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Indirect Role of T Cells in Development of Polioencephalitis and Encephalomyelitis Induced by Encephalomyocarditis Virus

DAVID J. TOPHAM,[1]* ADEKUNLE ADESINA,[1] MOHAN SHENOY,[2] JOHN E. CRAIGHEAD,[1] AND SUBRAMANIAM SRIRAM[2]

Department of Pathology[1] and Department of Neurology,[2] College of Medicine, University of Vermont, Burlington, Vermont 05405

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Infection of female BALB/c mice with encephalomyocarditis virus results in the development of a paralytic syndrome in 7 to 10 days postinoculation. Previous studies had suggested the involvement of an immune component in the development of central nervous system pathology. We have examined the effects of T-cell depletion on the development of polioencephalitis (neuronal necrosis and inflammation of the brain and brain stem) and the relative contribution of the $CD4^+$ and $CD8^+$ subsets following the establishment of viremia. We show that monoclonal antibody depletion of T cells is effective in the reduction of polioencephalitis when given prior to viral inoculation. However, administration of the antibodies 12 h or more after viral inoculation failed to alter the development of polioencephalitis or encephalomyelitis. We conclude that T cells are involved in the development of central nervous system disease during the initial stages of infection but are not responsible for the later progression of disease.

Picornaviruses are plus-stranded RNA viruses that have well-known tropisms for a number of organ systems in animals and humans. The M variant of encephalomyocarditis (EMC-M) virus was first isolated in 1959 during an epidemic affecting Panamanian swine (21). EMC-M virus has since provided a model for the study of a number of diseases associated with picornavirus infection. The outcome of a particular infection depends on the age, sex, and strain of the animal and can result in the development of insulitis (2, 5, 6, 14), myocarditis (2, 7, 15, 32), polymyositis (7, 19), encephalomyelitis (clinical presentation of paralysis resulting from demyelination in the spinal cord and the proximal nerve roots) (27), and in the present study, polioencephalitis characterized by neuronal necrosis and inflammation of the brain and brain stem.

Our earlier studies suggested that the demyelinating lesions in the spinal cord had an immune component to their pathogenesis. Immunosuppression of mice with anti-CD4 or anti-CD8 antibodies prior to viral infection resulted in a reduced incidence of clinical paralysis and was supported histologically by decreased demyelination in the spinal cord (27).

For this article we have extended the studies mentioned above to include the effects of T-cell depletion on polioencephalitis and to determine the relative contribution of CD8 and CD4 subsets of T cells when viremia has been established in the animal. Our experiments show that depletion with monoclonal antibodies is effective in the prevention of polioencephalitis when antibodies are given prior to viral infection. However, depletion of T cells with monoclonal antibodies as early as 12 h after viral inoculation fails to protect against either polioencephalitis or development of encephalomyelitis. Since treatment of animals with monoclonal antibodies is sufficient to achieve functional inactivation and depletion of T cells within 24 h of administration (4, 24), this suggests that the involvement of T cells is transient and indirect. T cells are clearly required during the initial stages of infection with EMC-M virus for the development of central nervous system disease, but their presence is not absolutely required during the entire course of infection.

MATERIALS AND METHODS

Animals. Inbred female BALB/c mice were purchased from Cumberland Farms (Clinton, Tenn.). Infected animals were housed separately in the animal colony at the University of Vermont.

Virus. Our original stock of EMC virus is a fourth-passage homogenate of a heart from a CD1 strain mouse and is designated EMC-M2 H4. The virus was passed once more in CD1 heart, designated EMC-M2 H5, and used for all further experiments.

Cells and media. L929 and Ehrlich-Lettre ascites cells were obtained from the American Type Culture Collection (ATCC CCL-1 and CCL-77, respectively). All tumor cell lines were maintained in minimum essential medium (Sigma Chemical Co., St. Louis, Mo.) supplemented with 0.1 mM L-glutamine, 50 U of penicillin per ml, 50 μg of streptomycin per ml, and 5% fetal calf serum (Cell Culture Laboratories, Cleveland, Ohio).

Propagation of virus. T-150 flasks containing 20 to 30 ml of the appropriate medium were seeded with either L929 cells or Ehrlich ascites tumor cells, and the cells were grown until 100% confluent. At that time, cultures were washed with phosphate-buffered saline (PBS) and inoculated with 10 PFU of virus stock per cell. The virus was allowed to adsorb for 30 min, fresh medium was added, and the infected cells were incubated for 48 h at 37°C in 5% CO_2. The supernatant was harvested and frozen at −70°C. Following three freeze-thaw cycles, the cellular debris was removed by centrifugation, and the clear supernatant was frozen at −70°C until needed. For in vitro proliferation assays and enzyme-linked immunosorbent assay (ELISA), the virus was further purified by using a modified version of the method of Ziola and Scraba (34). Inactivation of the virus was accomplished by irradiating the preparation with a General Electric G8T5 UV lamp at a distance of 6 in. (ca. 15 cm) for 10 min.

* Corresponding author.

Monoclonal antibodies. Purified ascites fluid preparation of antibodies was used in all experiments. Anti-CD4 antibody (GK1.5), anti-CD8 (2.43), and anti-Thy-1.2 (30-H-12) were derived from hybridoma cells obtained from the American Type Culture Collection. All antibodies are of the rat immunoglobulin G2b isotype and deplete the appropriate subsets in vivo. Animals received 1 mg of the indicated monoclonal antibody or antibodies or an equivalent volume of PBS at the times indicated.

Infection of experimental animals. Eight- to 12-week-old female BALB/c Cum mice were inoculated by intraperitoneal injection of 60 PFU of EMC-M2 H5 in 0.5 ml of PBS for clinical studies.

