JOURNAL OF VIROLOGY, Oct. 1988, p. 3589–3596
0022-538X/88/103589-08$02.00/0
Copyright © 1988, American Society for Microbiology
Vol. 62, No. 10

# Theiler's Virus Infection in Nude Mice: Viral RNA in Vascular Endothelial Cells

ANDREAS ZURBRIGGEN AND ROBERT S. FUJINAMI*

*Department of Pathology, University of California, San Diego, La Jolla, California 92093*

Received 25 April 1988/Accepted 22 June 1988

**Infection of athymic (*nu/nu*) mice with Theiler's murine encephalomyelitis virus results in an acute encephalitis which resembles poliomyelitis. Immunohistochemistry and in situ hybridization were used to delineate the presence of viral proteins and RNA in the nervous systems of nude mice infected with the Daniels strain of Theiler's virus. This system permits the analysis of a viral infection in the absence of an effective immune response. By immunohistochemistry, viral antigen was found in the processes and cell bodies of neurons and glial cells. Besides the presence of viral antigen in these cell types, by in situ hybridization, Theiler's virus RNA was also found in cells associated with vascular endothelium in the brains and spinal cords of these infected mice. Theiler's virus RNA-positive endothelial cells were observed not only near the primary lesions but also away from demonstrable lesions in normal-appearing regions in the central nervous system. Earlier work had suggested an intra-axonal dissemination for this virus (M. C. Dal Canto and H. L. Lipton, Am. J. Pathol. 106:20–29, 1982). Our findings are consistent with this model but also suggest an additional mechanism for virus spread within the central nervous system, i.e., by infecting vascular cells and crossing the blood-brain barrier. Lastly, after Theiler's murine encephalomyelitis virus infection, not only glial cells but also endothelial cells express major histocompatibility complex class II (Ia) antigen on their surface (M. Rodriguez, M. L. Pierce, and E. A. Howie, J. Immunol. 138:3438–3442, 1987). Our demonstration of Theiler's virus-infected endotheliumlike cells may explain interactions of virus products in stimulating antigen presentation.**

Theiler's murine encephalomyelitis virus (TMEV) is an animal model for demyelinating disorders such as multiple sclerosis (10). TMEV is a naturally occurring murine picornavirus. The natural infection of young mice generally produces an asymptomatic enteric illness. However, after intracerebral inoculation of SJL mice with the Daniels (DA) strain of TMEV, a biphasic disease of the central nervous system (CNS) (18) is observed. The acute stage of the disease is similar to poliomyelitis (24). In TMEV-infected mice, inflammation, necrosis of neurons, and proliferation of glial cells parallel virus replication. Further, viral antigen has been found in the cytoplasm of neurons and astrocytes (29). Similarly, in monkeys infected with human poliovirus (HPV), viral antigens have been detected within inflammatory, glial, and neuronal cell types as well as in vascular endothelial cells [E. Kovacs, R. K. Baratawidjaja, and N. A. Labzoffsky, Letter, Nature (London) **200**:497–498, 1963]. In contrast, in more recent studies with mice, Dal Canto et al. (8) detected poliovirus antigens only in neurons.

At 1 to 3 months after the acute phase of TMEV infection, the surviving animals develop a chronic disease marked by spasticity of the limbs. The pathological signs have been characterized by extensive meningeal and perivascular infiltrates with concomitant demyelination, primarily in the spinal cord (9). These cellular infiltrates consist of macrophages, lymphocytes, monocytes, and plasma cells (10). Within 1 month after infection, TMEV antigens can be localized within macrophages, astrocytes, and oligodendrocytes in immunocompetent mice. Later, TMEV antigens are present mainly in oligodendrocytes in the spinal cord (29). The developing white matter lesions resulting from TMEV infection are similar to the lesions in the autoimmune CNS disease experimental allergic encephalomyelitis (EAE) (9).

Several mechanisms have been proposed to explain the cause of demyelination in mice after TMEV infection. An immune system-mediated mechanism has been suggested by experiments in which early immunosuppression eliminated the mononuclear cell infiltration in the spinal cord white matter and decreased or prevented demyelination (19). Roos et al. (32) found that the timing of the immunosuppressive therapy administered to mice was critical for inhibition of demyelination to occur. For example, treatment of mice with antithymocyte serum or cyclophosphamide at the time of infection inhibited or diminished demyelination. However, treatment of infected mice continuously over a 5-week period or initiation of immunosuppressive therapy 5 weeks after infection did not decrease demyelination (32).

Other observations favor a mechanism of demyelination in which TMEV plays a more central role. For example, TMEV can be detected in small amounts in mouse spinal cord even 2.5 years after infection (20), and the demyelinating lesions are associated with an ongoing CNS infection (7). Treatment of mice with myelin fractions after TMEV infection did not lessen the demyelination as compared with that in untreated infected mice (16). In contrast, mice immunized with spinal cord homogenate and adjuvant, a method used to induce EAE, and treated with myelin fractions had decreased demyelination as compared with untreated mice with EAE. This result suggested a mechanism of demyelination different from that observed for EAE (16).

Nude mice provide a useful model in which infection can be elucidated in the absence of mature T lymphocytes. Nude or athymic mice are congenitally deficient in T cells and are more susceptible to viral infections (15). In these mice, electron microscopic studies have indicated that the DA

* Corresponding author.

strain of TMEV causes a lytic infection of cells in the absence of functional immune cells (33). Rosenthal et al. (34) have demonstrated the presence of antigen in lytically infected oligodendrocytes of TMEV-infected nude mice. These cells were identified as infected oligodendrocytes by their connections to myelin sheaths and the presence of viral antigen in the cytoplasm.

