Cell, Vol. 98, 629–640, September 3, 1999, Copyright ©1999 by Cell Press

Measles Virus Infection in a Transgenic Model: Virus-Induced Immunosuppression and Central Nervous System Disease

Michael B. A. Oldstone,*‡ Hanna Lewicki,* Diane Thomas,* Antoinette Tishon,* Samuel Dales,† John Patterson,* Mari Manchester,* Dirk Homann,* Denise Naniche,* and Andreas Holz*

*Division of Virology
Department of Neuropharmacology
The Scripps Research Institute
10550 North Torrey Pines Road
La Jolla, California 92037

†Department of Cell Biology
Rockefeller University
New York, New York 10024

Summary

Measles virus (MV) infects 40 million persons and kills one million per year primarily by suppressing the immune system and afflicting the central nervous system (CNS). The lack of a suitable small animal model has impeded progress of understanding how MV causes disease and the development of novel therapies and improved vaccines. We tested a transgenic mouse line in which expression of the MV receptor CD46 closely mimicked the location and amount of CD46 found in humans. Virus replicated in and was recovered from these animals' immune systems and was associated with suppression of humoral and cellular immune responses. Infectious virus was recovered from the CNS, replicated primarily in neurons, and spread to distal sites presumably by fast axonal transport. Thus, a small animal model is available for analysis of MV pathogenesis.

Introduction

Measles virus (MV) is among the most contagious agents of infection in humans (Griffin and Bellini, 1996; Oldstone, 1998). Despite the development and use of an excellent vaccine, over 40 million persons become infected, and over one million die each year (Markowitz and Katz, 1994; Griffin and Bellini, 1996; Oldstone, 1998). MV typically infects both the immune system and central nervous system (CNS) (McChesney and Oldstone, 1989; Markowitz and Katz, 1994; Griffin and Bellini, 1996). MV causes a profound suppression of the immune system (McChesney and Oldstone, 1989; Markowitz and Katz, 1994; Griffin and Bellini, 1996), which, when in association with secondary infections, is the major cause for the high morbidity and mortality. In addition, the virus can produce encephalomyelitis during its acute phase of infection and uncommonly cause postinfectious hyperallergic encephalomyelitis, subacute encephalitis, and persistent infection of neurons, a disease called subacute sclerosing panencephalitis (SSPE) (ter Meulen et al., 1983; Griffin and Bellini, 1996). Despite much work in this area, precisely how MV enters cells, disorders the immune system, and spreads in the CNS to cause disease is largely unclear. Without a suitable small animal model in which one can dissect and define these phenomena at the molecular level, such questions remain unanswered.

With the recent knowledge that human CD46 molecule and any one of its four major isoforms serve as a receptor for MV (Dorig et al., 1993; Naniche et al., 1993; Manchester et al., 1994), attention has been directed to generating transgenic mice with this component (Horvat et al., 1997; Rall et al., 1997; Thorley et al., 1997; Blixenkrone-Moller et al., 1998; Mrkic et al., 1998). Rodents lack the CD46 molecule and are generally nonpermissive to MV infection (Dorig et al., 1993; Naniche et al., 1993; Manchester et al., 1994; Horvat et al., 1997; Rall et al., 1997; Thorley et al., 1997; Mrkic et al., 1998) unless the virus undergoes multiple blind passages through mouse brain cells (Liebert and Finke, 1995). Such rodent-passaged MVs are phylogenetically distant from other MV isolates and are tropic only for the CNS (Liebert and Finke, 1995; Rima et al., 1995). However, insertion and expression of CD46 in rodent cells convert them from a state of resistence to permissivity for MV infection after a single passage (Dorig et al., 1993; Naniche et al., 1993; Manchester et al., 1994). Unfortunately the CD46 transgenic models generated so far have been limited either by negligible expression in one of the two sites needed for study of MV pathogenesis, the CNS and immune system (Rall et al., 1997; Blixenkrone-Moller et al., 1998), as well as a failure to generate a substantial MV infection in vivo (Horvat et al., 1997; Thorley et al., 1997; Mrkic et al., 1998) or specificity for MV (Blixenkrone-Moller et al., 1998).

We sought to utilize a CD46 transgenic mouse model without these limitations. A yeast artificial chromosome (YAC) library was used to obtain a full-length CD46 genomic clone; the YAC flanking sequence was removed, after which the entire CD46 gene was microinjected into fertilized mouse eggs (Yannoutsos et al., 1996). The resulting transgenic mice expressed all four major isoforms of CD46: BC1, BC2, C1, and C2. We noted that the locations and amounts of CD46 mimicked that in humans (Liszewski et al., 1991; Russell et al., 1992), and overall expression was greater than previously reported for the other transgenic models (Horvat et al., 1997; Rall et al., 1997; Thorley et al., 1997; Blixenkrone-Moller et al., 1998; Mrkic et al., 1998).

Following intracerebral (i.c.) inoculation of newborn mice and intraperitoneal (i.p.) or intraveneous (i.v.) inoculation of adult mice, MV replicated in cells of the immune system and CNS and infectious virus were recoverable from both by cocultivation on Vero cells. Virus was found primarily in T-enriched areas of the spleen and circulating PBL, and CD8, CD4 T cells, B cells, and macrophages were able to release virus. Further, the immune system's function was suppressed. In the CNS, MV replicated primarily in neurons, then spread rapidly throughout the nervous system. T lymphocyte

‡To whom correspondence should be addressed (e-mail: mbaobo@scripps.edu).

