Proc. Natl. Acad. Sci. USA
Vol. 94, pp. 4659–4663, April 1997
Medical Sciences

A transgenic mouse model for measles virus infection of the brain

GLENN F. RALL*†‡, MARIANNE MANCHESTER†§, LIA R. DANIELS§, ERIC M. CALLAHAN*, ALEC R. BELMAN*, AND MICHAEL B. A. OLDSTONE§

*The Fox Chase Cancer Center, Division of Basic Science, 7701 Burholme Avenue, Philadelphia, PA 19111; and §The Scripps Research Institute, Division of Virology, Department of Neuropharmacology, 10550 North Torrey Pines Road, La Jolla, CA 92037

Communicated by Hilary Koprowski, Thomas Jefferson University, Philadelphia, PA, February 17, 1997 (received for review November 25, 1996)

ABSTRACT **In addition to the rash, fever, and upper respiratory tract congestion that are the hallmarks of acute measles virus (MV) infection, invasion of the central nervous system (CNS) can occur, establishing a persistent infection primarily in neurons. The recent identification of the human membrane glycoprotein, CD46, as the MV receptor allowed for the establishment of transgenic mice in which the CD46 gene was transcriptionally regulated by a neuron-specific promoter. Expression of the measles receptor rendered primary CD46-positive neurons permissive to infection with MV–Edmonston. Notably, viral transmission within these cultures occurred in the absence of extracellular virus, presumably via neuronal processes. No infection was seen in nontransgenic mice inoculated intracerebrally with MV–Edmonston. In contrast, scattered neurons were infected following inoculation of transgenic adults, and an impressive widespread neuronal infection was established in transgenic neonates. The neonatal infection resulted in severe CNS disease by 3–4 weeks after infection. Illness was characterized initially by awkward gait and a lack of mobility, and in later stages seizures leading to death. These results show that expression of the MV receptor on specific murine cells (neurons) *in vivo* is absolutely essential to confer both susceptibility to infection and neurologic disease by this human virus. The disparity in clinical findings between neonatal and adult transgenic mice indicates that differences exist between the developing and mature CNS with respect to MV infection and pathogenesis.**

Humans are the only natural host for measles virus (MV) infection, and the unavailability of small animals that are susceptible to wild-type isolates has limited our understanding of the pathogenesis of the acute infection and its potential complications. These complications include transient immunosuppression and rare but lethal infection of the central nervous system (CNS) (1, 2). The neurodegenerative disease, subacute sclerosing panencephalitis (SSPE) (3), occurs in approximately 1/100,000 cases of acute measles infection, and clinical signs appear months to years after the primary infection. The disease is characterized by progressive dementia, ataxia, and seizures leading to death (3). While the disease is irreversible and fatal for children, it has recently been reported that long-term MV infection of the CNS occurs in approximately 20% of healthy individuals following an acute infection, suggesting that MV persistence is more common than previously thought, and that chronic CNS infection does not irrevocably lead to SSPE (4).

Previously, rodents have been used as models for MV CNS infection, but only with neuroadapted strains of MV. These strains are achieved by repeated intracerebral passage in newborn animals (5–8). The resultant "rodent-adapted" strains are genetically distant from their wild-type parent (ref. 9; W. Bellini, personal communication) and the disease caused in the rodent CNS is primarily an acute encephalitis that causes death within 1 week of inoculation (5–8). Persistent infection with the rodent-adapted strains is uncommon.

The recent identification of the human MV receptor, CD46, has provided an alternative way to characterize MV CNS infection. CD46 is a complement regulatory protein that protects host tissue from complement deposition by binding to complement components C3b and C4b (10). Its expression on murine fibroblast and lymphoid cell lines renders these otherwise refractory cells permissive for MV infection (11–13), and the expression of CD46 on primate cells parallels the clinical tropism of MV infection in humans and nonhuman primates (10). We postulated that expression of CD46 in specific murine tissues *in vivo* would confer susceptibility to human MV strains. Our expectation was that neuronal infection of these genetically engineered mice with nonrodent-adapted virus strains would more closely resemble MV infection of humans and allow a pathogenetic dissection of the human disease, SSPE.