In this report, we extend our earlier studies of TMEV infection in nude mice. Here, we demonstrate by immunohistochemistry the presence of viral antigen in hippocampal neurons and their processes and within glial cells. By in situ hybridization, TMEV nucleic acid was found not only in both of these cell types but also for the first time in cells associated with vascular endothelium, most probably endothelial cells. These TMEV RNA-positive cells were found both in close proximity to the primary lesions and at a distance from demonstrable lesions in normal-appearing regions in the CNS. This result suggests a new, previously undescribed mechanism of viral dissemination during TMEV infection.

## MATERIALS AND METHODS

**Cells.** BHK-21 cells were propagated in Dulbecco modified Eagle medium supplemented with 10% fetal bovine serum, 3 g of tryptose-phosphate broth per liter, 0.5% glucose, 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), 3.2 mM glutamine, 100 U of penicillin G per ml, 100 μg of streptomycin per liter, and 0.25 μg of amphotericin B (Fungizone; E. R. Squibb & Sons, Princeton, N.J.) per liter. These cells were routinely subcultured twice weekly. For immunocytochemistry and in situ hybridization experiments, cells were grown on poly-L-lysine coated slides. Cells were then infected with the DA strain of TMEV (33) at a multiplicity of infection of 0.1, fixed 16 to 20 h postinfection (p.i.) with 4% phosphate-buffered saline (PBS)–paraformaldehyde, washed with PBS, dehydrated with ascending concentrations of ethanol, air dried, and stored until use. Mock-infected BHK-21 cells similarly treated served as negative controls.

**Virus and plaque assay.** The DA strain of TMEV (12, 17) is a culture-attenuated virus strain and was routinely propagated in BHK-21 cells grown in supplemented Dulbecco modified Eagle medium.

TMEV was quantified by plaque assay on BHK-21 cells. Mice were euthanized. Tissues were obtained aseptically, transferred to preweighed tubes, and homogenized. The resulting tissue homogenates were frozen-thawed three times. Dilutions of tissue homogenates as well as control TMEV were added to cell monolayers in 35-mm plates. The tissue homogenate or control virus was allowed to adsorb for 1.5 h at room temperature, after which cells were overlaid with 0.5% agarose in medium 199 containing 1% fetal bovine serum. After 4 days at 37°C, cell monolayers were fixed and stained with crystal violet, and plaques were enumerated.

**Mice and tissues.** Female nude mice (5 weeks old) were obtained from Fred Rosen, University of California, San Diego, nude mouse facility. Fifteen nude mice were inoculated with $3 \times 10^5$ PFU of DA strain virus in the left cerebral hemisphere. For immunohistochemistry or in situ hybridization studies, animals were perfused 14 days p.i. with 4% PBS-paraformaldehyde. At this time, the majority of mice had clinical symptoms of poliomyelitis. Brains and spinal cords were removed and processed for paraffin embedding. Sections (4 μm) were cut and used for immunocytochemistry and in situ hybridization. Brains and spinal cords from mice which were sensitized with spinal cord homogenate-adjuvant and which had EAE served as controls (4).

**Antibodies.** Anti-DA strain virus antibodies were raised in rabbits. Tissue culture-prepared DA virus was purified on CsCl gradients (35). Rabbits were immunized intravenously with purified virus and boosted every 4 weeks for 6 months. The sera were tested by an enzyme-linked immunosorbent assay (28) and immunocytochemistry with DA virus-infected and mock-infected BHK-21 cells. All sera had high titers in the enzyme-linked immunosorbent assay, i.e., greater than 1/150,000. When sera were tested by immunocytochemistry, 1/8,000 was found to be an optimal dilution for the detection of viral antigen in infected BHK-21 cells. No nonspecific binding of these sera was found for mock-infected BHK-21 cells at dilutions of 1/100 or greater. In addition, positive sera were tested with Western blots (immunoblots) after denaturing polyacrylamide gel electrophoresis. The antisera reacted strongly with VP-1 and VP-2 and weakly with VP-3. Further, these antisera had the ability to neutralize infectious virus. Thus, the sera used for these experiments recognized conformational antigens as well as fixed antigens in addition to biologic epitopes, as determined by neutralization.

**Immunocytochemistry.** Infected and mock-infected BHK-21 cells as well as deparaffinized brain and cord sections were incubated with 5% normal goat serum for 30 min to block nonspecific binding. The anti-DA virus rabbit sera were added to the slides at a dilution of 1/8,000 for 24 h at 4°C, followed by a 1/50 dilution of biotinylated goat anti-rabbit immunoglobulin G (TAGO, Burlingame, Calif.) for 30 min at room temperature. The slides were incubated with an avidin-biotinylated horseradish peroxidase complex as prescribed by the vendor (Vector Laboratories, Burlingame, Calif.). The slides were treated with 0.05% diaminobenzidine–0.01% hydrogen peroxide for 10 min. The reaction was stopped by washing with PBS. After dehydration, cover slips were applied to the slides, and the slides were examined by light microscopy.

**In situ hybridization. (i) Preparation of DNA probes.** For this study, two different cDNA probes were used. A TMEV clone representing the 5′ noncoding portion of Bean strain viral RNA was kindly provided by Howard Lipton (27). The other clone was kindly provided by Michel Brahic (26) and represented a region of the viral polymerase from the GD VII strain of TMEV. The viral inserts were enzymatically cleaved from the vectors and gel purified. Both cDNAs were radiolabeled by the random hexanucleotide primer method (13) with [α-$^{35}$S]dCTP and [α-$^{35}$S]dATP (Dupont, NEN Research Products, Boston, Mass.). The probes were labeled to $10^8$ cpm/μg of DNA.