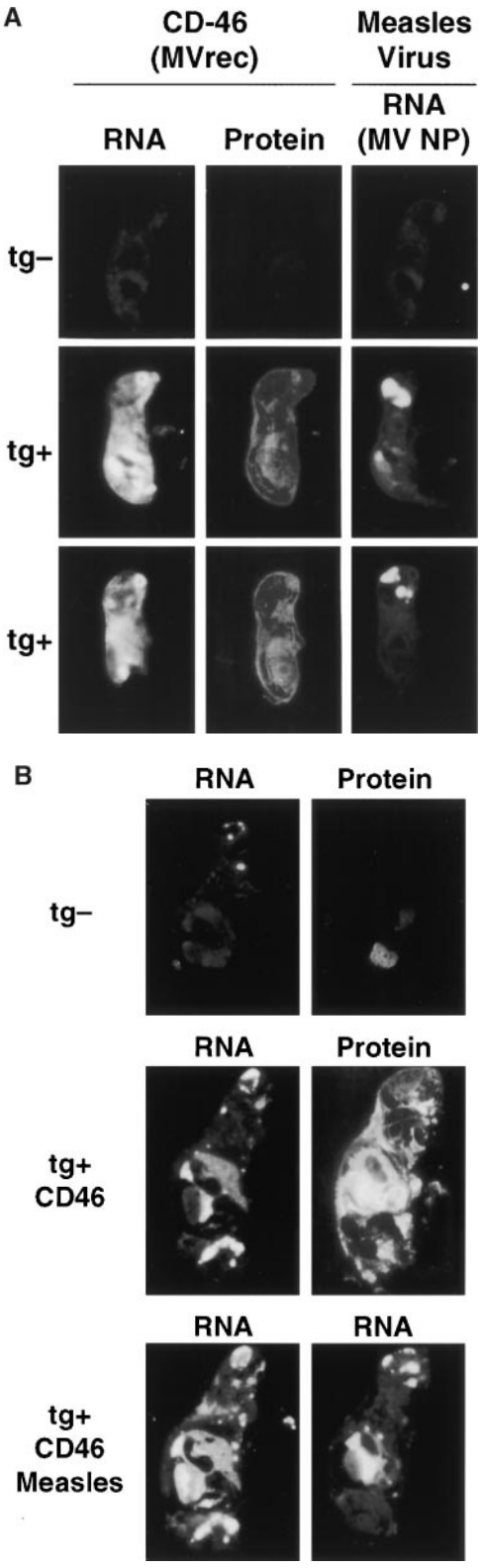


Figure 1. YAC-CD46 Mice Express CD46 Molecules in Multiple Tissues

Thirty micron whole-animal sections were made from (A) 10-day-old and (B) 65-day-old nontransgenic (tg^-) and YAC-CD46 transgenic (tg^+) mouse. CD46 RNA was detected using a ^{32}P-labeled riboprobe and CD46 protein with an antibody to CD46 and ^{125}I-labeled Staph A (see Experimental Procedures). MV (10^3 pfu) was given i.c. to newborn mice (A), and i.v. or i.p. to 65-day-old adult mice. Since results from i.p. and i.v. inoculations were similar, only the latter data are shown. Whole body in situ assay of control (normal, nontransgenic) mice showed no evidence of CD46 transcripts or protein, both of which are abundantly expressed throughout YAC-CD46 tg^+ mice. This profile for expression or absence of expression for CD46 transcripts and protein was identical for an additional two nontransgenic and three YAC-CD46 mice. Background ^{32}P in control mice represents binding of probe to hydroxylapatite in bone (Oldstone et al., 1986; Lipkin et al., 1989).

infiltration and apoptosis of neurons were associated with death usually by 21 days after infection. As such, this model reproduces multiple aspects of MV infection in humans.

Results

Generation of Transgenic Mice and Expression of CD46 RNA and Protein

CD46-expressing transgenic mice (Yannoutsos et al., 1996) YAC CD46 line 2 (YAC-CD46) contained 10 to 12 genome copies of CD46 and expressed all four of its major isoforms (Liszewski et al., 1991; Yannoutsos et al., 1996). After documenting integration and perpetuation of the CD46 gene for two generations, we determined the expression of CD46 RNA and protein of these mice using in situ hybridization within whole-animal sections and in Northern blots. Expression of CD46 transcripts and proteins detected within whole-animal sections (Figures 1A and 1B) was ubiquitous in YAC-CD46 transgenic mice but absent in the nontransgenic mice (6 of 6 mice studied in each group). When 4 μg RNA extracted separately from brain, spleen, lymph nodes, thymus, kidney, liver, gut, and lung was blotted on nitrocellulose and hybridized with a ^{32}P-labeled CD46 cDNA probe, Northern blot analysis revealed that these tissues from transgenic mice expressed the 4.5 to 4.8 kb CD46 transcript. By contrast, the same amount of RNA from nontransgenic mice did not contain any CD46 transcripts (3 of 3 mice studied in each group). In another transgenic line, neuron-specific enolase (NSE)-CD46, CD46 RNA was restricted to the brain (Rall et al., 1997), but we could not detect any CD46 transcripts or proteins in whole-animal sections of these mice. Even in brains of four NSE-CD46 transgenic mice, no ^{32}P-labeled CD46 was evident, although by RT–PCR CD46 was readily detected (data not shown; Rall et al., 1997), implying that the endogenous CD46 promoter more efficiently expressed CD46 than the NSE promoter. To compare the CD46 protein expression in brains of these two distinct transgenic lines, we quantitated CD46 protein on the surface of hippocampal neurons derived from either YAC-CD46 mice or NSE-CD46 mice. Neurons from YAC-CD46 mice contained at least 10-fold more CD46 molecules than neurons from NSE-CD46 mice. Data from two additional experiments showed that the concentration of CD46 in YAC-CD46 mice was 10- to 20-fold higher than in NSE-CD46 mice. Therefore, YAC-CD46 mice were used for the remainder of these studies.

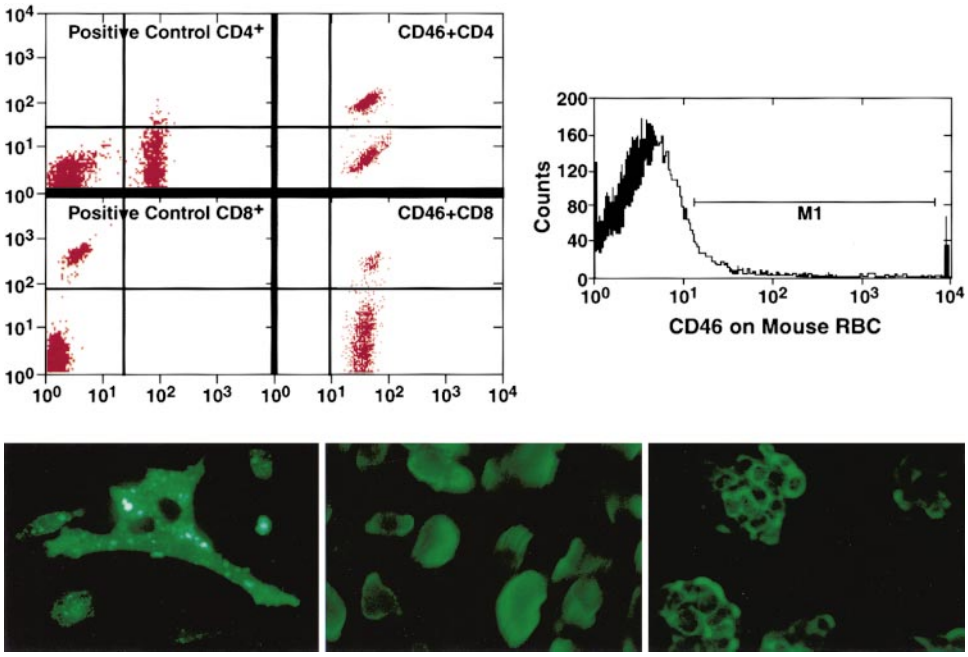


Figure 2. Expression of CD46 Molecules and Infectibility of Cells Harvested from YAC-CD46 Transgenic Mice

Upper left panel shows expression of CD46 protein on CD4 and CD8 lymphocytes obtained and sorted by FACS. Cells were stained with antibody to CD4 and cy-chromeE dye, antibody to CD8 and allophycocyanin, and antibody to CD46 conjugated to FITC. Antibodies to B cells and phycoerythrin were used to remove B cells. Positive controls appear as shown. Upper right panel shows CD46 on YAC-CD46 red blood cells (clear area) compared to their non-YAC-CD46 transgenic counterparts (shaded area). Lower panel shows expression of MV proteins in peritoneal macrophages (left), kidney cells (center), and skin fibroblasts (right) obtained from CD46 transgenic mice and infected in vitro with MV at MOI of 3. FITC-labeled antibody specific for MV identifies MV antigens. Non-MV-infected cells did not stain with these reagents. Viral progeny were found in supernatants after plaquing on permissive Vero cells. Titers of MV rarely exceeded 3 logs of virus/ml. Corresponding cells from tissues of nontransgenic mice were not infectible by MV. Monoclonal antibody E4.3 was used to stain CD46 on surfaces of cells and polyclonal antibody specific for MV to detect MV antigens (see Experimental Procedures).