MATERIALS AND METHODS

Cloning Procedures and Establishment of Transgenic Mice. Standard molecular techniques (14, 15) were used for the construction of the neuron-specific enolase (NSE)–CD46 transgene. The neuron-specific expression vector, NSE–Ex4, was obtained from S. Forss-Petter and J. G. Sutcliffe (The Scripps Research Institute, La Jolla, CA) and has been used successfully to express other genes in CNS neurons (16, 17). The CD46 cDNA (BC1 isoform) was obtained from John Atkinson (Washington University School of Medicine, St. Louis). The TA cloning kit (Invitrogen) was used to clone a PCR amplified CD46 gene, with *Hin*dIII sites inserted at the 5′ and 3′ termini. The entire CD46 gene was sequenced after PCR amplification to ensure that no sequence errors were introduced during the linker addition. Additionally, promoter–transgene junctions created by the subcloning were sequenced prior to microinjection. Fertilized oocytes were obtained from C57BL/6 × C3H/HeJ hybrid female mice. Purification of the transgene, preparation of mice, microinjection into fertilized C57BL/6 × C3H/HeJ hybrid oocytes, and embryo implants were carried out as described (17, 18).

Transgenic mice were identified by tail biopsy and subsequent DNA isolation. DNA (10 μg) was transferred onto filters (Nytran; Schleicher & Schuell) and probed with the simian virus 40 sequence located at the 3′ end of the NSE expression cassette.

Reverse Transcriptase (RT)-PCR Detection of Transgene Message. Brains and other organs were removed from saline-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

0027-8424/97/944659-5$2.00/0
PNAS is available online at **http://www.pnas.org**.

Abbreviations: MV, measles virus; CNS, central nervous system; SSPE, subacute sclerosing panencephalitis; NSE, neuron-specific enolase; RT, reverse transcriptase; pfu, plaque-forming units.

†G.F.R. and M.M. contributed equally to this work.
‡To whom reprint requests should be addressed.

perfused mice and were snap frozen in liquid nitrogen. The tissues were stored at −70°C before homogenization. Tissues were homogenized using a Vertishear (VirTis) for 30 sec in 1 ml Tri-Reagent/100 mg tissue (Molecular Research Center, Cincinnati), and RNA was purified according to the recommended protocol. Total RNA (500 ng) was reverse transcribed by the random priming method, using random hexamers (Pharmacia) and RT (Life Technologies, Gaithersburg, MD). The RNA–cDNA duplex was then subjected to 40 rounds of PCR (60°C annealing temperature; *Taq*I polymerase from Life Technologies) using 20-mer oligonucleotide primers that amplify a 390 bp, CD46-specific product: A, 5′-GAGAT CGACT CTAGA GGATC-3′; B, 5′-CAGGC GTCAT CTGAG ACAGG-3′. PCR products were visualized by ethidium bromide intercalation on a 0.8% agarose gel. In some cases, the PCR products were subsequently Southern blotted and probed with a ^{32}P-labeled CD46 fragment to ensure specificity of the PCR amplification (data not shown).

Detection of Protein Expression by Fluorescence-Activated Cell Sorter (FACS) Analysis. Adult transgenic and nontransgenic mice were saline perfused, and brains were removed. The hippocampus was dissected from adult mice and placed in ice-cold PBS until dissociation. The tissues were gently dissociated using fire-polished pipettes until a single-cell suspension was obtained. The suspension was passed through a cell strainer (Falcon) to remove clumps, then incubated with the mouse anti-CD46 monoclonal antibody E4.3 (19). The cells were extensively washed and incubated with a fluorescein-tagged, anti-mouse secondary antibody (Vector Laboratories). All reactions were incubated on ice for 30 min. The cells were then analyzed using a Becton Dickinson FACS 4. Dead cells were excluded by the addition of 1 ng/ml propidium iodide to the samples prior to flow cytometry. Nontransgenic neurons and transgenic neurons in the absence of the primary antibody served as negative controls; murine MC57 cells transfected with the CD46 construct were used as positive controls for staining.