**(ii) Preparation of BHK-21 cells and tissue sections for *in situ* hybridization.** Cells and tissue sections were processed as described by Wiley et al. (39). Briefly, after rehydration, the cells and tissue sections were rinsed twice in $H_2O$, incubated for 10 min with 0.2 M HCl, rinsed twice in $H_2O$, and immersed in 1% Triton-X 100 for 1.5 min. After being washed with PBS, the slides were treated for 20 min at 37°C with proteinase K at 1 μg/ml in 20 mM Tris hydrochloride–2 mM $CaCl_2$. The slides were postfixed with 4% PBS-paraformaldehyde for 4 min and washed with PBS. After dehydration through ascending ethanol, the slides were hybridized with 0.5 μg of probe per ml in 50% formamide–5× hybridization salts–5× Denhardt solution (21)–500 μg of salmon sperm DNA per ml–250 μg of HeLa cell RNA per ml–0.1% Triton-X 100–10 mM dithiothreitol.

Hybridization was carried out overnight at 37°C. Excess [$^{35}$S]cDNA was diluted by several changes of 2× and 0.1×

SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) (21). Washed slides were dehydrated, air dried, and immersed in NTB-2 emulsion (Eastman Kodak Co., Rochester, N.Y.). After 4 days of exposure, the slides were developed with Kodak D19 and fixed with Kodak standard fixer. The slides were counterstained with hematoxylin.

**(iii) Dual labeling for immunocytochemistry and in situ hybridization.** Selected paraffin sections were used for simultaneous detection of viral antigens and nucleic acid. For this purpose, immunocytochemistry was followed by in situ hybridization reactions. Immunocytochemistry was performed as described above with the exception that 0.04% diethylpyrocarbonate was added to the first and second antibody solutions to inhibit RNase in the sera (36). In addition, slides were treated with 0.1 M triethanolamine–0.25% acetic anhydride to reduce any nonspecific binding of the cDNAs to the diaminobenzidine product (2).

## RESULTS

To establish initial conditions for the immunohistochemical and in situ hybridization reagents, we first conducted studies on infected and mock-infected BHK-21 cells. Rabbit anti-DA virus sera were incubated with infected BHK-21 cells and processed for immunohistochemistry with diaminobenzidine (Fig. 1). Viral antigen was detected in the cytoplasm of infected BHK-21 cells. In these infected cells, cytoplasmic inclusion bodies were present in various sizes (Fig. 1a). Mock-infected BHK-21 cells were consistently negative.

When in situ hybridization studies were conducted with either cDNA (TMEV Bean- or GD VII-derived sequences), a similar cytoplasmic distribution of viral nucleic acid was observed (Fig. 1b). Most of the autoradiographic grains were localized in the cytoplasm of infected cells; very few grains were observed over the nucleus. Uninfected BHK-21 cells were negative and contained a few scattered background grains (Fig. 1c).

Having established the conditions for immunohistochemistry and in situ hybridization in the tissue culture system, we analyzed TMEV-infected, mock-infected, and EAE mouse brain and spinal cord sections for the presence of viral proteins and nucleic acids. The presence of TMEV antigen and RNA in the CNSs of nude mice at 3 days and at 1, 2 and 3 weeks p.i. was initially studied. At 3 days p.i., most of the viral RNA and antigen were localized near the site of injection within the brain. In contrast, the first appearance of TMEV products in the spinal cord was observed at 1 week p.i. The number of infected cells steadily increased, especially in the spinal cord, and by 3 weeks p.i. an enormous amount of viral antigen and RNA could be detected in the grey and white matter of the spinal cord. However, since only a small percentage (approximately 20%) of the infected nude mice survived up to 3 weeks p.i., a 2-week-p.i. time point was chosen for subsequent in-depth studies.

By immunohistochemistry, TMEV antigen was detected in the cytoplasm of infected neurons and glial cells from the brains of infected nude mice (Fig. 2a). Most of these cells were localized in and around the primary lesion in the brain, i.e., near the initial injection site in the cerebrum. Additionally, some distance away from the primary lesion, TMEV antigen was consistently found in pyramidal neurons in the hippocampus (Fig. 2b). TMEV antigen was also detected in the processes of these neurons (Fig. 2b). In these brain sections, TMEV antigens could not be demonstrated in cells around blood vessels or in endothelial cells. No mononuclear infiltrates were observed in the brain or in the spinal cord in these infected nude mice, although occasionally macrophagelike cells were found in areas of necrosis.

In the spinal cords of infected animals, only a few neurons and glial cells contained viral antigen, as determined by immunocytochemical methods. As found in the brain, no detectable TMEV antigens were observed around blood vessels or endothelial cells in the spinal cord. Similarly, no viral antigen was found in brain and spinal cord sections from control mice with EAE, although inflammatory cells and lesions were prominent features.

To demonstrate TMEV RNA, we performed in situ hybridization analysis on tissue sections with two different cDNA clones from opposite ends of the viral genome (26, 27). Both clones yielded similar hybridization results; however, the cDNA from the 5′ end of the BEAN strain consistently yielded less overall background signal under our conditions and therefore was used for most of these experiments.

In serial sections, the distribution of TMEV RNA determined by in situ hybridization was similar to that observed by immunocytochemistry for the detection of viral antigen. Most of the viral RNA was found in cells nearby or adjacent to the primary lesion in the brain (Fig. 3a). Neurons and their processes (Fig. 3b) and glial cells in the hippocampus contained viral RNA. As with the distribution of viral antigen, only a few neurons and glial cells in the spinal cord of infected mice contained viral RNA.