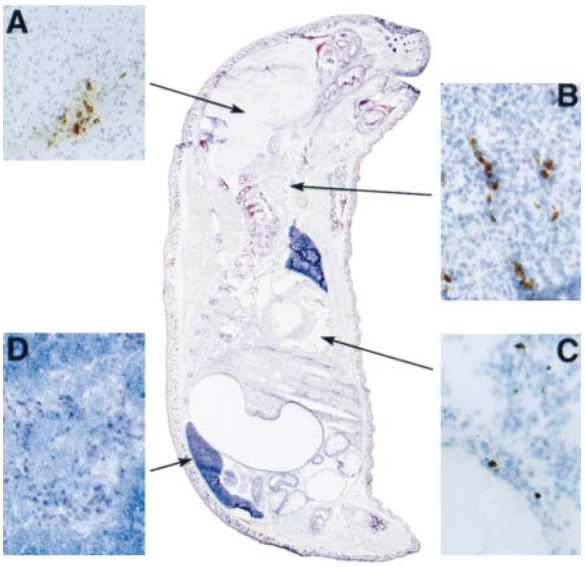
Multiple Cell Types from YAC-CD46 Mice Express CD46 Proteins and Are Infectible In Vitro by MV

Cells from the kidneys, skin, neurons, macrophages, and dendritic cells, as well as B lymphocytes, $CD8^+$, and $CD4^+$ T lymphocytes of YAC-CD46 mice expressed CD46 molecules on their surfaces (5 of 5 mice studied). These cells became infected with MV, expressed MV antigens, and released infectious viral progeny when cocultivated with permissive Vero cells (Figure 2). Cultured skin and kidney cells, macrophages, as well as lymphocytes that had been activated by mitogens or placed in mixed lymphocyte cultures produced low titers of infectious virus (10^2 to 10^3 pfu/ml) that were recoverable from the supernatant fluids. However, no released virus was detected in supernatants from cultured neurons, although infectious virus was formed upon cocultivation with Vero cells. Interestingly, and different from CD46 expression in humans (Liszewski et al., 1991), red blood cells from YAC-CD46 mice also expressed CD46 molecules on their surfaces as detected in a hemagglutinin assay and by reaction with anti-CD46 monoclonal antibodies (Figure 2).

MV Infects Neurons and Lymphoid Cells in the Blood, Spleen, and Lymph Nodes of YAC-CD46 Mice

After 10^3 pfu of MV was injected i.c. into neonates, and i.v. or i.p. into adult YAC-CD46 mice, MV RNA and protein were expressed in the CNS (Figures 1 and 3A); in cervical, mesenteric, and peribronchial lymph nodes; in the spleen (Figures 3B–3D); in Peyer's patches; and in peribronchial endothelial cells (5 of 5 mice studied). In spleens and lymph nodes, MV was expressed predominantly in T cell–enriched areas (Figure 3D).

We utilized double-labeling procedures to identify unique cells in the spleen and brain that expressed MV RNA and/or antigens (Figure 4) and cocultivation techniques to isolate infectious virus that was then quantitated (Figures 3 and 4). MV RNA was detected with a digoxigenin riboprobe and MV proteins by immunocytochemistry or Western blots using a human antibody to MV. To mark individual splenic cells, we used directly conjugated (FITC, PE) monoclonal antibodies to CD4 (L3T4, RM4-5), to CD8 (Ly-2, 53–6.7), to B cells (B220, CD45B), and to macrophages (MAC-1, CD11b) with fluorescence-activated cell sorter (FACS). To mark individual CNS cell populations, we employed monoclonal or monospecific rodent antibodies reactive with neurons (antibody to MAP-2), oligodendrocytes (antibody to MBP), astrocytes (antibody to GFAP), and microglia (antibody to F4/80). Five to six days after i.c. inoculation of MV into newborns, at least five spleens were harvested from YAC-CD46 mice and separated into lymphocyte and macrophage populations or into a lymphoid-enriched splenic population. FACS insured that purity of cell subsets studied exceeded 99%, and by staining 6 of 1400 $CD4^+$ T cells (0.4%), 15 of 1500



PFU/ml of MV recovered from cocultured cells using:

	d9	d14
CD46 tg + MV		
Spleen	2×10^2	1×10^5
Brain	2×10^4	6×10^4
non CD46 tg + MV	nil	nil

Figure 3. Expression of MV Antigens in Brain, Lymph Nodes, and Spleen of a 6-Day-Old YAC-CD46 Mouse and Recovery of Infectious MV from a Pool of Five 6-Day-Old mice Inoculated at Birth Intracerebrally with 10^3 pfu of MV

Whole animal sections of 10 μm were fixed and stained with antibody to MV in a peroxidase reaction assay (see Experimental Procedures for details). Inserts represent tissues from this mouse shown at higher magnifications.

(A) Brain at 200× magnification with the brown-colored cells containing MV antigens. At higher magnification or after double staining with cell-specific probes, cells were identified as neurons (see Figures 4–6).

(B) Cervical lymph node. Magnification, 400×.

(C) Peribronchial lymph node. Magnification, 400×.

(D) Spleen (magnification, 200×) with MV antigen in lymphocytes around central vessel. Similar results were obtained from three additional YAC-CD46 mice. Three similarly aged non-YAC-CD46 mice inoculated with MV failed to display immunochemical staining of any tissues when the same reagents were employed.

Lower table indicates day of recovery and amounts of infectious MV obtained from spleen and brain after cocultivation with Vero cells. Similar results occurred on two separate experiments. Infectious virus was not recovered from MV-inoculated non-CD46 tg mice.

$CD8^+$ T cells (1.0%), 3 of 3369 B cells (0.09%), and 2 of 510 MAC-1^+ monocytes/macrophages (0.4%) expressed MV gene products. By contrast, analysis of similar cell populations from non-YAC-CD46 mice infected with MV failed to express MV gene products. FACS-purified populations of $CD8^+$, $CD4^+$ T, and B lymphocytes; macrophages; and whole spleen lymphoid cells were placed in culture with Vero cells. After 6 but usually 8–10 days, small but definitive syncytia began to form in cultures containing $CD8^+$ T lymphocytes, B lymphocytes, and whole splenic lymphoid cells. After 10–12 days the presence of similar syncytia in both $CD4^+$ T lymphocyte and macrophage cultures was usually observed. Infectious virus was recovered by day 9 or later, and at day 14 over 10^5 pfu of virus per milliliter of culture supernatant was recorded from spleen, $CD8^+$ T, and $CD4^+$ T cell cultures. Isolation of MV from whole spleen or lymphoid subsets was noted 4–7 days after i.c. inoculation of newborn (ten mice) YAC-CD46 transgenic mice. Analysis of brain at similar harvested periods indicated recovery of two logs more infectious virus at day 9 than from the spleen (Figure 3). Immunochemical analysis indicated that MV expression was restricted almost entirely to neurons (Figure 4; >99% of 1000 cells counted) and was occasionally present in oligodendrocytes (<0.01%) and microglia (<0.5%). MV antigen was absent from $GFAP^+$ cells. Attempts to recover virus in the absence of cocultivation failed.

