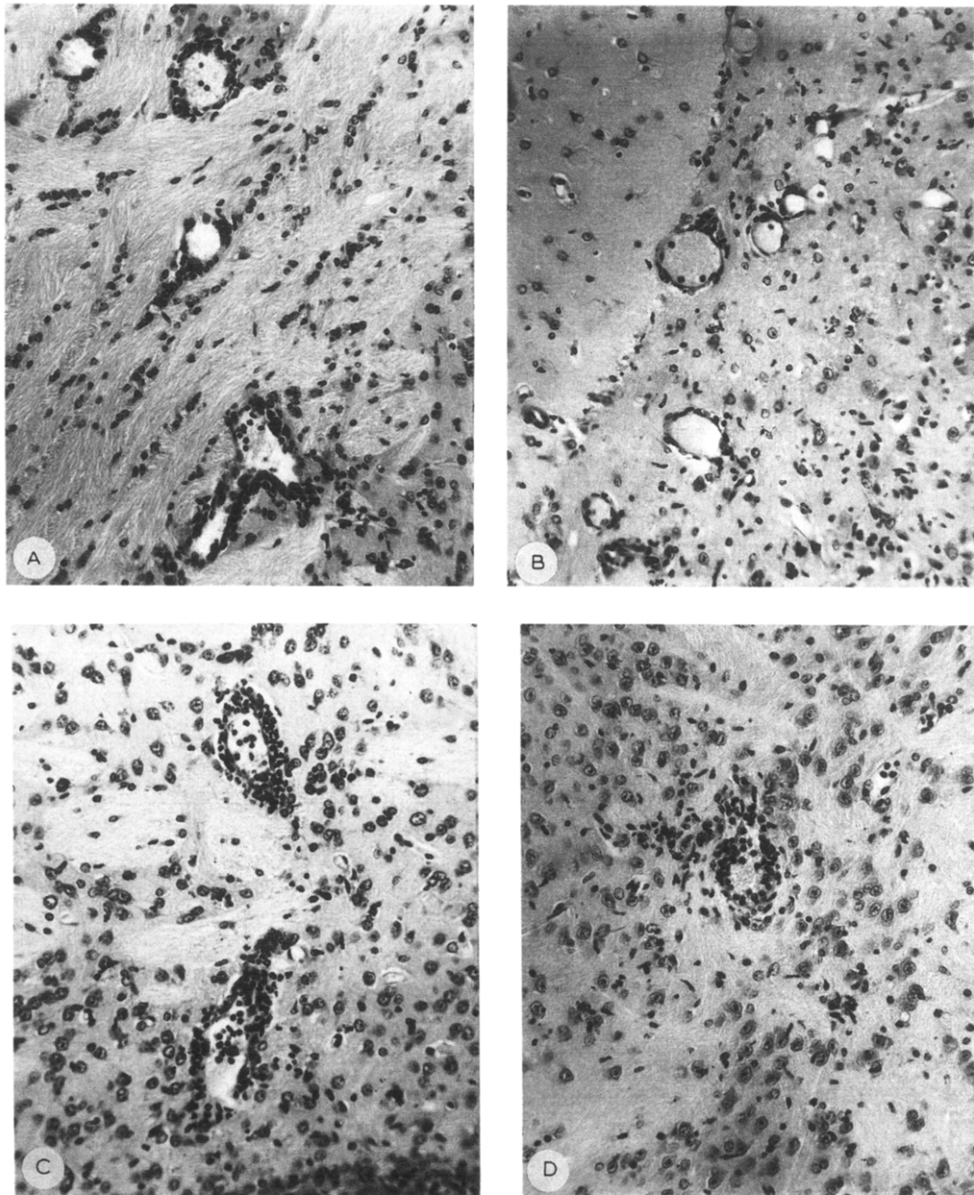


Fig. 1. Photomicrographs of cerebrum of mice showing the perivascular accumulation of mononuclear cells (H&E,  $\times 250$ ). All samples were taken at 5 days. *A*: Mice infected with JHMV that received nylon wool-purified T cells from JHMV-immunized donors. *B*: Mice infected with JHMV only. *C*: KLH-immunized mice injected with KFCA. *D*: KLH-immunized mice infected with JHMV and injected with KFCA.

cellular infiltration in the CNS that is initially evident by 4 days post-infection (Fig. 1A; Weiner, 1973). Thus, it is possible that the DTH-induced presence of monocytes within the CNS during the initial stages of JHMV infection was not of sufficient magnitude or duration to suppress virus replication or provide any substantial measure of protection.