Animal evaluation. Beginning 3 days after viral inoculation, the animals were examined daily. The neurological deficits were graded according to the following scale representing clinical advancement of disease: grade I, hunched posture or abnormal gait; grade II, mild to moderate paresis of hind limbs; grade III, complete hind-limb paralysis; grade IV, unable to move about cage or moribund; grade V, death. To calculate a mean clinical severity index for a group, the maximum scores of each animal in that group were summed and divided by the number of animals in the group.

FACS analysis. The fluorescence-activated cell sorter (FACS) was used to evaluate the effectiveness of the monoclonal antibodies in depleting the appropriate T-cell subsets in vivo. Briefly, single-cell suspensions were prepared from the spleen or lymph nodes of monoclonal antibody-treated and naive (control) animals. The appropriate dilutions in PBS of purified and biotinylated monoclonal antibody preparations specific for the major T-cell subset markers, CD4 (helper), CD8 (cytotoxic/suppressor), and fluorescein isthiocyanate-conjugated Thy-1.2 (pan-T cell) were added to separate tubes containing the cells. After allowing sufficient time for attachment, the cells were washed, centrifuged, and stained with streptavidin-phycoerythrin secondary (TAGO). Control wells received the secondary only. After a 30-min incubation at 4°C, the cells were again washed, centrifuged, and then fixed with 1% formaldehyde for analysis in the cell sorter.

Morphological studies. Mice were sacrificed between days 13 and 15 postinoculation. The brain and spinal cord were harvested after perfusion with 4% buffered paraformaldehyde. The brain was sectioned coronally at three levels: (i) at the olfactory tubercle showing the caudate nucleus, (ii) posterior to the optic chiasma showing the hippocampus and the basal ganglia, and (iii) at the level of the floccular nodular lobe of the cerebellum and brain stem. The sections were embedded in paraffin and stained with hematoxylin and eosin. Brains were chosen at random from each group for scoring. The various regions of the brain, including the nonolfactory neocortex; subregions of the olfactory cortex including the hippocampus, the pyriform cortex, amygdaloid nucleus, and the enterorhinal cortex; basal ganglia; cerebellum; and the brain stem, were examined blindly for lesions. Each area was scored on a scale of 0 to 4 for each of the following pathological parameters: (i) neuronolysis (necrosis), (ii) perivascular inflammation, and (iii) microglial nodules. A score of 0 indicates no lesions, +1 indicates minimal severity or equivocal lesions, +2 indicates definite lesions but a mild degree of severity, +3 indicates moderate severity, and +4 indicates very severe pathology. A total histological score for a given region of the brain was obtained by the summation of the scores for the individual parameters. The scores for each region were averaged arithmetically and tested for significance by the Wilcoxan rank sum test (see Fig. 2). A total pathological score for each parameter (necrosis, perivascular inflammation, and microglial nodules) was obtained by summation of the scores for all regions of the brain and averaging arithmetically for each treatment group (see Fig. 3). The averages were tested for significance by the Wilcoxan rank sum test.

Antibody titer to EMC virus. Animals were bled at the time of sacrifice, and the serum was measured for antibody against known EMC virus-positive and -negative control antiserum. A standard ELISA protocol was used with purified EMC viral antigen (23). Values represent individual animals and are expressed relative to values for a pooled EMC-M antiserum (see Fig. 4).

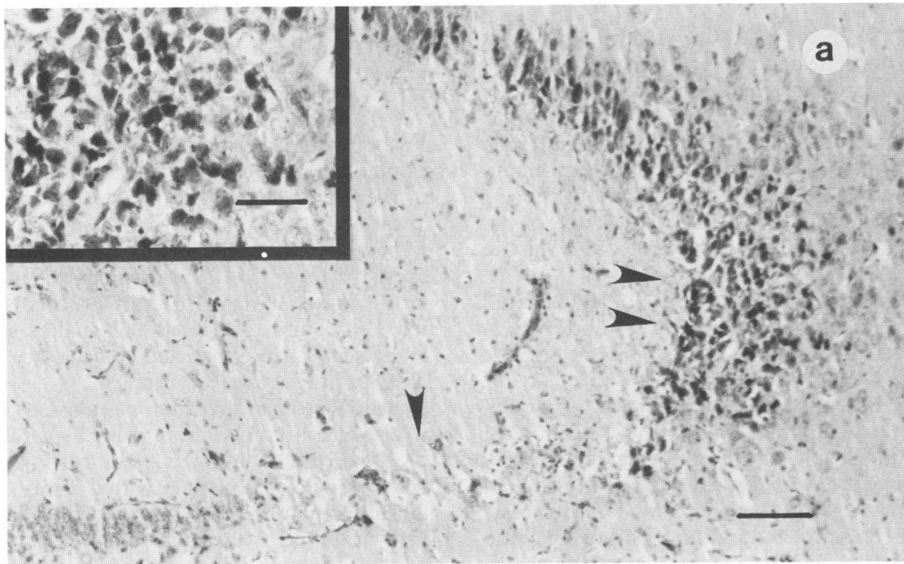
Lymphocyte proliferation response to EMC virus. Splenocytes were obtained from animals at the time of sacrifice (day 15 postinoculation). Spleen cells (4×10^5) were cultured with 0.5 mg of UV-inactivated virus per well in 96-well microtiter plates for 96 h. Purified anti-CD4 or anti-CD8 (1 μg) was added to the indicated wells at the beginning of the culture. During the last 18 h of the culture, tritiated thymidine was added and the degree of proliferation was assessed by scintillation counting. Values are expressed in counts per minute (see Table 3).