Interestingly, in a few vessels in all infected mice, a small but consistent number of endothelial cells or cells associated with these blood vessels contained large numbers of grains (Fig. 3c). These cells clearly contained TMEV RNA. Some of these positive blood vessels were localized within the primary lesion in the brain, but other vessels away from this area which contained no inflammatory cells and no observable pathological changes also contained TMEV RNA. In addition, a few cells lining spinal cord vessels contained TMEV RNA. No positive hybridization signal could be demonstrated in brain and spinal cord sections from mice with EAE (Fig. 3d) or mock-infected control mice. Thus, in contrast to the immunocytochemistry data, infection of cells associated with blood vessels could be readily demonstrated.

The presence of virus in the brains and spinal cords of nude mice was confirmed by a plaque assay at 1, 2, and 3 weeks p.i. (three to four mice per time point) (Table 1). At 1 week after inoculation, infectious virus titers averaged 812 PFU/mg in the brain and 75 PFU/mg in the spinal cord. This number diminished somewhat at 2 weeks p.i. to 460 PFU/mg of brain tissue and 65 PFU/mg of spinal cord tissue. By 3 weeks, the titers were 556 PFU/mg in brain tissue and 626 PFU/mg in spinal cord tissue.

For simultaneous detection of viral antigen and RNA, immunocytochemistry was combined with in situ hybridization. Clearly, both viral antigen and RNA could be demonstrated in the same cell. In general, viral antigen and RNA were localized. However, many cells were found negative for viral antigen by immunocytochemistry yet contained TMEV RNA. All endothelial cells and cells associated with blood vessels in the brain and spinal cord that contained large amounts of viral RNA were found negative for viral antigen by the dual detection system, thus confirming the results of our previous experiments.

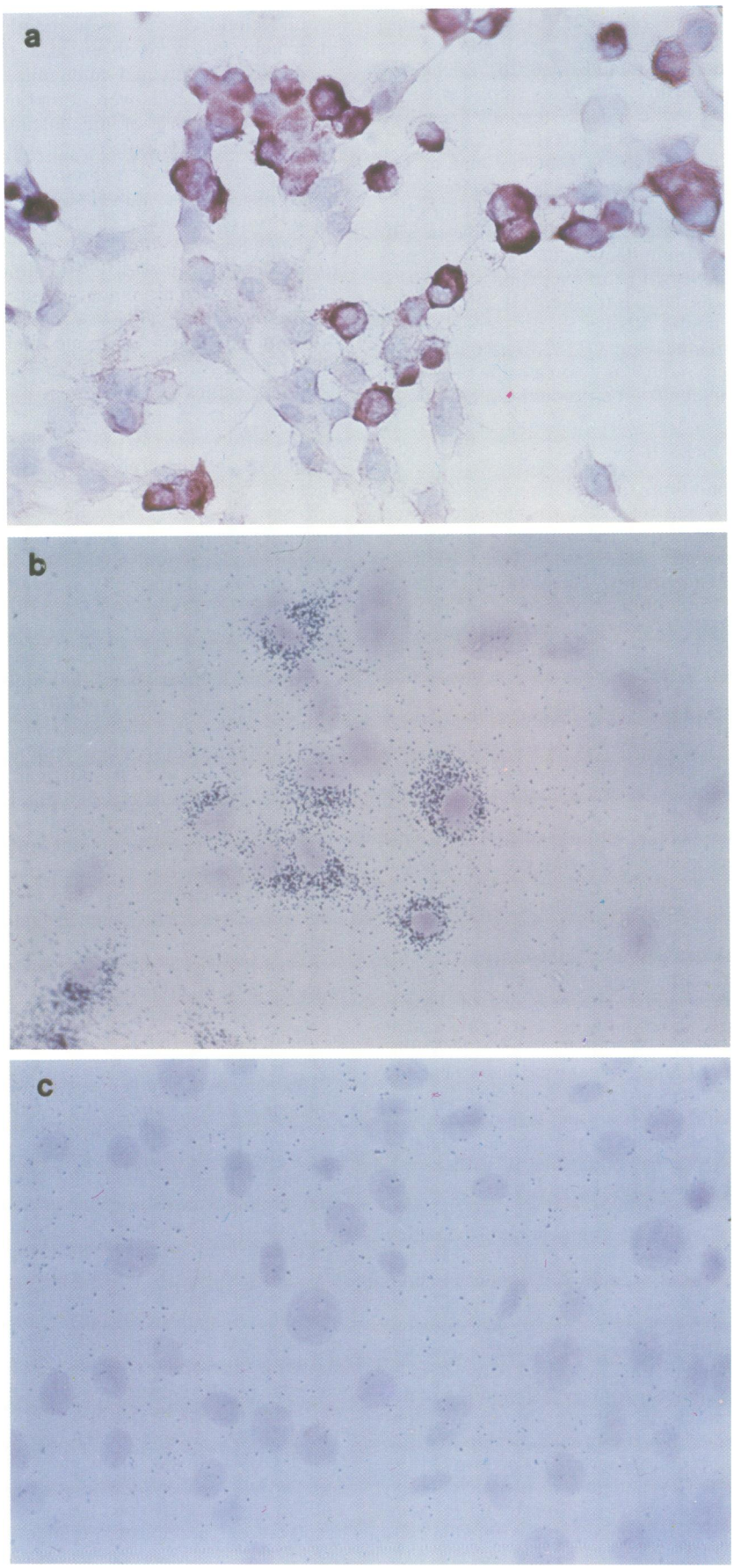


FIG. 1. (a) TMEV antigen in the cytoplasm of infected BHK-21 cells. Anti-DA avidin-peroxidase stain. (b) Cytoplasmic distribution of TMEV nucleic acid in infected BHK-21 cells demonstrated by in situ hybridization. (c) Mock-infected BHK-21 cells after in situ hybridization; only a few scattered grains were noted. Magnification for all three panels, ×215.