MV Initiates CNS Disease in YAC-CD46 Mice

After i.c. inoculation of 10^3 or more pfu of MV, viral transcripts and antigens were detected in groups of neurons throughout the recipient's CNS. As shown in Figure 4A (lanes 1 and 2) and Figure 4C, viral RNA was expressed in the brain 6 days after infection of newborn YAC-CD46 transgenic mice. From Figure 4C, it is evident that MV RNA was present in multiple sites of the brain. No MV transcripts were detected in brains of non-CD46 mice inoculated with equivalent doses of virus (Figure 4A, lanes 3–5 and 4B). Higher resolution light microscopy demonstrated that MV antigens resided in neurons throughout the optic tegmentum (D), cerebral cortex (E, G–I), hippocampus (J), and cerebellum (K). Electron microscopy revealed abundant MV nucleocapsids in infected neurons (Figure 5). Further, MV nucleocapsids appeared to move into neuritic processes (axons) containing normal and disorganized microtubules (Figures 5A, 5B, 5E–5G). Infectious MV was recovered from brains after cocultivation with Vero cells (Figures 3 and 4F). Accompanying MV replication and spread was a generalized astrocytosis (Figure 4L), activation of microglia, and infiltration primarily of $CD4^+$ T cells (M) and some $CD8^+$ T cells (N) into the brain parenchyma. FVB/N ($H\text{-}2^q$) mice were subject to these sequela of MV infection; over 94% died within 3 weeks postinfection after displaying lethargy, weakness, occasional paraplegia, and seizures. Expression of MV transcripts or protein was observed in 20 out of 20 $CD46^+$ transgenic mice. By contrast, 10 out of 10 non-$CD46^+$ transgenics failed to display MV transcripts or proteins or replicate MV after similar virus inoculation. A similar degree and timing of mortality uniformly followed MV infection in other strains of CD46 transgenic mice crossed to the F2 level, including BALB ($H\text{-}2^d$), SWR/J ($H\text{-}2^q$), SJL ($H\text{-}2^s$), PLJ ($H\text{-}2^u$), C3H ($H\text{-}2^k$), and C57BL/6 ($H\text{-}2^b$) mice crossed to the C57BL/6 background to seven generations. Yet, none of the sex- and strain-matched nontransgenic littermates became ill following MV inoculation (i.c., 10^3 to 10^5 pfu) or expressed MV transcripts or proteins (three to eight mice per group).

Activation of astrocytes and microglia along with infiltration of T lymphocytes after MV infection suggested that cytokines and/or chemokines were being synthesized in the CNS. To evaluate this possibility, both total

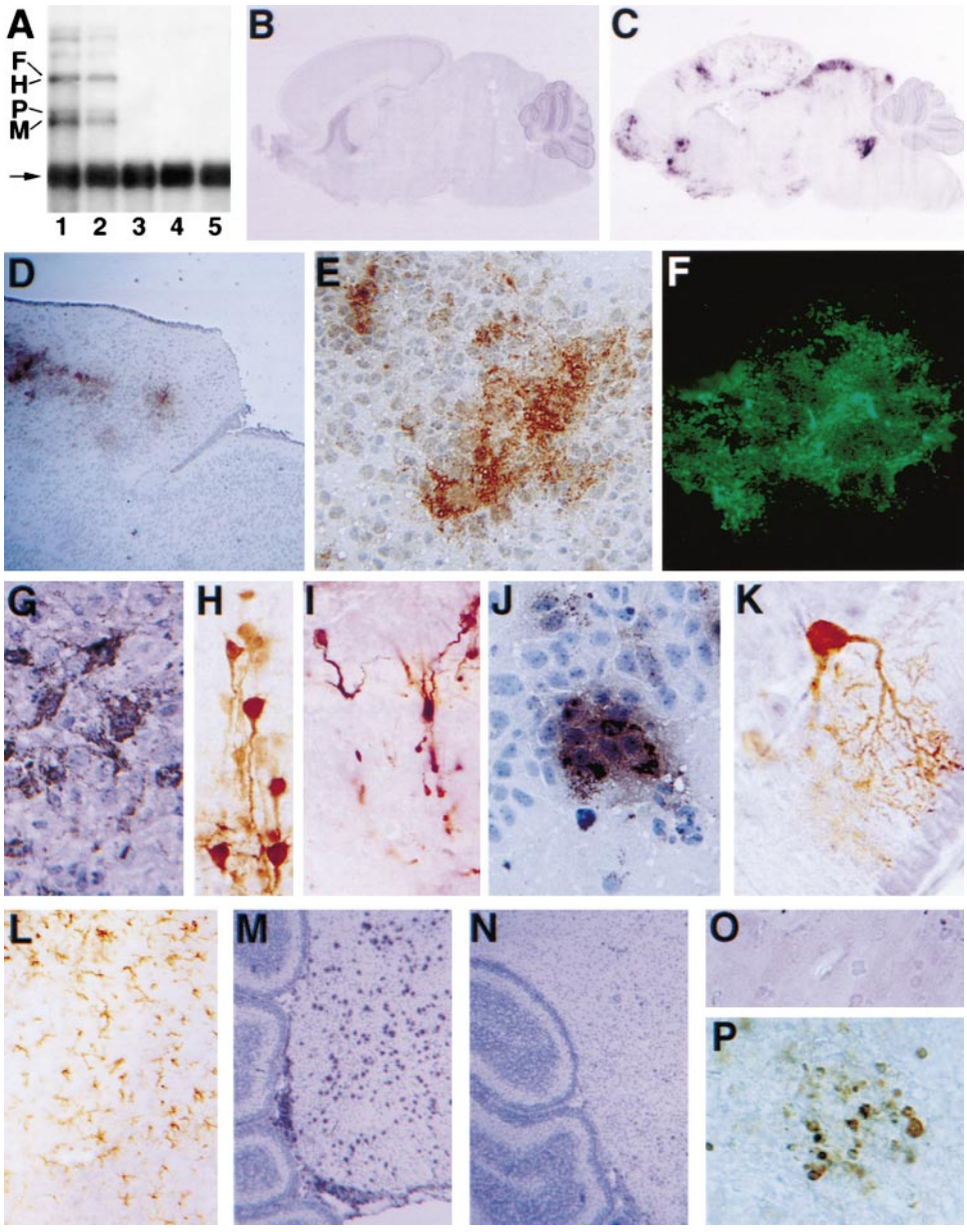


Figure 4. Specificity and Expression of MV in the CNS after Infection of YAC-CD46 Transgenic Mice

MV RNA ([A], lanes 1 and 2 and [C]) and protein (D, E, G–K) were expressed in neurons throughout the brains of infected YAC-CD46 mice. MV was isolated when brain tissue components were cocultivated on Vero cells. Insert shows staining of a syncytia located in Vero cells with monoclonal antibody to MV (F) (see Experimental Procedures). No MV RNA was detected in nontransgenic mice given a similar inoculum of MV ([A], lanes 3–5 and [B]). Infection was accompanied by generalized activation of astrocytes ([L] antibody to GFAP), brisk $CD4^+$ T cell (M) and modest $CD8^+$ T cell infiltration (N). Newborn YAC-CD46 mice were inoculated i.c. with 10^3 pfu of MV; their brains were harvested 6 to 10 days later as a source of isolated RNA or tissue fixed in 2% paraformaldehyde, sectioned on a vibratome, and stained for expression of MV RNA or antigens as described in Experimental Procedures. (A) shows MV transcripts in brains from two $CD46^+$ transgenic mice (lanes 1 and 2) but not from two nontransgenic littermates (lanes 3 and 4) or from a normal mouse (lane 5) infected with 10^5 pfu of MV. Hybridization was with a MV probe in which the viral N was deleted (see Experimental Procedures). In (B) no MV transcripts appear in a nontransgenic mouse tested by in situ hybridization, whereas (C) shows the presence of MV transcripts. For (B) and (C), MV N probe was used. Expression of MV antigens is observed in (D) optic tigmentum; (E, G–I) separate areas in the cerebral cortex; (J) hippocampus; (K) Purkinje neuron in the cerebellum. Results were similar in brains from three other MV-infected YAC-CD46 mice inoculated with 10^3 pfu and in groups of three mice inoculated with either 10^4 or 10^5 pfu of MV. (L) shows activated astrocytes near the hippocampal area as revealed by GFAP stain. Corresponding astrocytosis was found in areas throughout the CNS. (M) and (N) display infiltrating $CD4^+$ and $CD8^+$ T lymphocytes, respectively, in an area by the cerebellum. Tissue sections were 5 μm apart. A similar preponderance of $CD4^+$ T cells occupied the cerebral cortex. (P) shows apoptosis of neurons found in MV-infected YAC-CD46 mice. By contrast, inoculation of MV into nontransgenic littermates did not produce MV replication (A and C) and did not show presence of MV antigens in neurons (data not shown), T cell infiltration, or astrocytosis, and apoptosis was negligible (O).