RESULTS

Polioencephalitis induced by EMC virus. (i) Administration of antibodies prior to viral infection. In vivo administration of anti-CD4 and anti-CD8 antibodies results in the rapid depletion of the appropriate T-cell subset from the spleen, lymph nodes, and peripheral blood (4, 31). While our earlier studies had focused on reduction of the demyelinating lesions with this form of therapy, it was not clear whether this immunosuppression affected the polioencephalitic part of the disease. In order to determine the effects of systemic T-cell depletion on polioencephalitis, BALB/c mice were treated intraperitoneally with 1 mg of anti-CD4 or anti-CD8 24 h prior to viral inoculation. Control animals received PBS. On day 13 postinoculation, the animals were sacrificed. The degree of polioencephalitis in all areas of the brain was determined as described in Materials and Methods.

The neuropathology of affected animals was characterized by prominent neuronal necrosis ranging from nuclear pyknosis to karyolysis. Shrunken amphophilic cytoplasm of neurons in the pyramidal layer of the hippocampus (Fig. 1a) and perivascular mononuclear infiltration in the fascia dentata were also seen. Other regions of the olfactory cortex, including the pyriform cortex, enterorhinal cortex, and the amygdaloid nucleus, were involved to a lesser degree than the hippocampus (Fig. 2). Focal necrosis with microglial activation and hypertrophy of endothelial cells was seen in the basal ganglia (Fig. 1c). Neuronolysis of the granular cells of the cerebellum was occasionally seen, but the involvement of the brain stem was rare. In addition, focal meningitis in areas of neuronal necrosis was often present (Fig. 1e). The involvement of the various regions of the brain, in terms of frequency, is in the order hippocampus > basal ganglia > pyriform cortex > enterorhinal cortex > cerebellum (Fig. 2).

Animals that received either anti-CD8 or anti-CD4 antibodies 24 h before inoculation had relatively fewer lesions in all areas examined compared with the group receiving PBS (Fig. 2). Anti-CD4 was particularly effective ($P < 0.01$) in reducing the development of lesions in the hippocampus (Fig. 1b), basal ganglia (Fig. 1d), enterorhinal cortex, and cerebellum. Significant reductions in each of the pathological parameters (necrosis, perivascular inflammation, and micro-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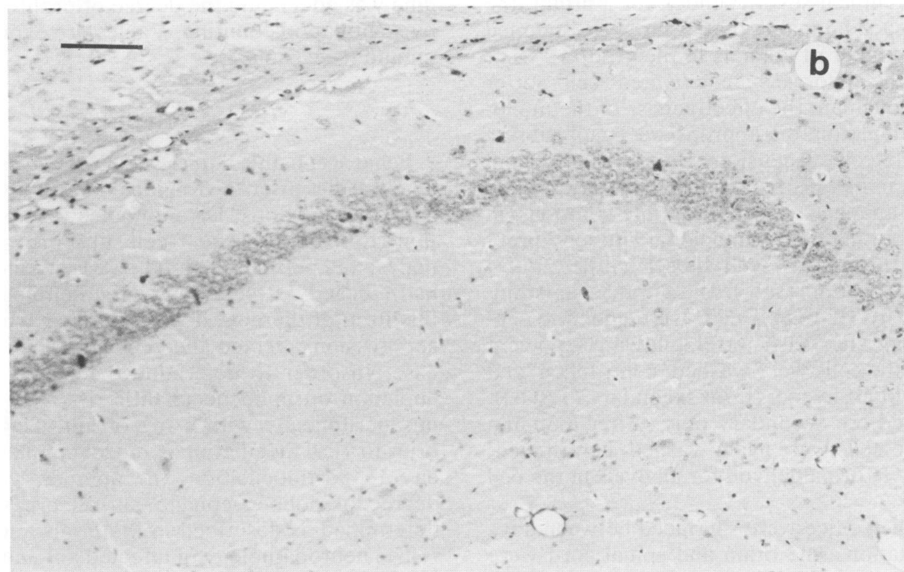
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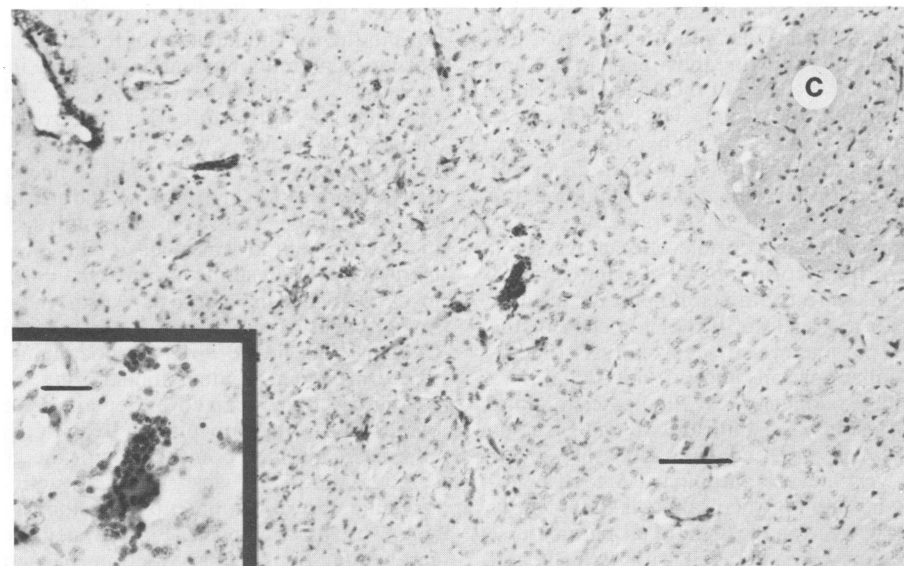
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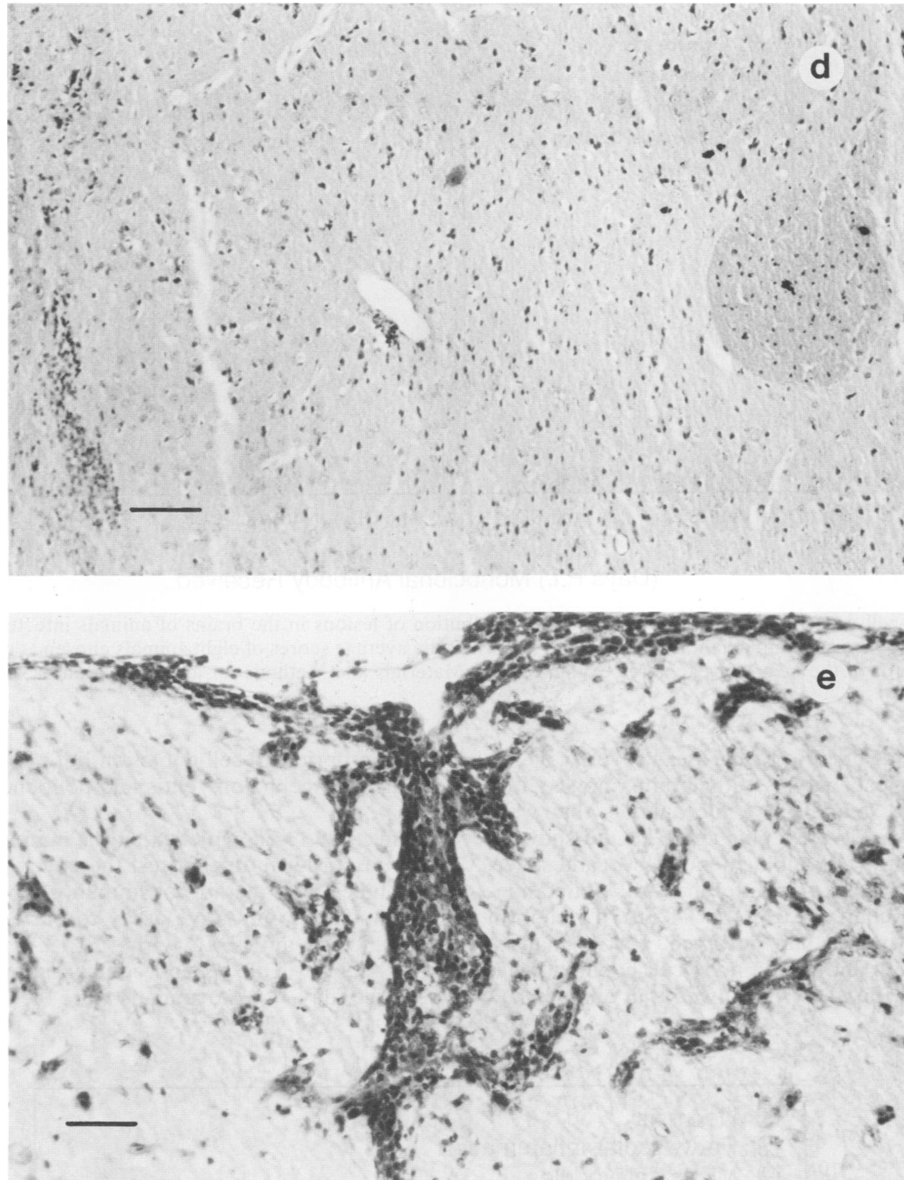