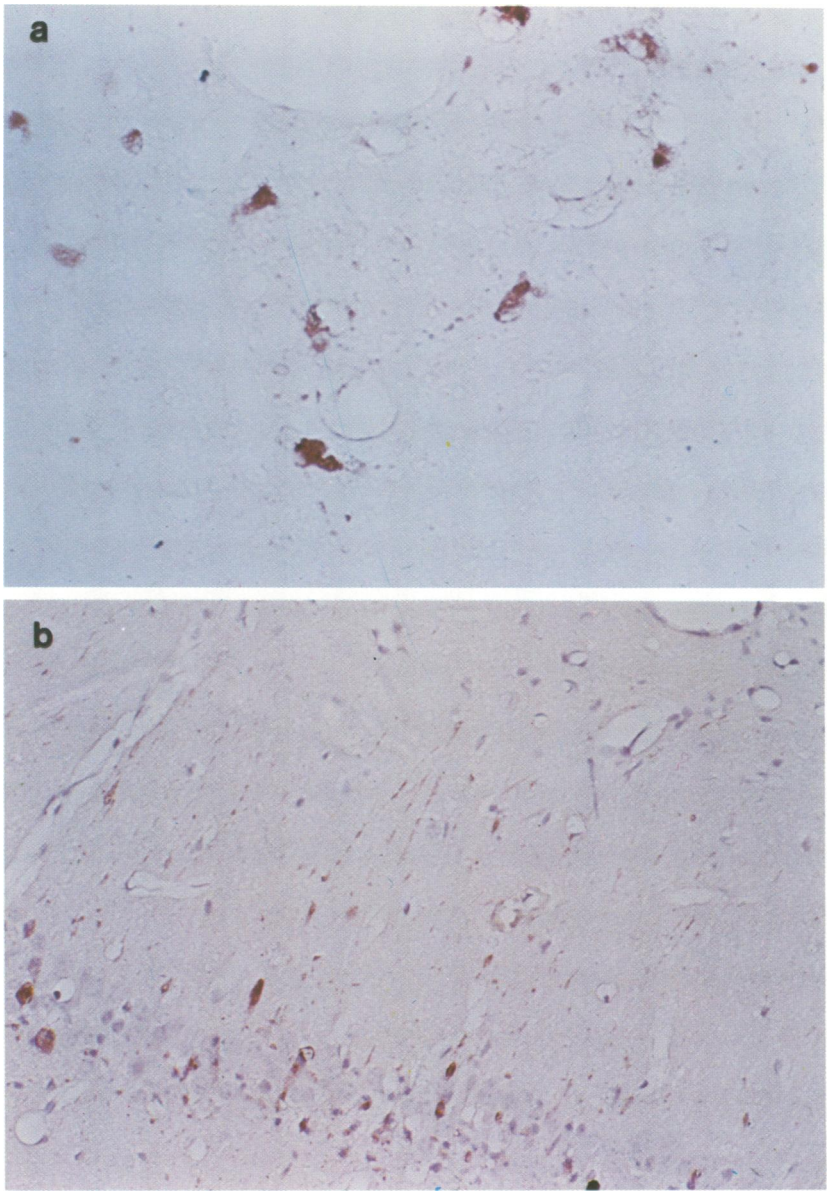


FIG. 2. (a) TMEV antigen in infected glial cells in the brain of a TMEV-infected mouse. Anti-DA avidin-peroxidase stain. Magnification, ×215. (b) TMEV antigen in pyramidal neurons and their processes in the hippocampus. Anti-DA avidin-peroxidase stain. Magnification, ×86.

## DISCUSSION

In the present study, TMEV antigen and RNA were detected in the CNSs of infected athymic (*nu/nu*) mice by immunocytochemistry and in situ hybridization. Most of the viral antigen and RNA were found in the cytoplasm of infected neuronal and glial cells in the brain. Some of these infected cells were found in association with large necrotic lesions. In addition, TMEV antigen and RNA were common in pyramidal cells in the hippocampus. The viral RNA as well as the antigen colocalized in the cytoplasm of the cell body and nerve processes. A few virally infected neurons and glial cells were found in the spinal cord. There appeared to be a gradient of infected cells from the initial site of injection in the brain to a small number of discrete cells in the spinal cord, suggesting dissemination from the brain. This idea was confirmed by a viral plaque assay in which the virus titer in the brain at the early time points was consistently higher than that in the spinal cord (Table 1).