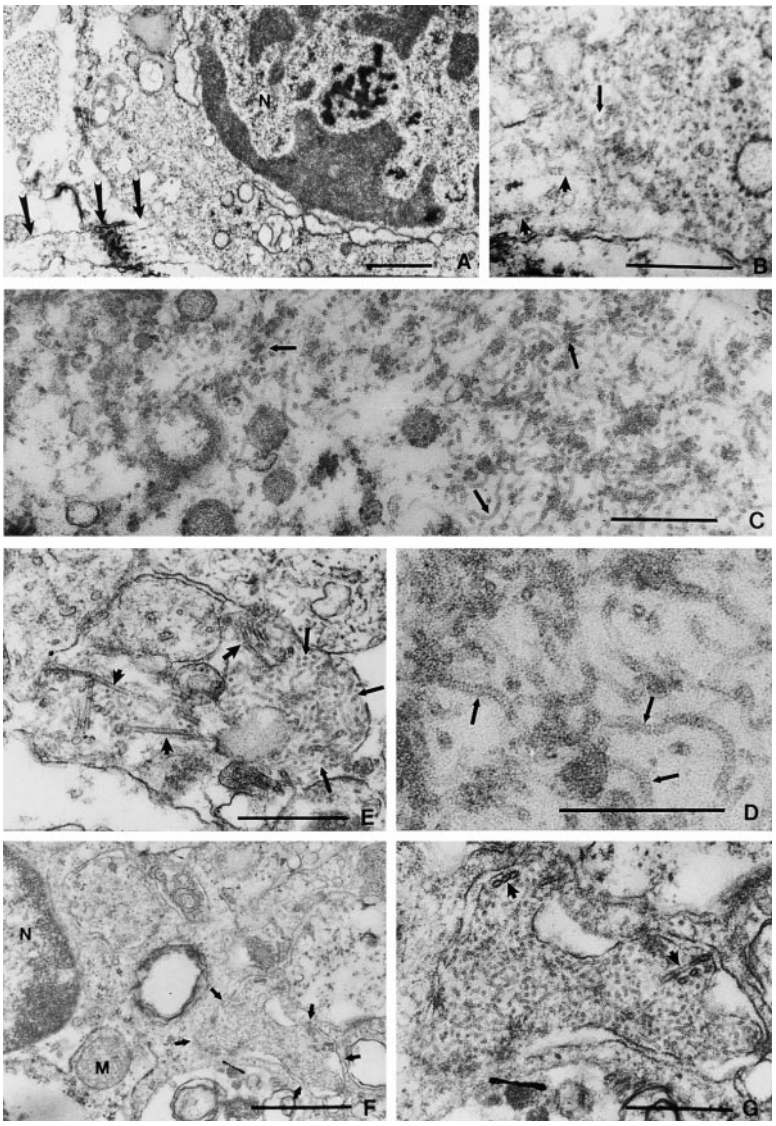


Figure 5. Selected Examples Illustrate Virus Nucleocapsids within Neurons

(A) The arrows point to a neurite emerging from a neuron in which the nucleus (N) is pycnotic.

(B) A region of this neurite shown at a higher magnification to demonstrate nucleocapsids (arrow) and microtubules indicated by short arrows.

(C) Illustrates massive accumulations of nucleocapsids (arrows) in the cytosol of a neuron.

(D) Selected region of (C) at a higher resolution demonstrates the helical configuration (arrows) of individual nucleocapsids.

(E) Section through a neurite illustrates presence of microtubules (short arrows) and nucleocapsids (arrows).

(F) Portion of the nucleus (N) and cytoplasm including a mitochondrion (M) of a neuron contiguous to a neurite process indicated by arrows, in which nucleocapsids are evident.

(G) The same process shown at a higher resolution contains microtubules (short arrows) and numerous nucleocapsids. The length of magnification bars is as follows: (A and F) 1.0 μm; (B, C, E, and G) 0.5 μm; (D) 0.25 μm.

RNA and poly(A)-enriched RNA were isolated from brains of MV-infected and uninfected YAC-CD46 mice (10^3 and 10^5 pfu, four mice per group). By RPA, when compared to non-MV-infected YAC-CD46 mice at 6 days postinfection, brains of YAC-CD46 MV-infected mice had a 6-fold enhanced expression of IL-12 (p40), an 11-fold enhancement of LT-β, and a 17-fold enhancement of TNFα. There was also a 3-fold increase in IL-1α, 7-fold increase of IL-1β, and 2-fold increase of both IL-1r and TNFβ. Continued enhanced elevation of TNFα, LT-β, and IL-12 was noted at 10 days post–MV infection. By contrast, IFNγ and IL-10 were not elevated in MV-infected mice. Several of these cytokinea (i.e., TNFα and IL-1) have been associated with apoptosis (Ashkenazi and Dixit, 1998; Thornberry and Lazebnik, 1998). To determine whether apoptosis accompanied MV infection, the in situ TUNEL assay was utilized. As a representative sample in Figure 4P indicates, 50- to 100-fold more apoptotic cells in CD46 mice infected with MV than in nontransgenic mice inoculated with MV or YAC-CD46 mice receiving a needle wound in their brains (Figure 4O). Apoptotic cells were identified as being primary neurons by double labeling. CC chemokines, such as Rantes, MIPα and β, and MCP-1, or CXC chemokines, such as IP-10 and TCA-3, which attract T cells (Zingoni et al., 1998), or MIG, which is induced by IL-12, were also detected in brains of YAC-CD46 mice inoculated with MV. In contrast, chemokines, such as EOTXN, which attracts eosinophils (Rolling, 1997), LTN, and MIP-2 were not.

MV Spreads Rapidly throughout the CNS via Axonal Transport

Initial studies with polyclonal SSPE antibody documented the rapid and efficient spread of MV antigens within axonal tracts throughout the CNS in seven out of seven mice studied (Figure 6). Spread to distal sites was noted by the second to third day after infection with 10^3 pfu of MV. We then compared the identity of MV proteins in neuronal cell bodies to that in axonal tracts using monoclonal antibodies. Monoclonals specific for the

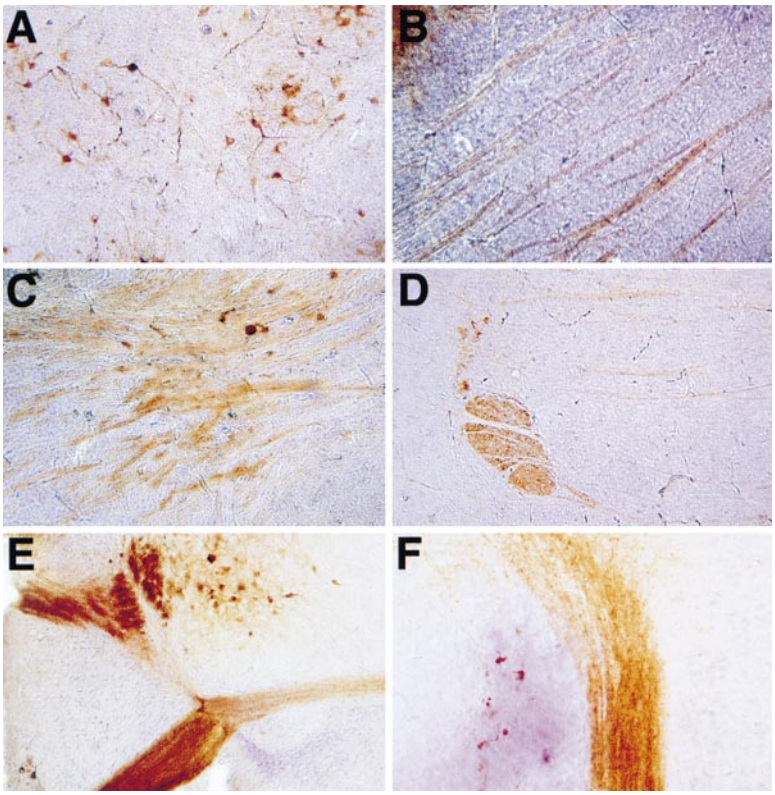


Figure 6. Spread of MV throughout the CNS Is Likely by Rapid Axonal Transport and via MV RNP Complexes