FIG. 1. Photomicrographs of brain sections from mice. (a) Hippocampus from a mouse infected with EMC virus and treated once prior to inoculation with PBS. There is focal destruction of the fascia dentata (arrow). Inset from the pyramidal cell layer (double arrow) shows prominent neuronal necrosis. Bar = 100 μm (50 μm in inset). (b) Same area shown in panel a but from a mouse treated with anti-CD4 antibody 1 day prior to inoculation is without histopathologic features. Bar = 100 μm. (c) Basal ganglia from a mouse infected with EMC virus and treated once prior to inoculation with PBS. There is a well-circumscribed area of increased cell density and prominent capillaries related to microglial activation and hypertrophy of endothelial cells. Inset shows a perivascular cuff of inflammatory cells from the center of the lesion. Bar = 100 μm (25 μm in inset). (d) Same area shown in panel c but in a mouse treated with anti-CD4 antibody 1 day prior to inoculation is without histopathologic features. Bar = 100 μm. (e) Leptomeninges and superficial cortex from a mouse infected with EMC virus and treated once prior to inoculation with PBS. There is prominent leptomeningeal infiltration by inflammatory cells with extension to the Virchow-Robin space. Bar = 50 μm. All sections were hematoxylin and eosin stained.

glial nodules) were noted for treatment with either anti-CD4 or anti-CD8 (Fig. 3).

(ii) Effects of anti-T-cell antibodies given after viral inoculation. In order to assess the role of T cells after the establishment of EMC virus infection, mice were inoculated with 60 PFU of EMC-M virus and given anti-CD4, anti-CD8, or a combination of anti-CD4 plus anti-CD8 (1 mg of each antibody) at specific times after viral infection. Antibodies given at days 2 and 4 or 3 and 5 postinfection had no apparent effects on disease. Administration of antibodies even as early as 12 h after viral infection with an additional dose at 72 h did not alter the course of clinical disease (Table 1). For example, when treatment was first given between 12 and 24 h postinfection and again at 72 h, animals that received PBS had an incidence of clinical paralysis of 75%, with a mean clinical severity index of 2.5; mice that were treated with anti-CD4 had an incidence of 70%, with a mean severity index of 2.0; those that received anti-CD8 antibody