During the acute stage of the disease in immunocompetent mice, viral RNA was found mostly in neurons, but during the chronic phase, viral RNA was primarily detected in the glial cells in the spinal cord (3). How TMEV spreads from the brain to the spinal cord is still not fully understood. Different investigators have demonstrated, using a variety of methods, that neurons and their dendrites and axons in the brain and spinal cord contain virus or viral antigens (11, 30, 37). Here we demonstrate the distribution of viral RNA in immunoincompetent mice. Dal Canto and Lipton (11), using ultrastructural immunohistochemical methods, found viral particles mainly in neurons during the acute stage of the disease. In an immunoperoxidase study by Rodriguez et al. (30), viral antigens were observed in dentrites and axons of the hippo-

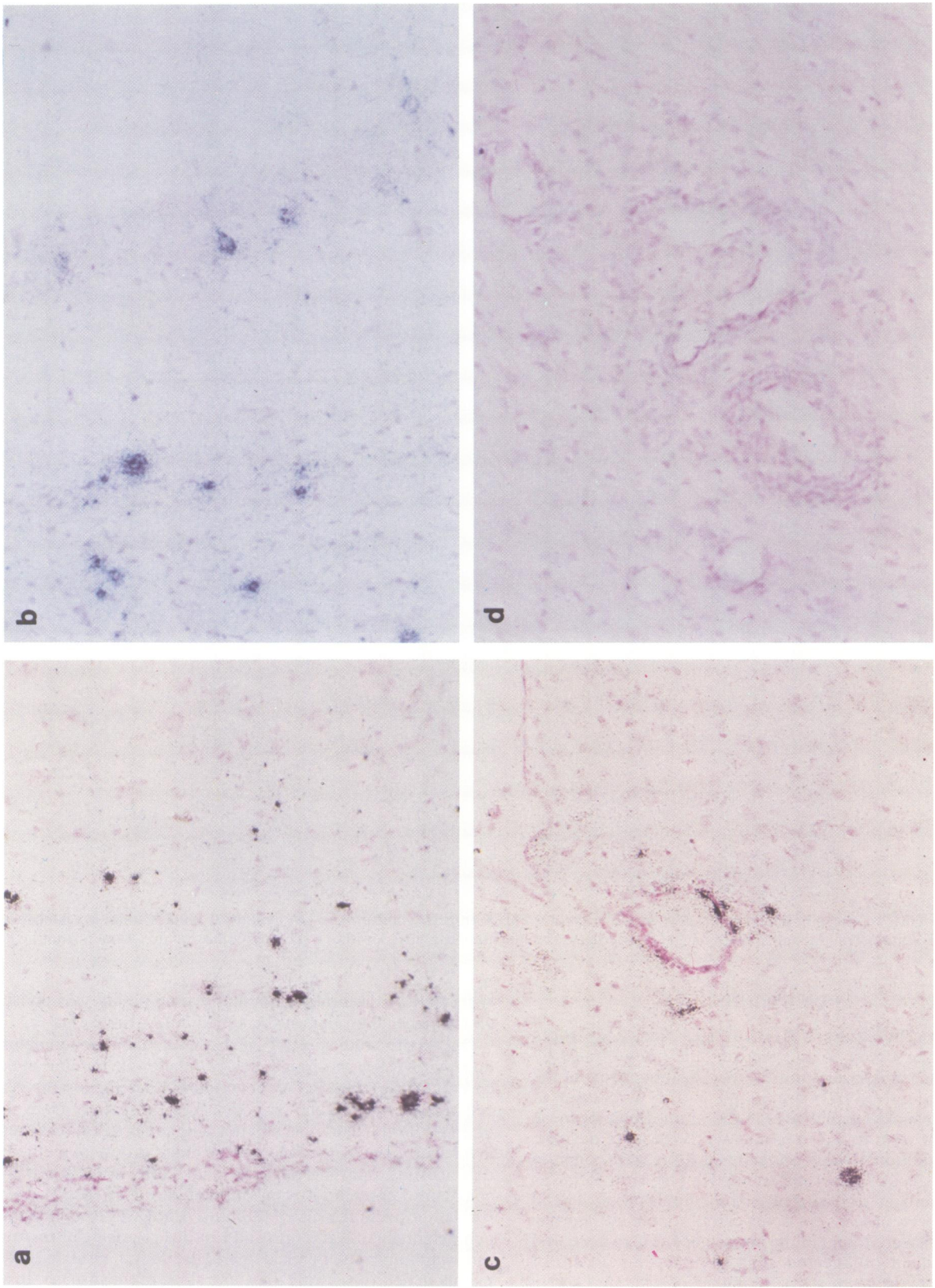


FIG. 3. (a) TMEV RNA demonstrated by in situ hybridization in the vicinity of the primary injection site in the brain of an infected mouse. (b) TMEV nucleic acid demonstrated by in situ hybridization in neurons and their processes in the hippocampus. (c) TMEV RNA demonstrated by in situ hybridization in vascular endothelial cells in the brain. (d) Lack of positive hybridization in an inflammatory lesion from a mouse with EAE after in situ hybridization. Magnification for all four panels, ×86.

TABLE 1. Quantitation of TMEV by a plaque assay[a]

| Wk p.i. | PFU/mg of: | |
|---|---|---|
| | Brain tissue | Spinal cord tissue |
| 1 | 50 | 0 |
| | 50 | 0 |
| | 550 | 0 |
| | 2,600 | 300 |
| 2 | 200 | 30 |
| | 220 | 30 |
| | 700 | 50 |
| | 700 | 150 |
| 3 | 350 | 300 |
| | 600 | 700 |
| | 720 | 880 |

[a] The assay was done after intracerebral inoculation of $3 \times 10^5$ PFU per mouse. Nude mice were euthanized at 1, 2, and 3 weeks p.i., and brains and spinal cords were removed, homogenized, and plaqued on BHK-21 cells as described in Materials and Methods. Each value represents data from a single animal.

campal pyramidal cells. Following in situ hybridization, viral RNA was detected in neurons and their processes (37). All these studies clearly demonstrate neuronal infection with TMEV and suggest spread of virus in the CNS via axoplasmic and dendritic flow.