(A) Polyclonal antibody (SSPE) to MV-stained neuronal cell bodies in the cerebral cortex. (B–F) MV N antigens expressed in axonal tracts (B and C) leading to caudate nucleus of the extra pyramidal system (D) and axonal tracts in the corticospinal pyramidal tract (E and F). Similar observations were noted in an additional six MV-infected YAC-CD46 mice. No staining of CNS was noted in control non-YAC-CD46 mice inoculated with MV.

two viral glycoproteins HA and F can detect these antigens on the membranes of infected cells, and those against the N protein identify the antigen within the cell. MV HA, F, and N were detected in neurons of YAC-CD46 mice examined 2, 5, and 7 days post–MV infection of newborn mice (10^3 pfu, 10^5 pfu). In contrast, only the N antigen was found within axonal tracts throughout the CNS at comparable times. Electron microscopic examination did not reveal any budding virus in neurons but indicated presence of nucleocapsids within axons (Figures 5A, 5B, 5E–5G). No evidence of formed virions was noted in axons. These findings supported the concept that infectious virus was not being spread via the fluid phase but was likely transported as MV ribonucleoprotein complexes toward termini of neurites.

MV Infects and Can Be Isolated from Spleens and PBL of Adult YAC-CD46 Mice and Suppresses Cell-Mediated and Humoral Immune Responses

In the last series of experiments, we analyzed MV infection of the immune system in adult YAC-CD46 mice. As shown in Figure 7, the expression of CD46 molecules on the surface of PBL was quantitatively similar in cells obtained from humans (A) and from YAC-CD46 transgenic mice (B); both were over 100-fold greater than amounts expressed by the β-actin (βa) promoter-generated BC1 isoform of CD46 (C). Infectious MV was recovered from YAC-CD46 PBL or spleens (Figure 3; Figures 7D and 7E) obtained 2 to 5 days after i.v. or i.p. injection when cocultivated on Vero cells, but not from βa-CD46 transgenic mice. Infectious MV was recovered from purified populations (FACS) of $CD8^+$, $CD4^+$, and B lymphocytes and from $F4/80^+$ macrophages. These observations were uniformly observed in four 6- to 8-week-old YAC-CD46 mice inoculated i.v. with MV. Lymphoid tissues or cells were obtained at day 3 or 7 after MV inoculation. In the next series of experiments, we noted that MV suppressed the ability of YAC-CD46 mice to mount both a cellular and humoral immune response. As shown in Figure 7F, when MV was inoculated in YAC-CD46 mice primed 60 to 90 days earlier with lymphocytic choriomeningitis virus (LCMV), splenic lymphocytes removed 3 days later were unable to mount a MHC-restricted cytotoxic T lymphocyte response to LCMV-infected targets. In contrast, similarly harvested lymphocytes from YAC-CD46 mice that were not infected with MV are able to kill LCMV-infected targets. Killing was MHC restricted, as lysis by cytotoxic T cells (CTLs) was only noted with LCMV-infected MHC class I matched but not with MHC class I mismatched target cells. Suppression of cell-mediated CTL immune response was also noted during primary virus infection. All three YAC-CD46 $H\text{-}2^b$ mice receiving 1×10^5 pfu of vaccinia virus (VV) i.p. mounted a MHC-restricted (to $H\text{-}2^b$ targets but not $H\text{-}2^q$ VV infected targets) virus-specific CTL response. Specific ^{51}Cr release in a 5 hr assay being 30%, 23%, 10% at effector to target (E:T) ratios of 50:1, 25:1, and 12.5:1. In contrast, when 1×10^6 pfu of live measles virus was given i.v. 3 days after VV priming, none of the mice (3/3) released >1% ^{51}Cr even at the highest 50:1 E:T ratio. When LCMV, instead of VV, was used to generate a primary day 7 CTL response, similar suppression of the cell-mediated immune response was noted in some but not all inoculated mice, likely reflecting the known vigor of the LCMV CTL response.

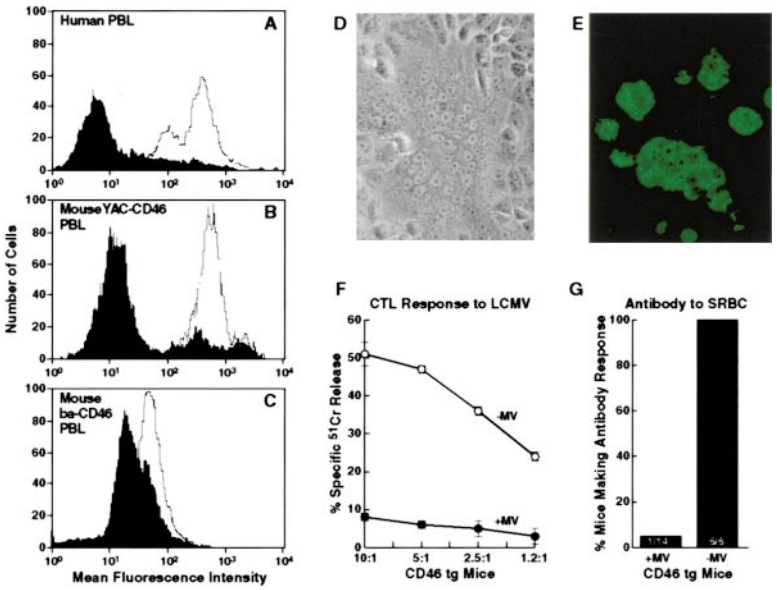


Figure 7. MV Induces Immunosuppression in YAC-CD46 Transgenic Mice

PBL from humans (A) and from YAC-CD46 transgenic mice (B) expressed equivalent levels of CD46 molecules on their surfaces. Such expression was over 100-fold greater than carried by PBL from β-actin (βa)-generated transgenic mice (mouse βa-CD46 PBL, [C]). (D) MV^+ syncytia formed when PBL harvested 6 days after i.v. infection (10^4 pfu of MV) from YAC-CD46 transgenic mice were cocultivated with Vero cells. Specificity for MV was documented when syncytia was stained with fluorochrome labeled monospecific antibody to MV ([E]; see Experimental Procedures). (F) Injection of MV into mice previously primed to LCMV or into mice primed to SRBC (G) prevents lymphocyte function in a LCMV-specific CTL assay or generation of antibodies to SRBC (numbers in bar graph indicate number of mice making a response over the total numbers in each group). See Experimental Procedures for details.

MV-infected YAC-CD46 mice were also unable to generate an antibody response to sheep red blood cells (SRBC) (Figure 7). Thirteen out of 14 of such mice given 1×10^6 SRBC i.v. 5 days after virus infection failed to mount antibody responses to the SRBC antigens 12 days later. In contrast, all six YAC-CD46 littermates treated the same way except inoculated with vehicle generated antibody responses with a mean endpoint sera titer dilution of 1/448 (range 1/256 to 1/1024).