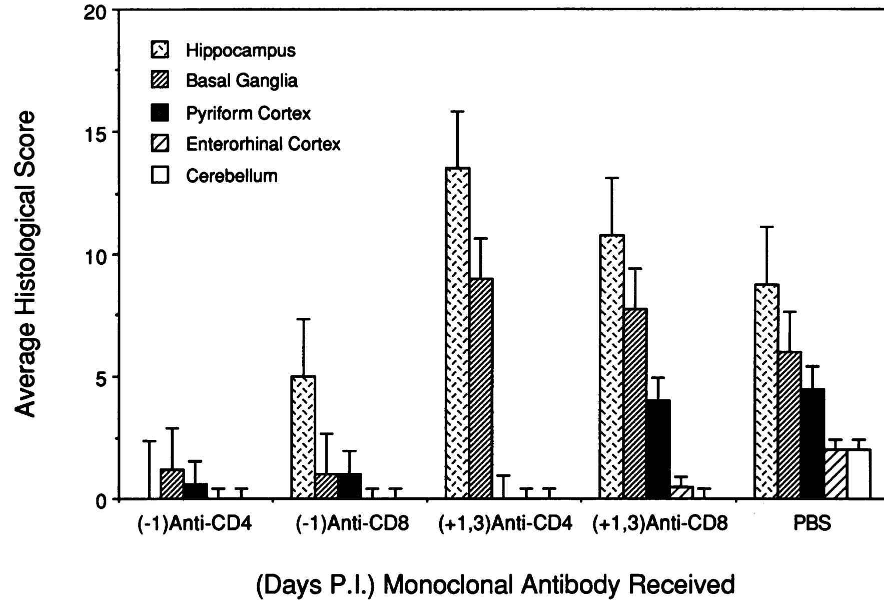

FIG. 2. Effects of T-cell depletion on the development and distribution of lesions in the brains of animals infected with EMC-M virus. Animals were sacrificed 13 days postinoculation (P.I.). Values represent the average scores of eight animals chosen at random, and error bars indicate the standard error of the mean for each area examined. See Materials and Methods for an explanation of scoring.

had an overall incidence of 80%, with a mean severity of 2.9. Furthermore, histological analysis of the brains showed little or no protection (Fig. 2 and 3). While animals that were given a combination of anti-CD4 plus anti-CD8 did show an apparent reduction in the overall incidence (46%) and severity (1.8) of clinical paralysis, this difference was not statistically significant (chi-square = 1.85, $P > 0.10$). To confirm that T-cell depletion indeed had occurred, FACS analysis was done on spleen cell and lymph node cell populations at the time of sacrifice. The analysis showed that animals had the appropriate T-cell depletion but nevertheless developed paralytic encephalomyelitis and polioencephalitic signs (Table 2).

Effects of T-cell depletion on the immune response to EMC virus. In order to further test the effectiveness of anti-T-cell antibodies as a method of immunosuppression, an in vitro lymphocyte-proliferative assay to purified EMC virus antigen and an anti-EMC virus ELISA were performed in T-cell-subset-depleted and control animals. At the time of sacrifice (day 14), the animals were bled and the serum was

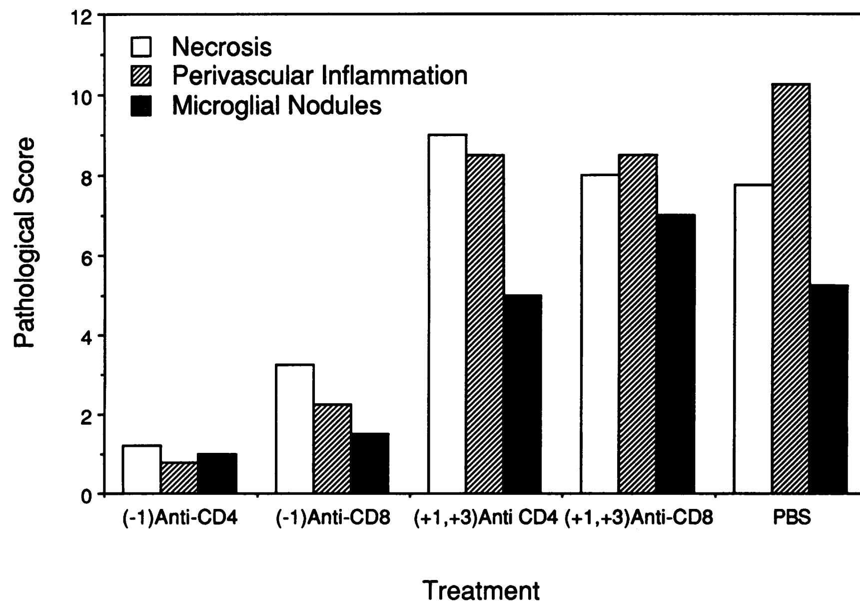

FIG. 3. Effects of T-cell depletion on the specific type of lesions developed in the brain during EMC-M virus infection. See Materials and Methods for an explanation of scoring.

TABLE 1. Development of paralytic symptoms in mice receiving anti-T-cell antibodies after infection with EMC virus

Expt no.	No. of mice with symptoms[a]/no. inoculated at:							
	12 and 72 h with:				Days 3 and 5 with:			
	Anti-CD4	Anti-CD8	Anti-CD4 and anti-CD8	PBS	Anti-CD4	Anti-CD8	Anti-CD4 and anti-CD8	PBS
1	5/6	6/6	2/5	6/6	4/5	5/5	5/5	5/5
2	3/3	3/6	4/7	3/6	6/6	5/6	5/5	6/6
3	4/8	7/8	1/3	6/8	ND[b]	ND	ND	ND
Total (%)	12/17 (70)	16/20 (80)	7/15 (46)	15/20 (75)	10/11 (91)	10/11 (91)	10/10 (100)	11/11 (100)
Mean clinical severity	2.0	2.9	1.8	2.5	3.0	3.1	2.8	3.1

[a] Animals developing hind-limb paralysis.
[b] ND, not done.

measured for anti-EMC virus antibody. Animals that had received anti-CD8 or PBS had titers that were comparable to those of control animals, while those that had received anti-CD4 or a combination of anti-CD4 along with either anti-CD8 or anti-Thy-1.2 had significantly lowered values (Fig. 4). These results are consistent with the role of the CD4 subset in the development of an antibody response. This is further supported by the observations on the proliferative response to EMC virus antigens. Animals that received anti-CD4 antibody either before or after viral infection had a significantly lowered proliferative response compared with untreated animals or animals that received anti-CD8 antibody. This proliferative response was mediated by the CD4 subset of T cells, since addition of anti-CD4 antibody abrogated the response (Table 3).