Primary Neuron Explant Cultures. Embryonic mice (day 16–17) were used for the preparation of dissociated hippocampal neuron cultures following the original protocol of Banker and Goslin (20) with minor modifications (17, 21). Briefly, hippocampi were dissected from embryos and placed in ice-cold plating medium (DMEM supplemented with 10% fetal bovine serum/10^4 units/ml penicillin/10 mg/ml streptomycin). The tissues were washed three times with cold PBS and digested with trypsin–EDTA (Sigma) at 37°C for 15 min. The trypsin was inactivated with plating medium, and the hippocampi were mechanically dissociated with fire-polished pipettes and passed through a cell strainer (Falcon) to obtain a single-cell suspension. The neurons were spun at 150 × *g* for 7 min onto a serum cushion, resuspended, counted, and plated onto poly-L-lysine coated (Sigma), round glass coverslips (Carolina Biological Supply) at a density of 500 cells per mm^2.

Four hours after the neurons were added to the coverslips, the slips were placed on top of a confluent primary astrocyte feeder layer (17), in serum-free neurobasal medium (Life Technologies) supplemented with growth factors (B27 additive; Life Technologies). The cocultures were maintained at 37°C in 5% CO_2.

Neuronal Infection and Virus Detection Assays. Forty-eight hours after culturing, neurons were infected with MV–Edmonston at a multiplicity of infection of 1 or 3 in an inoculum volume of 500 μl. After 1 hr of gentle rocking, the inoculum was removed, cells were washed in PBS, neurobasal medium was added back to the cultures, and the cultures were incubated until harvesting.

Infectious center assays and plaque assays were performed as described (17). Briefly, infected nontransgenic or transgenic neurons were harvested at various times after infection by gentle scraping from coverslips. The cells were counted (dead cells excluded by trypan blue) and serially diluted in cold PBS. The cells were then seeded onto a confluent layer of Vero cells, and allowed to incubate for 1 hr with gentle rocking. Thereafter, 4 ml of molten 0.5% agarose/1× Medium 199 was gently added and allowed to solidify. Six days later the cells were fixed with 4% formaldehyde and the remaining cells stained with 1% crystal violet. For plaque assays, 10-fold serial dilutions of supernatants were used to infect Vero cell monolayers, and subsequently processed as described above.

Neurons were also plated on Transwell tissue culture inserts (Costar) at a density of 500 cells/mm^2. These neurons were transferred into cultures that had been infected with MV–Edmonston 2 days previously. At various times, the neurons on the Transwells were fixed with 1:1 methanol:acetone and stained as described below.

Infection of Mice and Immunohistochemical Staining for MV. NSE–CD46 transgenic and nontransgenic mice, both adults (>4 weeks of age) and neonates (1–10 days postnatally) were inoculated intracerebrally with MV–Edmonston at 10^5 plaque-forming units (pfu) in a volume of 30 μl along the midline. All mice were observed daily and mice were sacrificed throughout the infection.

Frozen brain sections (10 μm) were prepared on a cryomicrotome and fixed in 50% acetone/50% ethanol for 10 min followed by 95% ethanol for 20 min. Tissues were blocked with normal goat serum and incubated with a human SSPE antiserum. Antibody detection was performed using an avidin–biotin–peroxidase detection kit (Vectastain Elite, Vector Laboratories). Slides were mounted with an aqueous medium and photographed using a Nikon microscope at ×100 power.

RESULTS

Establishment and Characterization of Transgenic Mice. Transgenic mice were generated that expressed human CD46 (BC1 isoform) under the transcriptional control of the NSE promoter, which restricts expression to CNS neurons in brains of transgenic mice (16, 17) (Fig. 1). The linearized, gel-purified NSE–CD46 transgene was microinjected into approximately 200 fertilized $(C57BL/6 \times C3H/HeJ)_{F2}$ one-cell stage embryos. From this microinjection, 56 mice were born, 4 of which contained the transgene as determined by tail biopsy analysis. Progeny mice derived from these founders used in the following experiments were backcrossed 3–4 generations to the C57BL/6 or C3H/HeJ backgrounds.