#### *Prolonged nonviral-induced response*

Histological examination of mice protected from a lethal JHMV infection via the adoptive transfer of CD4<sup>+</sup> T cell clones showed a dramatic increase in the mononuclear infiltration into the brain parenchyma compared to mice receiving JHMV only. The increase was noted by 5 days post-infection (unpublished data). KLH, which resulted in the most vigorous DTH response as measured by footpad swelling, also induced the most vigorous cellular infiltration into the CNS (data not shown). To approximate the prolonged mononuclear infiltration seen during infection, KLH was emulsified in Freund's complete adjuvant (KFCA), and injected into KLH-immunized mice. This resulted in a prolonged bilateral accumulation of mononuclear cells in the CNS that was not localized to the area of injection (Fig. 1C). This vigorous response to KFCA in KLH-immunized mice had no apparent adverse clinical effects, and no deaths occurred. By contrast, naive mice receiving KFCA exhibited little perivascular cuffing or cellular infiltration in the CNS. Table 3 (experiment No. 3) shows that KFCA also elicited a vigorous DTH response in the footpad ( $70.0 \pm 11.0 \times 10^{-2}$  mm) in KLH-immunized mice, compared to naive mice ( $11.3 \pm 8.0 \times 10^{-2}$  mm). The phenotype of the T<sub>DTH</sub> cell reactive to KFCA is CD4<sup>+</sup> and Thy-1<sup>+</sup> (data not shown), identical to the cell responsible for the DTH response to KLH (Table 2). KFCA injected i.c. into KLH-immunized mice co-infected with JHMV produced a prolonged cellular infiltration and perivascular accumulation of mononuclear cells consistent with a typical DTH response (Fig. 1D); however, it was unable to either confer survival or suppress virus replication (Table 3, experiment No. 3). This further indicates that a DTH-induced accumulation of monocytes, even throughout the peak period of virus replication in the CNS, was not protective. In addition, no change in survival time was noted in KLH-immunized mice receiving KFCA i.c. with a concurrent JHMV infection compared to naive mice.

## **Discussion**

Infection of mice with JHMV results in an acute encephalomyelitis with both acute and chronic ongoing demyelination (Lampert et al., 1973; Stohlman and Weiner, 1981; Erlich et al., 1987). Although demyelination has been reported in immunosuppressed mice (Weiner, 1971), relatively little is known about the contribution of the immune system in prevention of lethal infection, clearance of infectious virus or establishment or maintenance of latent JHMV infection in the CNS. We have sought to determine if the protection afforded JHMV-infected mice by the adoptive transfer of JHMV-specific CD4<sup>+</sup> T cells (Stohlman et al., 1986) is

due to the DTH-induced accumulation of antigen-nonspecific monocytes within the CNS. DTH responses are initiated by the interaction of CD4<sup>+</sup> T cells with antigen in an *I-A*-restricted manner, resulting in both T proliferation and the release of a variety of lymphokines. This is followed by an increase in vascular permeability and the local accumulation of bone marrow-derived antigen-nonspecific monocytes which produce a variety of monokines and hydrolytic enzymes (Nash and Gell, 1981; Liew, 1982; Askanase and Van Loveren, 1983; Geczy, 1983).