The suppression of an antibody response along with the FACS analysis demonstrates the profound immunosuppression in vivo following treatment with anti-T-cell antibodies and shows a lack of correlation of postinoculation immunosuppression with the development of either polioencephalitis or encephalomyelitis.

DISCUSSION

In summary, our studies have shown the following. (i) Treatment with anti-CD8 or anti-CD4 antibodies prior to viral inoculation reduces the severity of polioencephalitis and encephalomyelitis; anti-CD4 is more effective than anti-CD8 antibody. (ii) Immunosuppression with anti-T-cell antibodies given as soon as 12 h postinoculation does not alter the overall severity of polioencephalitis, encephalomyelitis, or clinical paralysis. While these studies continue to draw a link between infection by a neurotropic virus and the immune system in the development of central nervous system disease, they fail to demonstrate a direct involvement of T cells in the pathogenesis of the disease.

In vivo therapy with monoclonal antibodies to T-cell subsets results in the depletion of more than 90% of the appropriate T-cell subset from peripheral blood, spleen, and lymph nodes within 24 h of administration (4, 24). This phenomenon has been used to study the effector mechanism of a number of T-cell-mediated autoimmune diseases. Anti-CD4 antibodies have been shown to prevent or alter the development of experimental allergic encephalomyelitis (24, 25), murine myasthenia gravis (3), and NZB × NZW disease

TABLE 2. FACS analysis on lymph nodes of EMC virus-infected animals treated with monoclonal antibodies to specific T-cell subsets

Time of administration and antibody	% Positive cells[a]		
	Thy-1.2$^+$	CD4$^+$	CD8$^+$
1 and 3 days postinoculation			
Anti-CD4	42.8	1.6	28.3
Anti-CD8	77.1	68.1	1.0
1 day preinoculation			
Rat immunoglobulin G	85.2	42.9	23.9
Anti-CD4	56.3	2.9	17.0
Anti-CD8	71.1	56.1	1.2
None (PBS)	87.9	63.7	18.9

[a] Animals were sacrificed 14 days after inoculation and tested for T-cell depletion.

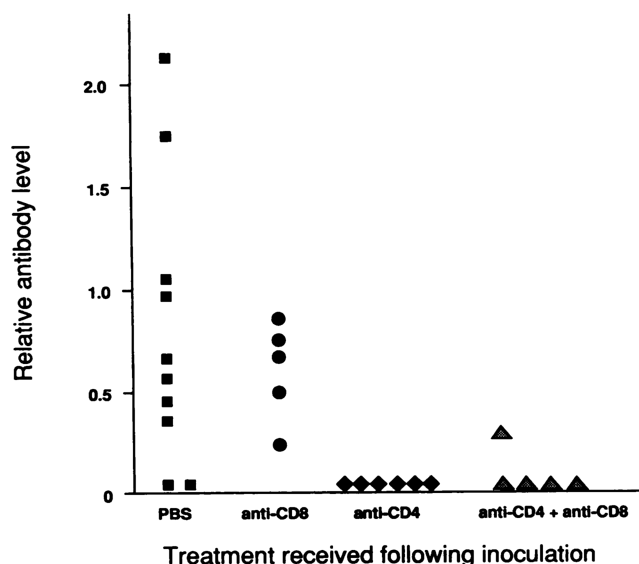

FIG. 4. Antibody levels in sera of animals that received anti-T-cell-subset-specific antibody following inoculation with EMC virus. Antibody levels were measured on day 14 postinoculation and are expressed relative to levels in positive, pooled antiserum.

TABLE 3. Proliferative response of splenocytes to UV-inactivated viral antigen from animals that were injected with EMC virus and received anti-T-cell-subset-specific antibodies

Time of administration and monoclonal antibody (in vivo)	cpm[a] after following treatment (in vitro):			
	None	EMC-M (0.5 μg) with following antibody:		
		None	Anti-CD4	Anti-CD8
1 day preinoculation				
Anti-CD4	1,561	2,799	2,146	2,463
Anti-CD8	8,340	33,823	1,936	66,956
1 day postinoculation				
Anti-CD4	1,969	2,263	4,622	2,935
Anti-CD8	1,505	10,807	1,053	18,898
None (PBS)	3,715	31,506	3,811	40,249

[a] Data are from a single representative assay.

(31). Thus, it was our belief that if T cells were responsible for directly mediating the central nervous system pathology that develops following infection with EMC virus, depletion of lymphocytes at any time prior to the development of clinical disease should likewise reduce or prevent the ultimate expression of this viral disease.

Viral infection of the central nervous system appears to involve at least two broad forms. For example, acute encephalitis due to herpes or arbovirus infection is a disease of the gray matter with perivascular parenchymal infiltration of inflammatory cells and neuronophagia (1, 17). The disease is thought to be caused by a direct cytopathic effect of the virus on neurons and glial cells, since virus is present in areas of injury and necrosis (1, 17, 18). In contrast, postinfectious encephalomyelitis is a disease of the white matter with perivascular inflammation and demyelination (26). The latter is thought to be autoimmune in nature, since the virus is not found in the brain and there is no antibody to viral antigens detected in the central nervous system (8, 18, 26). In prior studies on experimental models of polioencephalitis and encephalomyelitis it appeared that an immune component was involved in the development of the disease (8). Thus, nude mice show decreased severity of encephalitic lesions following Venezuelan equine encephalitis virus (9) and in the degree of demyelination following Semliki Forest virus infection (10, 16, 29). In the latter studies, reconstitution of nude mice with naive cells led to reexpression of disease (10, 29).