Tissue expression of the NSE–CD46 mRNA was analyzed by RT-PCR on total RNA isolated from at least four transgenic and three nontransgenic mouse tissues per line. The results showed that brain homogenates from lines 18 (data not shown) and 52 (Fig. 2) expressed CD46 mRNA, whereas nontransgenic littermates did not. The omission of the enzyme from the

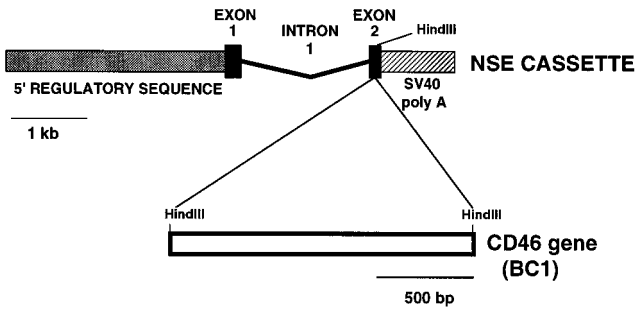

FIG. 1. Characterization of NSE–CD46 transgenic mice. The NSE–CD46 construct consists of 2.8 kb of 5′ NSE regulatory sequence, NSE exon 1 and intron 1, and 15 bp of NSE exon 2 adjacent to a unique *Hin*dIII site. Polyadenylylation signals are provided by the simian virus 40 sequence at the 3′ end of the construct, which also facilitates identification of transgenic mice by slot-blot analysis of genomic DNA.

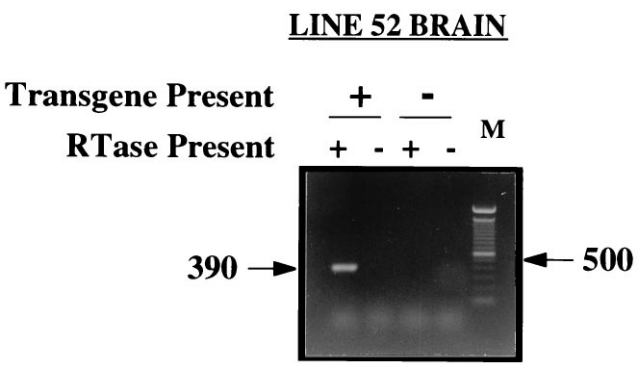

FIG. 2. Amplification of CD46 message in transgenic brains. Total RNA was isolated from four line 52 transgenic (+) and three nontransgenic (−) littermate brain homogenates, reverse transcribed and PCR amplified (5) using 20-mer oligonucleotides within the CD46 sequence. Samples were incubated with and without RT to verify amplification of a cDNA–RNA substrate. Representative results from one transgenic and one nontransgenic brain are shown.

RT reaction was done to ensure that a cDNA–RNA duplex was the template for PCR amplification. CD46 mRNA was not detected in other organs surveyed from any of the four line 52 mice, including liver, thymus, kidney, lung, heart, spleen, and testes, although a PCR product was consistently generated from line 18 thymus, lung, and spleen (data not shown).

To determine if the CD46 protein was expressed on the surface of mouse neurons, the CNS hippocampus was dissected from adult brain, and cells were dissociated and subjected to FACS analysis using fluorescent antibodies. The hippocampus is a neuron-dense structure; therefore, this procedure routinely yields preparations that are >90% neurons as determined by staining with a NSE antibody (G.F.R., unpublished data). The single cell populations from CD46-expressing mice were incubated sequentially with the anti-CD46 monoclonal antibody, E4.3 (19), and a fluorescein isothiocyanate-conjugated secondary, followed by FACS cytometry. All transgenic samples were compared with hippocampal suspensions from nontransgenic littermates. The histograms shown in Fig. 3 are representative of such experiments, which were verified twice with different homogenates. The data consistently showed moderate CD46 expression in line 18, compared with high expression in line 52 preparations. Line 21, which had no detectable expression of CD46 protein, is shown as a negative control. Based on the number of cells that were initially gated, we estimate that approximately 70% of cells in the line 52 hippocampal preparations express the CD46 protein.