In another system, type 2 HPV, also a picornavirus, spread through the mouse CNS after neuronal infection via axonal flow (14). HPV antigens were exclusively localized within neurons and their processes by ultrastructural immunohistochemical methods (8). In earlier studies HPV reached the CNS along peripheral nerves (1), suggesting axonal transport of HPV. Our results with nude mice are consistent with these findings.

Besides neuronal infection, our findings strongly suggest TMEV infection of vascular cells in the brain and spinal cord. Cells associated with blood vessels and endothelial cells within the lesions but distinct from any detectable pathological changes were infected, as demonstrated by the presence of TMEV RNA. No autoradiographic grains could be detected in vascular endothelial cells in the CNS from mice with EAE or mock-infected mice. Thus, TMEV was able to infect vascular endothelial cells and cells associated with blood vessels. This observation supports a previously unrecognized dissemination of TMEV within the CNS and a pathway for viral entrance into the CNS. This latter route may be one which the virus uses during natural infection (25). In monkeys HPV antigens were found to be present inside vascular endothelial cells by immunofluorescence staining [Kovacs et al., Letter, Nature (London) **200**:497–498, 1963]; however, in mice, presumably not the natural host, HPV antigens were not found (8).

In our study, endothelial cells contained viral RNA, yet viral antigen was not observed in these cells. This may be a question of sensitivity of the immunohistochemistry. It is known that viral nucleic acid can be detected earlier by in situ hybridization than viral antigens can be detected by immunohistochemistry (5). On the other hand, the synthesis of all major TMEV capsid proteins in the CNS has been reported to be restricted at the level of RNA replication (6). This observation is also supported by our dual-labeling results. In some cells both viral antigen and RNA could be demonstrated, but consistently more cells containing autoradiographic grains than immunocytochemistry-positive cells were found. Studies are ongoing to delineate this point.

Our results strongly suggest that viral RNA in endothelial cells could play a role in antigen presentation. Some investigators (23, 38) could not demonstrate major histocompatibility complex class II (Ia) antigen expression on glial cells, neurons, and endothelial cells in lesions from rats with EAE. However, during TMEV infection, the production of Ia antigen within glial cells and endothelial cells was reported to increase (31). Other viruses, including coronaviruses, have the ability to induce Ia antigen expression on cultured astrocytes (22). During TMEV infection, Ia expression on endothelial cells may be linked to the presence of viral RNA, which could stimulate antigen-presenting functions and explain, in part, Ia expression on the surfaces of endothelial cells after TMEV inoculation. The correlation between direct virus infection and major histocompatibility complex class II expression within the same cells still needs to be clarified. Dual-labeling experiments to verify the presence of virus within cells expressing Ia are required and are in progress.

## ACKNOWLEDGMENTS

We thank Peggy Farness and Susan McClanahan for excellent technical assistance, Diana Ferris for manuscript preparation, and Lindsay Whitton for helpful suggestions.

This work was supported by Public Health Service grant NS23162 from the National Institutes of Health and by National Multiple Sclerosis Society grants GR 1780, GR 2029 (to R.S.F.), and FG 755-1 (to A.Z.). A.Z. is a Fellow of the National Multiple Sclerosis Society and the Schweizerische Stiftung für medizinisch-biologische Stipendien.

## LITERATURE CITED

1. **Bodian, D., and H. A. Howe.** 1941. The rate of progression of poliomyelitis virus in nerves. Bull. Johns Hopkins Hosp. **69**:79–85.
2. **Brahic, M., A. T. Haase, and E. Cash.** 1984. Simultaneous *in situ* detection of viral RNA and antigens. Proc. Natl. Acad. Sci. USA **81**:5445–5448.
3. **Brahic, M., W. G. Stroop, and J. R. Baringer.** 1981. Theiler's virus persists in glial cells during demyelinating disease. Cell **26**: 123–128.
4. **Brown, A. M., and D. E. McFarlin.** 1981. Relapsing experimental allergic encephalomyelitis in the SJL/J mouse. Lab. Invest. **45**:278–284.
5. **Cash, E., M. Chamorro, and M. Brahic.** 1985. Theiler's virus RNA and protein synthesis in the central nervous system of demyelinating mice. Virology **144**:290–294.
6. **Cash, E., M. Chamorro, and M. Brahic.** 1986. Quantitation, with a new assay, of Theiler's virus capsid protein in the central nervous system of mice. J. Virol. **60**:558–563.
7. **Chamorro, M., C. Aubert, and M. Brahic.** 1986. Demyelinating lesions due to Theiler's virus are associated with ongoing central nervous system infection. J. Virol. **57**:992–997.
8. **Dal Canto, M. C., R. L. Barbano, and B. Jubelt.** 1986. Ultrastructural immunohistochemical localization of poliovirus during virulent infection of mice. J. Neuropathol. Exp. Neurol. **45**: 613–618.
9. **Dal Canto, M. C., and H. L. Lipton.** 1975. Primary demyelination in Theiler's virus infection: an ultrastructural study. Lab. Invest. **33**:626–637.
10. **Dal Canto, M. C., and H. L. Lipton.** 1977. Animal model of human disease: multiple sclerosis. Am. J. Pathol. **88**:497–500.
11. **Dal Canto, M. C., and H. L. Lipton.** 1982. Ultrastructural immunohistochemical localization of virus in acute and chronic demyelinating Theiler's virus infection. Am. J. Pathol. **106**:20–29.
12. **Daniels, J. B., A. M. Pappenheimer, and S. Richardson.** 1952. Observations on encephalomyelitis of mice (DA strain). J. Exp. Med. **96**:517–535.
13. **Feinberg, A. P., and B. Vogelstein.** 1983. A technique for

radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **132**:6–13.