Our earlier observations on the demyelinating myelitis had suggested that this syndrome represented a variant of postinfectious encephalomyelitis, with the CD8 population of T cells playing a direct role in the demyelination (27). We have now shown that administration of either anti-CD4 or anti-CD8 antibody prior to viral inoculation is also effective in reducing the development of polioencephalitis; what is puzzling is our inability to alter disease of either the spinal cord or brain with T-cell subset depletion shortly after viral infection, which makes a direct role of the T cells seem unlikely. It appears that the $CD8^+$ T cells play a role in promoting demyelination of the spinal cord and the subsequent development of clinical paralysis (27) at some time during the early stages of the viral infection but are not involved after the first 12 h. Likewise, the $CD4^+$ population of T cells, while having a role early in the infectious process, are not directly responsible for the tissue pathology that develops later in the course of infection.

In other viral diseases that are thought to be immune mediated, EMC-M virus was found to induce a diabetes mellitus-like syndrome in infected male BALB/c ByJ mice which could be prevented by the preinoculation administration of anti-CD4 antibody (14). On the other hand, anti-CD4 antibodies worsened Theiler's murine encephalomyelitis virus disease when they were given prior to viral infection (30) whereas anti-CD8 antibodies reduced the degree of demyelination when given either before or after viral infection (22). In coxsackievirus B3-induced myocarditis, the role of T-cell subsets appears to be strain dependent; anti-CD4 antibodies are protective in AJ mice, whereas anti-CD8 antibodies are protective in BALB/c mice (15). It is not clear, however, whether immune mechanisms similar to those we have observed operating in the present model are involved in these other viral diseases.

The outcome of the infection of cells, in terms of pathogenesis, is influenced both by the cytokine response of the cells to the infecting virus and by the response of the cells to the cytokines. For example, differences have been noted between diabetogenic and nondiabetogenic variants of EMC virus in terms of the interferon-inducing capabilities (33). The immune effects of T cells in the EMC-M virus model appear to be indirect and could be mediated through the secretion of cytokines such as gamma interferon or tumor necrosis factor alpha. It is known that the receptor for the majority of rhinovirus (a picornavirus) serotypes is the cellular adhesion molecule ICAM-1 (13, 28), which is involved in the immune response and is upregulated by the cytokines gamma interferon, interleukin 1, and tumor necrosis factor alpha (11). It is thought that these cytokines influence the number and state of the viral receptors on the plasma membrane such that viral tropism is affected (21a). It is possible that a similar mechanism is operating in the EMC virus model. The differential effects of CD4 and CD8 subset depletion may be related to the differences in the production of lymphokines by T cells and the regulatory role of T cells on the production and secretion of cytokines by immune and nonimmune cells in response to the viral infection. T-helper cells ($CD4^+$), for example, are distinguished into subtypes according to the secretion of lymphokines (20). In addition, it has been shown that CD8 cells modulate the final differentiation of T-helper cells into subsets (12), and so depletion of $CD8^+$ cells could also affect $CD4^+$ lymphocyte lymphokine secretion.

Thus, it appears that picornaviruses may have evolved mechanisms which allow them to take advantage of the immune response against them to promote their spread and replication. This suggests a mechanism by which T cells influence viral infection by modulating the production of cytokines in vivo. Removal of T cells by administration of monoclonal antibodies could upset the balance of cytokine induction required for pathogenic infection and could cause protection. These possibilities are currently under investigation.

Our results establish a link among lymphocytes, virus, and the ultimate expression of disease, although the nature of the association is not known. While it is conventionally assumed that viral tropism to the brain and the development of polioencephalitis and encephalomyelitis is independent of the host immune system, our observations suggest that, at least in EMC virus-induced disease, host T cells play a role in the outcome of infection.