MV Infection and Spread in Primary Neuron Cultures in the Absence of Extracellular Virus Production. We next established explant cultures of hippocampal neurons from transgenic and nontransgenic embryos to better evaluate the infectivity and spread of MV in primary neurons. The number of infected cells during the course of infection was measured both by determining the percent of neurons that were immunopositive and by establishing the number of neurons that could transmit infectious virus as determined by infectious center assays. The data are shown in Fig. 4. Only neurons from NSE–CD46 mice, but not from nontransgenic mice, could be infected with MV–Edmonston. Approximately 2% of the transgenic neurons were immunopositive 48 hr after infection, increasing to 22% by 144 hr. Fig. 5 *A* and *B* show representative photomicrographs of such infected neurons taken 48 and 96 hr after infection with 1×10^6 pfu of MV–Edmonston (multiplicity of infection = 3). Transmission of the virus was associated with neuron clusters, suggesting that the virus spreads in the cultures via neuronal processes. This was confirmed by (*i*) the absence of extracellular virus from infected cell supernatants, (*ii*) the re-isolation of the virus from individual neurons by infectious center assays, and (*iii*) expression of viral antigens in neuronal processes (Figs. 4 and 5 *A* and *B*). The amount of recovered virus paralleled the number of cells expressing viral antigen, indicating that infectious virus was produced in those cells that express viral proteins, despite the absence of extracellular viral particles in the medium.

To confirm that low levels of extracellular virus do not account for the spread of MV within our cultures, we evaluated whether MV could be transmitted between neurons separated by a permeable membrane. Under these conditions, CD46-expressing neurons could not be infected when exposed to supernatant produced by the physically separate, MV-infected monolayer (data not shown). In contrast, neuronal infection was easily detected upon exposure to supernatants of physically separate neurons infected with lymphocytic choriomeningitis virus, which produces extracellular virus from neurons (G.F.R., unpublished observations). These results indicate that cell–cell contact is required for the spread of MV in CD46 transgenic neurons and that transmission is independent of the production of extracellular virions.

MV Infects Mouse Transgenic Neurons *in Vivo*, Spreads Within the CNS, and Results in Neurologic Disease. To determine whether expression of CD46 made neurons permissive for MV infection *in vivo*, NSE–CD46 mice were inoculated intracerebrally with 1×10^5 pfu of MV–Edmonston. Immunohistochemical staining of brain sections using human SSPE serum revealed scattered infection of

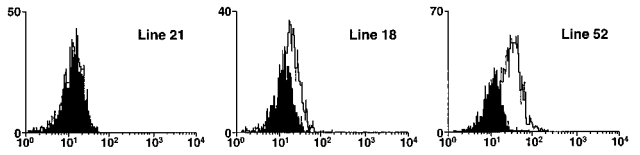

FIG. 3. Cell surface expression of CD46 on dissociated, transgenic neurons. Hippocampi from NSE–CD46 transgenic mouse lines 21, 18, and 52 were removed, dissociated, and incubated with the anti-CD46 monoclonal antibody E4.3 (19). The solid areas in each panel represent neurons from nontransgenic littermate controls stained using the identical procedure. Each panel depicts fluorescence intensity (*x* axis) vs. cell number (*y* axis). These results were subsequently confirmed with two additional hippocampal preparations.

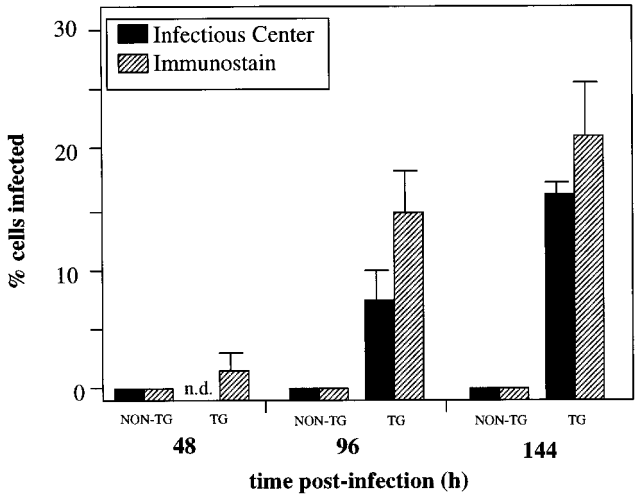

FIG. 4. Detection of MV in primary neuron cultures. Primary neurons cultured from transgenic (TG) (line 52) and nontransgenic (NON-TG) embryonic hippocampi were infected with MV–Edmonston at a multiplicity of infection = 3, 48 hr after culturing. At various times after infection, neurons were harvested for infectious center analysis or immunostained with SSPE serum. Supernatants were also collected for plaque assay. For the immunostaining, 10 fields per time point were counted, and standard deviations are shown.