We have determined that JHMV elicits a vigorous DTH response in C57BL/6 mice that is mediated by an Lyt-1<sup>+</sup>, CD8<sup>-</sup>, CD4<sup>+</sup>, Thy-1<sup>+</sup> cell and shows the appropriate genetic restriction to *I-A*. We have further shown that the adoptive transfer of these nylon wool-purified polyclonal DTH-inducer T cells protects mice from a lethal infection without reducing the virus titer in the CNS, suggesting that they are functionally similar to the CD4<sup>+</sup> T<sub>DTH</sub> cell clones (Stohlman et al., 1986). To determine if the accumulation of antigen-nonspecific monocytes in the CNS provides protection to the host from a lethal JHMV infection, DTH reactions were induced in the CNS with non-JHMV antigens concomitant with a lethal JHMV infection. Transient responses early in infection were unable to suppress virus replication or confer survival to lethally infected mice, suggesting that the presence of monocytes early in infection did not alter the outcome of a lethal infection. KFCA was retained in the brain parenchyma and induced a persistent and panencephalitic DTH response in KLH-immunized mice. Mice exhibited perivascular cuffs of mononuclear cells resembling the response in JHMV-specific CD4<sup>+</sup> T cell clone recipients (Stohlman et al., 1986). However, in spite of clear evidence of a DTH response to KFCA by both footpad swelling and histological examination of the CNS, these mice also succumbed to a lethal JHMV infection. This indicates that the presence of increased numbers of monocytes throughout the infection was also unable to alter the course of the lethal disease. These data also suggest that the lymphokines released by the DTH-inducer T cells, the monokines and hydrolytic enzymes released by the monocytes and the loss of vascular permeability do not contribute directly to survival from a lethal JHMV infection.

Cells of the monocyte/macrophage lineage have been implicated as the pivotal cells in natural resistance to a number of viral infections, including two other strains of mouse hepatitis virus (Mogensen, 1979). These studies have relied on either the *in vitro* growth of virus in macrophages, the ability of macrophages to suppress virus growth in a second susceptible cell type or the *in vivo* effects of anti-macrophage treatments such as carrageenan or silica particles (Allison, 1974; Mogensen, 1979). Although there are numerous virus infections in which macrophages are believed to play a role in survival from lethal infections, it has recently been shown that mononuclear phagocytes do not play an important role in the clearance of lymphocytic choriomeningitis virus (Lehman-Grube et al., 1987). JHMV replicates equally well *in vitro* in macrophages from both resistant and susceptible strains of mice (Stohlman et al., 1982b). This is in contrast to both the MHV-2 and MHV-3 strains, whose *in vitro* replication in macrophages correlates with mouse strain-specific resistance (Bang and Warwick, 1970; Virelizier and Allison, 1976). In addition, macrophages from naive mice can suppress the replication of JHMV in a second

susceptible cell line *in vitro*; however, we suggested that this suppression was not of critical importance in survival, since macrophages from both susceptible and resistant strains of mice suppressed virus replication to the same extent (Stohlman et al., 1982b).

It has been suggested that DTH responses may be superfluous or even detrimental to the host due to the local increase in extracellular fluids and tissue injury induced by the accumulation of monocytes (Liew, 1982; Askenase and Van Loveren, 1983; Townsend, 1985). Our study suggests not only that the antigen-nonspecific components of the DTH response are not detrimental, but also that they do not play a major role in either viral clearance or protection from a lethal infection. Mice undergoing either transient or prolonged DTH responses in the CNS to nonviral antigens had the same mean survival time as animals receiving JHMV only, suggesting that these responses may in fact be superfluous to the immunological events that interact to provide protection from a lethal infection.

The data in this report demonstrate that the nonspecific components of the DTH response do not provide a substantial measure of protection; however, the mechanism of protection afforded by the JHMV-specific T cell clones is not clear. In addition, mice surviving infection in the absence of virus reduction via the adoptive transfer of DTH-inducer T cell clones, nylon wool-purified DTH-inducer T cells or neutralizing monoclonal antibodies have persistent JHMV infections with ongoing chronic demyelination (Buchmeier et al., 1984; Stohlman et al., 1986; unpublished observation). Neither the DTH-inducer T cell clones or the nylon wool-purified T cells from mice immunized with JHMV exhibit demonstrable cytotoxic activity against virus-infected targets expressing MHC class I or class II molecules. Histological analysis of the CNS of mice protected by the adoptive transfer of T cell clone 4B10 suggests that mice are protected from death due to the inhibition of viral replication in neurons (unpublished data). A similar phenomenon has been previously described following protection mediated by the adoptive transfer of anti-JHMV monoclonal antibodies (Buchmeier et al., 1984). These mechanisms may be different since protection afforded by DTH-inducer T cell clones does not alter the antiviral antibody response (Stohlman et al., 1986); however, the effect, *i.e.*, the reduction of neuronal infection appears to be similar. With the demonstration that the antigen-nonspecific components of the DTH response do not constitute a major protective mechanism in JHMV infection, efforts are now being directed toward understanding the role of viral-specific immunity in survival from lethal JHMV infection.

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