14. **Jubelt, B., S. J. Goldfarb, M. J. Paradise, and M. G. Close.** 1986. Fast axonal transport of human poliovirus (HPV). Neurology **36**(Suppl. 1):204.
15. **Kindred, B.** 1979. Nude mice in immunology. Prog. Allergy **26**: 137–238.
16. **Lang, W., C. Wiley, and P. Lampert.** 1985. Theiler's virus encephalomyelitis is unaffected by treatment with myelin components. J. Neuroimmunol. **9**:109–113.
17. **Lehrich, J. R., B. G. W. Arnason, and F. Hochberg.** 1976. Demyelinating myelopathy in mice induced by the DA virus. J. Neurol. Sci. **29**:149–160.
18. **Lipton, H. L.** 1975. Theiler's virus infection in mice: an unusual biphasic disease process leading to demyelination. Infect. Immun. **11**:1147–1155.
19. **Lipton, H. L., and M. C. Dal Canto.** 1976. Theiler's virus-induced demyelination: prevention by immunosuppression. Science **192**:62–64.
20. **Lipton, H. L., J. Kratchovil, P. Sethi, and M. C. Dal Canto.** 1984. Theiler's virus antigen detected in mouse spinal cord 2 1/2 years after infection. Neurology **34**:1117–1119.
21. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
22. **Massa, P. T., and V. ter Meulen.** 1987. Analysis of Ia induction on Lewis rat astrocytes in vitro by virus particles and bacterial adjuvants. J. Neuroimmunol. **13**:259–271.
23. **Matsumoto, Y., and M. Fujiwara.** 1986. In situ detection of class I and II major histocompatibility complex antigens in the rat central nervous system during experimental allergic encephalomyelitis: an immunohistochemical study. J. Neuroimmunol. **12**:265–277.
24. **Olitsky, P. K.** 1945. Certain properties of Theiler's virus, especially in relation to its use as model for poliomyelitis. Proc. Soc. Exp. Biol. Med. **58**:77–81.
25. **Olitsky, P. K., and R. W. Schlesinger.** 1941. Histopathology of CNS of mice infected with virus of Theiler's disease (spontaneous encephalomyelitis). Proc. Soc. Exp. Biol. Med. **47**:79–83.
26. **Ozden, S., F. Tangy, M. Chamorro, and M. Brahic.** 1986. Theiler's virus genome is closely related to that of encephalomyocarditis virus, the prototype cardiovirus. J. Virol. **60**:1163–1165.
27. **Pevear, D. C., M. Calenoff, E. Rozhon, and H. L. Lipton.** 1987. Analysis of the complete nucleotide sequence of the picornavirus Theiler's murine encephalomyelitis virus indicates that it is closely related to cardioviruses. J. Virol. **61**:1507–1516.
28. **Rice, G. P. A., and R. S. Fujinami.** 1986. Measles virus, p. 370–383. *In* H. U. Bergmeyer (ed.), Methods of enzymatic analysis. VCH Verlagsgesellschaft, Weinheim, Federal Republic of Germany.
29. **Rodriguez, M., J. L. Leibowitz, and P. W. Lampert.** 1983. Persistent infection of oligodendrocytes in Theiler's virus-induced encephalomyelitis. Ann. Neurol. **13**:426–433.
30. **Rodriguez, M., J. L. Leibowitz, H. C. Powell, and P. W. Lampert.** 1983. Neonatal infection with the Daniels strain of Theiler's murine encephalomyelitis virus. Lab. Invest. **49**:672–679.
31. **Rodriguez, M., M. L. Pierce, and E. A. Howie.** 1987. Immune response gene products (Ia antigens) on glial and endothelial cells in virus-induced demyelination. J. Immunol. **138**:3438–3442.
32. **Roos, R. P., S. Firestone, R. Wollmann, D. Variakojis, and B. G. W. Arnason.** 1982. The effect of short-term and chronic immunosuppression of Theiler's virus demyelination. J. Neuroimmunol. **2**:223–234.
33. **Roos, R. P., and R. Wollmann.** 1984. DA strain of Theiler's murine encephalomyelitis virus induces demyelination in nude mice. Ann. Neurol. **15**:494–499.
34. **Rosenthal, A., R. S. Fujinami, and P. W. Lampert.** 1986. Mechanism of Theiler's virus-induced demyelination in nude mice. Lab. Invest. **54**:515–522.
35. **Rueckert, R. R., and M. A. Pallansch.** 1981. Preparation and characterization of encephalomyocarditis (EMC) virus. Methods Enzymol. **78**:315–325.
36. **Shivers, B. D., R. E. Harlan, D. W. Pfaff, and B. S. Schachter.** 1986. Combination of immunocytochemistry and in situ hybridization in the same tissue section of rat pituitary. J. Histochem. Cytochem. **34**:39–43.
37. **Stroop, W. G., J. R. Baringer, and M. Brahic.** 1981. Detection of Theiler's virus RNA in mouse central nervous system by *in situ* hybridization. Lab. Invest. **45**:504–509.
38. **Vass, K., H. Lassmann, H. Weckerle, and H. M. Wisniewski.** 1986. The distribution of Ia antigen in the lesions of rat acute experimental allergic encephalomyelitis. Acta Neuropathol. **70**: 149–160.
39. **Wiley, C. A., R. S. Schrier, J. A. Nelson, P. W. Lampert, and M. B. A. Oldstone.** 1986. Cellular localization of human immunodeficiency virus infection within the brains of acquired immune deficiency syndrome patients. Proc. Natl. Acad. Sci. USA **83**:7089–7093.