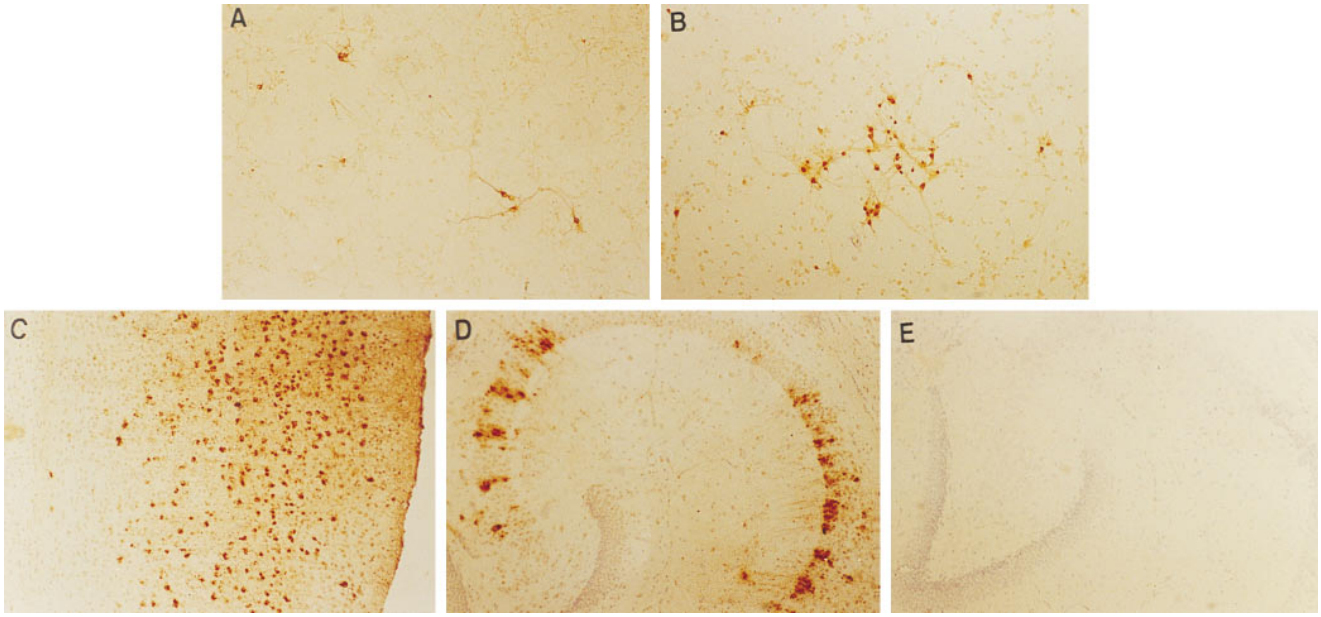

FIG. 5. Spread of MV in primary neurons and *in vivo*. (*A*) CD46+ primary neurons, infected for 48 hr with MV–Edmonston. (*B*) CD46+ primary neurons 96 hr after infection. Coverslips were fixed with 1:1 acetone:methanol and immunostained with a human SSPE serum as described. (*C–E*) NSE–CD46 neonatal (<24 postnatal) transgenic and nontransgenic mice were inoculated intracranially with 10^5 pfu of MV–Edmonston and brain sections were immunostained for MV as described. Immunohistochemical staining for MV antigens in the cortical region (*C*) and in the hippocampus (*D*) of a representative, NSE–CD46 transgenic mouse infected with MV 10 days previously (×100). (*E*) Hippocampus of a nontransgenic mouse, inoculated 10 days previously (×100).