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REFERENCES

1. **Albrecht, P.** 1968. Pathogenesis of neurotropic arbovirus infections. Curr. Top. Microbiol. Immunol. **43**:44–91.
2. **Babu, P. G., S. Huber, S. Sriram, and J. E. Craighead.** 1985. Genetic control of multisystem autoimmune disease in EMC infected Balb/c Cum and Balb/c ByJ mice. Curr. Top. Microbiol. Immunol. **122**:154–161.
3. **Christadoss, P., and M. J. Dauphinee.** 1986. Immunotherapy for myasthenia gravis: a murine model. J. Immunol. **36**:2437–3442.
4. **Cobbold, S. P., A. Jayasurya, A. Nash, T. D. Prospero, and H. Waldman.** 1984. Therapy with monoclonal antibody by elimination of T cell subsets in vivo. Nature (London) **312**:549–551.
5. **Craighead, J. E.** 1966. Pathogenicity of E & M variant of the EMC virus. II. Lesions of the pancreas, parotid gland, and the lacrimal gland. Am. J. Pathol. **48**:375–386.
6. **Craighead, J. E.** 1981. Viral diabetes mellitus in man and experimental animals. Am. J. Med. **70**:127–134.
7. **Cronin, M. E., L. A. Love, F. W. Miller, P. R. McClintock, and P. H. Plotz.** 1988. The natural history of EMC virus induced myositis and myocarditis. J. Exp. Med. **168**:1639–1648.
8. **Dal Canto, M. C., and S. G. Rabinowitz.** 1981. Experimental models of virus induced demyelination. Ann. Neurol. **11**:109–121.
9. **Dal Canto, M. C., and S. G. Rabinowitz.** 1981. CNS demyelination in Venezuela equine encephalitis. Experimental model of virus induced myelin injury. J. Neurol. Sci. **48**:397–418.
10. **Dal Canto, M. C., S. G. Rabinowitz, and T. C. Johnson.** 1979. Virus induced demyelination: production of viral temperature sensitive mutants. J. Neurol. Sci. **42**:155–168.
11. **Frohman, E., T. Frohman, M. Dustin, B. Vayuvegula, B. Choi, A. Gupta, S. van den Noort, and S. Gupta.** 1989. The induction of intercellular adhesion molecule 1 (ICAM-1) expression on human fetal astrocytes by interferon-γ, tumor necrosis factor α, lymphotoxin, and interleukin 1: relevance to intracerebral antigen presentation. J. Neuroimmunol. **23**:117–124.
12. **Gajewski, T. F., J. Joyce, and F. W. Fitch.** 1989. Anti-proliferative effect of gamma interferon in immune regulation. 1. Differential selection of TH1 and TH2 murine helper T cell clones using rIL-2 and gamma-IFN. J. Immunol. **143**:14–22.
13. **Greve, J., G. Davis, A. Meyer, C. Forte, S. Connolly Yost, C. Marlor, M. Kamarck, and A. McClelland.** 1989. The major human rhinovirus receptor is ICAM-1. Cell **56**:839–847.12.
14. **Haynes, M. K., S. A. Huber, and J. E. Craighead.** 1987. Helper-inducer T-lymphocytes mediate diabetes in EMC-infected BALB/c ByJ mice. Diabetes **36**:877–881.
15. **Huber, S., A. Weller, M. Herzum, P. A. Lodge, M. Estrin, K. Simpson, and M. Guthrie.** 1988. Mechanism of experimental picornavirus induced autoimmunity in myocarditis. Etiology, pathogenesis and treatment. Pathol. Immunopathol. Res. **7**:279–291.
16. **Jenkins, H. G., E. M. Tansey, F. Macefield, and H. Ikeda.** 1988. Evidence of T cell factor as a cause of demyelination in mice following Semliki forest virus infection. Brain Res. **459**:145–149.
17. **Johnson, R. T.** 1987. Pathogenesis of acute viral encephalitis and post infectious encephalomyelitis. J. Infect. Dis. **155**:359–364.
18. **Johnson, R. T., D. S. Burke, M. Elwell, C. J. Leake, A. Nisalak, C. H. Hoke, and W. Lorsomurdee.** 1985. Japanese encephalitis immunocytochemical studies viral antigens and inflammatory cells in fatal cases. Ann. Neurol. **18**:567–573.
19. **Miller, F. W., L. A. Love, T. Biswas, P. McClintock, and P. H. Plotz.** 1987. Viral and host genetic factors influence EMC virus induced polymyositis in adult mice. Arthritis Rheum. **30**:544–561.
20. **Mosman, T. R., H. Cherwinski, M. W. Bond, M. A. Giedlin, and R. L. Coffman.** 1986. Two types of murine helper T cell clones. 1. Definition according to profiles of lymphokines activities and secreted proteins. J. Immunol. **136**:2348–2357.
21. **Murnane, T. G., J. E. Craighead, H. Mondragon, and A. Shalokov.** 1960. Fatal disease of swine due to EMC virus. Science **131**:498–499.

21a. **Palmenberg, A.** Personal communication.

22. **Rodriguez, M., and S. Sriram.** 1988. Successful therapy of Theiler's virus induced demyelination with monoclonal anti Lyt-2 antibody. J. Immunol. **140**:2950–2955.
23. **Shanely, J. D.** 1980. Enzyme-linked immunosorbent assay for immunoglobulin G antibody to encephalomyocarditis virus. J. Clin. Microbiol. **12**:663–665.
24. **Sriram, S., L. Carroll, S. Fortin, S. Cooper, and G. Ranges.** 1988. In vivo immunomodulation by monoclonal anti CD4 antibody. II. Effect on T cell response to MBP and EAE. J. Immunol. **141**:464–468.
25. **Sriram, S., and C. A. Roberts.** 1986. Treatment of established CR-EAE with anti L3T4 antibodies. J. Immunol. **135**:4464–4472.
26. **Sriram, S., and L. Steinman.** 1984. Postinfectious and postvaccinial encephalomyelitis. Neurol. Clin. N. A. **2**:341–353.
27. **Sriram, S., D. J. Topham, S.-K. Huang, and M. Rodriguez.** 1989. Treatment of encephalomyocarditis virus-induced central nervous system demyelination with monoclonal anti-T-cell antibodies. J. Virol. **63**:4242–4248.
28. **Staunton, D., V. Merluzzi, R. Rothlein, R. Barton, S. Marlin, and T. Springer.** 1989. A cell adhesion molecule, ICAM-1, is the major surface receptor for rhinoviruses. Cell **56**:849–853.
29. **Suckling, A. J., S. Pathak, S. Jagelman, and H. E. Webb.** 1978. Virus associated demyelination. A model using a virulent Semliki Forest virus infection of mice. J. Neurol. Sci. **39**:147–154.
30. **Welsh, C. J., P. Tonks, A. A. Nash, and W. F. Blakemore.** 1987. The effect of L3T4 T cell depletion on the pathogenesis of Theiler's virus murine encephalomyelitis virus on CBA mice. J. Gen. Virol. **68**:1659–1678.
31. **Wofsy, D., and W. E. Seaman.** 1985. Successful therapy of autoimmunity in NZB × NZW mice with monoclonal antibody to L3T4. J. Exp. Med. **161**:378–381.
32. **Woodruff, J. F.** 1980. Viral myocarditis: a review. Am. J. Pathol. **101**:425–484.
33. **Yoon, J.-W., P. McClintock, T. Onodera, and A. Notkins.** 1980. Virus-induced diabetes mellitus. XVIII. Inhibition of a nondiabetogenic variant of encephalomyocarditis virus. J. Exp. Med. **152**:878–892.
34. **Ziola, B. R., and D. G. Scraba.** 1974. Structure of the mengo virion. Virology **57**:531–542.