Discussion

In this paper, we describe a transgenic mouse model that reproduces many of the cardinal manifestations of MV infection. Criteria for virus-induced suppression of the immune response and of CNS disease with spread of virus throughout the nervous system were met. Virus replicated in both the immune system and CNS with recovery of infectious progeny.

Success in preparing this model required a high level of MV receptor expression on neurons and on cells of the immune system. This was achieved by inserting the full-length genomic copy of CD46 into fertilized eggs to allow expression of its four major isoforms. As a consequence, formidable levels of CD46 proteins appeared on the surfaces of neurons, lymphocytes, macrophages, and dendritic cells often at amounts comparable to those naturally occurring in humans. When transgenic mice expressed only the BC1 isoform in neurons, under transcriptional regulation of the NSE promoter (Rall et al., 1997), 10- to 20-fold fewer CD46 molecules appeared on neurons than when the endogenous CD46 gene was used. The consequence for the infectious process was that although MV replicated in neurons of NSE-CD46 transgenic mice, the kinetics and spread of virus were more restricted than in YAC-CD46 mice.

As expected, the transcriptional regulation of CD46 BC1 by NSE yielded mice that failed to express CD46 on cells of the immune system (Rall et al., 1997), and such cells were resistant to MV infection. When the NSE promoter was replaced by that of βa or immediate early genes of cytomegalovirus (CMV) (J. P. and M. B. A. O., unpublished observations; Baskar et al., 1996), CD46 expression was noted on PBL but only at low levels (Figure 7). Neither these cells nor lymphoid cells from spleens and lymph nodes produced infectious MV in vivo. By contrast, YAC-CD46 lymphoid cells had the potential to preferentially express any or all isoforms of CD46 and possessed CD46 levels 100- to 150-fold greater than those expressed by cells from βa-CD46 or CMV-CD46 mice (J. P. and M. B. A. O., unpublished observations). PBL from YAC-CD46 mice and humans expressed levels of CD46 molecules at comparably high levels (Figure 7). This coincided with efficient MV infection of YAC-CD46 lymphoid cells and production of infectious progeny from lymphocytes and macrophages as MV does in humans (McChesney and Oldstone, 1989; Griffin and Bellini, 1996). Consistent with our findings that low or no CD46 expression (i.e., βa-CD46 and CMV-CD46 transgenic mice) does not support MV replication in vivo, others were unable to generate a CD46 animal model of MV infection in vivo using various cDNAs of CD46 (Horvat et al., 1997; Thorley et al., 1997). Such cDNA constructs lack introns and regulatory sequences that likely are beneficial for transgene expression. A direct correlation between levels of transgene expression and the production of disease has been described for other systems (Games et al., 1995; Rockenstein et al., 1995). Recently, Mrkic et al. (1998) and Blixenkrone-Moller et al. (1998) utilized a YAC strategy to express CD46 as was done here. However, in these other models either lack of MV specificity (Mrkic et al., 1998), absent or minimal to modest virus replication in and failure to recovery virus from the immune system (Mrkic et al., 1998), lower CD46 expression levels, or need to damage the hosts interferon system (Blixenkrone-Moller et al., 1998) for MV replication was noted. The explanation for differences between CD46 genome models of others and ours is not totally clear but may relate to the quality and quantity of YAC-CD46 sequences introduced into

fertilized eggs or site(s) of DNA integration. In our YAC-CD46 model, the ability to infect mice correlated directly with the presence and expression levels of CD46 (see Figures 1, 3, 4, and 7). Further, all seven mouse strains tested (FVB/N, SWR/J, C3H, BALB/c, C57BL/6, PL/J, and SJL/J) were susceptible to MV infection when they expressed CD46 molecules. This universal susceptibility of multiple mouse strains contrasts to the selective susceptibility of primarily the C3H (H-2^k) strain reported for mouse brain passaged MV (Liebert and Finke, 1995).

Analysis of the CNS infection in YAC-CD46 mice indicated that MV replicated primarily in neurons and spread throughout the neural axis along axons likely by fast axonal transport. Abundant documentation was obtained for MV transcription, translation, and recovery of infectious virus. This was supported by electron microscopy showing copious accumulation of MV nucleocapsids in neuronal cell bodies without detection of any budding virus. MV could be rescued from infected brains upon cocultivation with Vero cells. These observations of abundant nucleocapsid formation, absence of budding, and requirement for cocultivation to isolate infectious virus parallel the observations made with persistent MV infection in brains of patients with SSPE (ter Meulen et al., 1983; Griffin and Bellini, 1996) and provide the opportunity to dissect the molecular parameters of this human disease in our murine model. In preliminary studies, intranasal route of MV infection also allowed spread and replication of MV.

Two days after infection, MV clearly spread to neurons at multiple sites throughout the brain, including into cells of the cerebellum, hippocampus, striatum, and cerebral cortex. MV nucleocapsids were noted in the prominent axonal tracts, including those connecting the extrapyramidal and cerebellar systems and the cortico-spinal pyramidal tract (Figures 5 and 6). Immunochemical analysis indicated MV HA and F in the neuronal soma but absent from axons; this, in concert with the absence of virions in axons, lack of virus budding, and our inability to recover free virus from brain tissue suggested that infection likely spread via an infectious ribonucleoprotein complex. This conclusion was strengthened by observing MV nucleocapsid complexes within axons (Figure 5). Others using in vitro assays have shown infectivity of MV RNP complexes for cells (Rozenblatt et al., 1979; Griffin and Bellini, 1996).

An abundance of $CD4^+$ T lymphocytes along with $CD8^+$ T cells infiltrated into the brain parenchyma. Interestingly, the enhanced frequency of $CD4^+$ T over that of $CD8^+$ T cells in brain lesions mirrors in vivo as well as in vitro observations of human CTL in MV infection (Long and Jacobson, 1989), suggesting that MV may block the MHC class I antigen presentation pathway (Oldstone, 1997). In vitro, effects of MV on antigen-presenting dendritic cells have been noted (Fugier-Vivier et al., 1997; Grosjean et al., 1997; Schnorr et al., 1997). The lymphocyte infiltration we describe was associated with astrocytosis, proliferation of microglia, elevation of selected Th1 cytokines, and chemokines. The chemokines expressed (Rantes, MIPα and β, MCP-1, IP-10, and TCA-3) are those known to attract or emanate from T cells (Rolling, 1997; Zingoni et al., 1998). Mortality resulting from MV infection may be related to the effect of cytokine(s) on immature neurons (Trgovcich et al., 1997; Tanaka et al., 1998) and the susceptibility of neurons in newborns to apoptosis (Trgovcich et al., 1997; Tanaka et al., 1998; Griffin and Hardwick, 1999), CNS toxins (Eastman et al., 1994), and/or lack of protection because of immaturity of the newborn immune system (Mosier, 1974). In our model, MV replication in neurons of newborn mice caused an acute CNS disease with death within 3 weeks. By contrast, challenge of adults with MV, while resulting in neuronal infection failed to produce acute illness or death. Recently, using Rag2 knockout $\times$ NSE-CD46 transgenic mice, Lawrence et al. (1999) noted that abolition of the CD4/CD8 T cell response enhanced the adults' susceptibility, resulting in a clinical and pathologic picture similar to MV infection in the newborn. These results are consistent with the interpretation that the immune response protected adult mice but not newborns against the spread of MV infection within the CNS, although other factors such as varying susceptibilities of neurons during development and aging might be involved in CNS pathogenesis (Johnson et al., 1972; Trgovcich et al., 1997; Tanaka et al., 1998; Griffin and Hardwick, 1999). Thus, the acute CNS disease in newborns infected with MV may be pharmacologically mediated and treatable with reagents that block the apoptosis-promoting cytokines. Alternatively, during MV infection, enzymes such as the double-stranded RNA adenosine deaminase may be induced by cytokines (Patterson et al., 1995), and this enzyme aberrantly edits glutamate receptors so as to interfere with Ca^{2+} flux, thereby causing neuronal dysfunction and death (Lomeli et al., 1994). These possibilities are now being explored.