neurons in transgenic mice infected as adults (data not shown), compared with widespread infection in transgenic mice infected within 48 hr of birth (Fig. 5 *C* and *D*). In contrast, no staining for MV antigens in nontransgenic brains infected at any age was detected (Fig. 5*E*). In all infected transgenic mice studied (six line 52 transgenic mice, greater than 5 weeks of age), MV antigen was largely restricted to the cytoplasm and neuronal processes. In all neonates infected with MV (four litters infected within 24 hr of birth), a clinical illness appeared within 15–20 days after infection, characterized by a ruffled appearance, lack of mobility, awkward gait, and tremors. This condition rapidly progressed to frequent seizures and paralysis. Death occurred usually within 3 days of the onset of symptoms. Immunohistochemical analysis of brains taken throughout infection indicate a progressive spread of virus throughout the CNS. Attempts to re-isolate MV from infected brain homogenates by plaque assay were not successful; similar difficulties in isolating infectious virus from brain tissue have been observed with SSPE (22–24). MV replication in neurons was observed in NSE–CD46 transgenic mice on both C57BL/6 and C3H/HeJ backgrounds, and we have recently replicated the time course of sickness and death using as few as 10^3 pfu.

DISCUSSION

Our data indicate that expression of CD46 confers MV tropism to mouse neurons *in vivo*. Infection of neurons is productive, since the infection rapidly disseminates throughout the CNS, but transmission of the virus does not occur via an extracellular route. This finding is consistent with the well-established clinical observation that infectious virus cannot be directly isolated from SSPE brain tissues, despite a widespread MV infection. The absence of complete viral particles may be due to the fact that viral genomes isolated from the CNS of autopsied SSPE patients often reveal extensive mutations within the envelope-associated genes, including the matrix, hemagglutinin, and fusion genes (3). Such mutations may prohibit the formation of enveloped virions and likely explains the absence of extracellular virus in brain homogenates. Experiments are currently underway to determine the basis for these mutations and to test the hypothesis that the virus is transmitted trans-synaptically, obviating the need for the production of complete viral particles.

Our findings also describe, to the best of our knowledge, the first evidence that expression of human CD46 in transgenic mice confers susceptibility to MV-induced disease. Previous studies using the mouse hydroxymethylglutaryl coenzyme A reductase promoter to drive widespread expression of CD46 in transgenic mice did not result in *in vivo* infection or disease, although cells explanted from those transgenic mice could support MV replication (25). In our study, the appearance of disease in infected neonates but not adults suggests that differences between the developing and mature CNS might influence the susceptibility of a neuron to infection, and therefore be an important determinant of the outcome of infection. This age dependence of viral expression has been previously noted with two rodent-adapted MV strains (8). While both the mouse-adapted MAEd strain and the hamster neurotropic strain HNT induced 100% mortality in suckling mice, the lethality was reduced to 20–30% in weanling mice. The authors concluded that the maturation of CNS cells converted the virulent infection to a primarily defective infection. Our results support this finding, but are even more dramatic: in our system, no transgenic mouse more than 10 days of age developed CNS disease. The "age-dependent" hypothesis is further supported by clinical findings, since SSPE occurs in children who were acutely infected when very young and not in acutely infected adults. Furthermore, in preliminary experiments, the MV–Chicago isolate was also able to infect NSE–CD46-expressing neurons *in vivo*. These results with MV–Edmonston and MV–Chicago suggest the feasibility of using the CD46 receptor with other promoters to express MV in the respiratory tract and immune system to create mouse models that may mimic the human acute disease to study the pathogenesis of, and immune response to, measles infection.

Finally, persistent infection of the adult CNS is evidently more common than previously thought, since approximately 20% of the adults who experienced a normal MV infection and

died of nonviral causes were positive for MV antigens (4). This raises the possibility that a persistent measles infection of the CNS may be associated with currently poorly understood CNS disorders. The NSE–CD46 model should be of value in dissecting the events leading to neuronal susceptibility to infection, neuron–measles interactions, and the pathogenesis of MV infection within the brain.

We thank M. Horwitz, J. Price, and E. Katari for assistance in the establishment of transgenic mice, and I. Schulman and C. Evans for helpful discussions. This work was supported by National Institutes of Health Grants AI39466 and MH19185 (M.B.A.O.) and by fellowships from the Leiper Trust (G.F.R.) and the World Health Organization (M.M.).

1. Norrby, E. & Oxman, M. (1990) in *Virology,* eds. Fields, B. N. & Knipe, D. M. (Raven, New York), pp. 1013–1045.
2. Griffin, D. E. & Bellini, W. J. (1996) in *Virology*, eds. Fields, B. N., Knipe, D. M. & Howley, P. M. (Lippincott–Raven, Philadelphia), pp. 1267–1312.
3. ter Meulen, V., Stephenson, J. R. & Kreth, H. W. (1983) *Compr. Virol.* **8,** 105–159.
4. Katayama, Y., Hotta, H., Nishimura, A., Tatsuno, Y. & Homma, M. (1995) *J. Gen. Virol.* **76,** 3201–3204.
5. Liebert, U. G. & Finke, D. (1995) *Curr. Top. Microbiol. Immunol.* **191,** 149–166.
6. Inagawa, D. & Adams, J. (1958) *Proc. Soc. Exp. Biol. Med.* **98,** 567–569.
7. Albrecht, P. & Schumacher, H. (1971) *J. Infect. Dis.* **124,** 86–93.
8. Griffin, D., Mullinix, J., Narayan, O. & Johnson, R. T. (1974) *Infect. Immun.* **9,** 690–695.
9. Rima, B. K., Earle, J. A. P., Backzo, K., Rota, P. A. & Bellini, W. J. (1995) *Curr. Top. Microbiol. Immunol.* **191,** 65–84.
10. Liszewski, M. K., Post, T. W. & Atkinson, J. P. (1991) *Annu. Rev. Immunol.* **9,** 431–455.
11. Naniche, D., Varior-Krishnan, G., Cervoni, F., Wild, T. F., Rossi, B., Rabourdin-Combe, C. & Gerlier, D. (1993) *J. Virol.* **67,** 6025–6032.
12. Manchester, M., Liszewski, M. K., Atkinson, J. P. & Oldstone, M. B. A. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 2161–2165.
13. Dorig, R. E., Marcil, A., Chopra, A. & Richardson, C. (1993) *Cell* **75,** 295–305.
14. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY).
15. Ausubel, F. M., Brent, R. & Kingston, R. E. (1987) *Current Protocols in Molecular Biology* (Greene/Wiley Interscience, New York).
16. Forss-Petter, S., Danielson, P. E., Catsicas, S., Battenberg, E., Price, J., Nerenberg, M. & Sutcliffe, J. G. (1987) *Neuron* **5,** 187–197.
17. Rall, G., Mucke, L. & Oldstone, M. B. A. (1995) *J. Exp. Med.* **182,** 1201–1212.
18. Hogan, B., Costantini, F. & Lacy, E. (1986) *Manipulating the Mouse Genome* (Cold Spring Harbor Lab. Press, Plainview, NY).
19. Cho, S.-W., Oglesby, T. J., Hsi, B.-L., Adams, E. M. & Atkinson, J. P. (1991) *Clin. Exp. Immunol.* **83,** 257–261.
20. Banker, G. & Goslin, K. (1991) in *Culturing Nerve Cells*, eds. Banker, G. & Goslin, K. (MIT Press, Cambridge, MA), pp. 251–281.
21. Pasick, J. M. M., Kalicharran, K. & Dales, S. J. (1994) *J. Virol.* **68,** 2915–2928.
22. Baublis, J. V. & Payne, F. E. (1968) *Proc. Soc. Exp. Biol. Med.* **129,** 593–597.
23. Katz, M., Oyanagi, S. & Koprowski, H. (1969) *Nature (London)* **222,** 888–890.
24. Payne, F. E., Baublis, J. V. & Itabashi, H. H. (1969) *N. Engl. J. Med.* **281,** 585–589.
25. Horvat, B., Rivailler, P., Varior-Krishnan, G., Cardoso, A., Gerlier, D. & Rabourdin-Combe, C. (1996) *J. Virol.* **70,** 6673–6681.