In our YAC-CD46 transgenic mice, MV can infect cells of the immune system, as evident by presence of viral gene products in $CD4^+$ and $CD8^+$ T lymphocytes, B lymphocytes, in the T-enriched area of the spleen lymph nodes, Peyer's patches, and circulating PBL and recovery of infectious MV by coculture of spleen lymphoid cells, $CD8^+$ and $CD4^+$ T cells, B cells, PBL, and macrophages on susceptible Vero cells (Figures 3 and 7). Adult YAC-CD46 mice, which ordinarily mount an effective cell-mediated CTL response after challenge with LCMV or VV, or a robust antibody response after immunization to SRBC, failed to do so when infected with MV, thus imitating suppression by MV noted in humans (McChesney and Oldstone, 1989; Griffin and Bellini, 1996). Further, in preliminary studies (Slifka et al., unpublished observations), MV-infected transgenic mice replicated 6-fold more *Listeria* monocytogenes in their spleens than noninfected transgenic mice, suggesting enhanced susceptibility to opportunistic infections. The fact that $CD8^+$, $CD4^+$, and B lymphocytes; macrophages; and dendritic cells from YAC-CD46 mice expressed high levels of CD46 molecules on their surfaces and can be infected with MV in vitro (Figure 2) in association with findings that $CD8^+$, $CD4^+$ T cells, and macrophages are infected in vivo indicates this model's usefulness for adoptive transfers to complement deficiencies in mice after selective knockout of immune system components. Thus, this model presents an opportunity to define in vivo the mechanism of immune system suppression by MV and to develop strategies for overcoming

that defect. A further application is testing of new MV vaccines that provide immunity without immunosuppression, a failing of the currently utilized vaccine (Markowitz and Katz, 1994; Griffin and Bellini, 1996). Lastly, infection of the YAC-CD46 transgenic model by MVs in which individual viral genes have been deleted by reverse genetics (Radecke et al., 1995) may provide a way to ascertain the role of individual MV genes in determining the parameters involved in the CNS disease and immunosuppression.

Experimental Procedures

Mice and Virus Used

Generation of the YAC-CD46 transgenic mouse line 2 and NSE-CD46 mouse line 52 has been described (Yannoutsos et al., 1996; Rall et al., 1997). Progeny YAC-CD46 mice were crossed with mice of the FVB/N, SWR/J, BALB/c, C3H, PLJ, and SJL/J backgrounds for a minimum of two generations. NSE-CD46 and YAC-CD46 transgenics were bred for seven generations onto C57BL/6 backgrounds. To generate mice expressing the human CD46 BC1 isoform cDNA under control of the human βa promoter, the human βa and CD46 sequences were isolated from the plasmid pK523 (kindly provided by John Atkinson, Washington University School of Medicine, St. Louis, MO), digested with PvuI, and partially digested with CspI to generate a 9.5 kb fragment. The fragment was cloned into the vector pBC SK^+ (Stratagene, La Jolla, CA) in which a CspI linker had been introduced into the XbaI-XhoI cloning sites. After digestion with XbaI and PvuL, the 9.5 kb fragment containing the βa promoter sequences, BC1 cDNA, and SV40 transcription terminator was isolated and injected into fertilized (C57BL/6 × C3H/HeJ) F1 eggs. The resulting offspring were crossed with C57BL/6 or C3H/HeJ mice. Founder animals and subsequent breeders were housed in the mouse hepatitis virus, pathogen-free breeding colony of The Scripps Research Institute (La Jolla, CA).

The primary virus used was MV Edmonston strain, whose origin, biological properties, and quantitation by plaquing on Vero cells are detailed elsewhere (McChesney and Oldstone, 1989; Manchester et al., 1994; Rall et al., 1997). Inocula were 10^3, 10^4, or 10^5 pfu of MV for YAC-CD46 and NSE-CD46 mice and 10^6 pfu of MV for βa-CD46 mice. MV was inoculated either intracerebrally (i.c.), intraperitoneally (i.p.), or intravenously (i.v.).

MV was recovered from brain and spleen by cocultivation on Vero cells. Briefly, a suspension of brain tissue was placed on a ficoll-hypaque gradient as described (Rall et al., 1997) and cocultivated with Vero cells (Manchester et al., 1994; Rall et al., 1997). Splenic tissue was passed through a 100 μm nylon mesh and RBC removed by treatment with NH_4Cl (Oldstone et al., 1991; von Herrath et al., 1994). Resultant lymphoid cells are washed and cocultivated on Vero cells. Cultures formed syncytia, and identity of MV was determined on infected cells by immunofluorescence using a specific antibody to MV (Manchester et al., 1994; Rall et al., 1997). Amounts of infectious virus made were determined by plaquing log dilutions of supernatant fluids on Vero cells. Technique for separation of $CD4^+$, $CD8^+$, B lymphocyte, and macrophage populations using fluorochrome-labeled monoclonal antibodies and FACS has been published (Tishon et al., 1988; Homann et al., 1998).

Characterization, use, and quantitation of LCMV and VV have been noted earlier (Oldstone et al., 1991).

DNA, RNA, and Protein Analysis

The transgene's presence was identified by Southern blot analysis (Rall et al., 1997). CD46 and MV RNA were detected by Northern blot analysis using standard procedures. Tissues were removed, snap frozen in liquid nitrogen, and used as the source of RNA or protein (Oldstone et al., 1991; Manchester, et al., 1994; Rall et al., 1997). Total RNA (10 μg/sample) was run on a 1% formaldehyde gel and transferred to Nytran membranes (Micron Separations, Inc., Westboro, MA). For detection of CD46 mRNA, a CD46 probe was prepared by random-primer labeling of a 1.6 kb DNA fragment from the CD46 gene (Liszewski et al., 1991). For detection of MV mRNA, a MV nucleocapsid (N) probe was prepared by random primer labeling of a 1.7 kb DNA fragment from the MV N gene (plasmid peNI, kindly provided by Dr. Martin Billeter, Zurich, Switzerland). Blots were probed with a random-primed, ^{32}P-labeled DNA probe for either CD46 or MV N RNA. RNA loading in each sample was quantified by hybridization of the same blots with a ^{32}P-labeled cDNA probe specific for cyclophilin. To detect CD46 and MV RNA in whole body sections, mice of various ages were sacrificed, placed in carboxymethlycellulose (CME), and frozen in a dry ice and alcohol bath. Sections (20 to 40 μm) were obtained on 3M tape and either air dried and directly hybridized with a ^{32}P riboprobe to detect RNA or transferred to a nylon membrane for detection of protein (Oldstone et al., 1986; Lipkin et al., 1989). Riboprobes for MV N and CD46 were prepared and labeled with ^{32}P. Briefly, a 289 bp CD46 molecule was obtained by digestion of pBSC CD46 (containing BC1 isoform) with BsaI and transcribed in vitro in the presence of ^{32}P UTP and T7 polymerase. The resulting 289 nucleotide sequence constituting the riboprobe was purified and used in hybridization.

To detect MV N gene, a 650 bp fragment of the gene was generated by PCR and inserted into a pSP71. Primer pairs A: 5′-GCTGAATTCAAT TAACCCTCACTAAAGGG-3′ and B: 5′-CGGCTCGAGGGGCCGTAACC GCCTTTGCT-3′ corresponding to the T3 region of the pBluescript KS (5′ end) and an internal MV NC gene sequence (3′ end), respectively, were used to amplify the 5′ end of the MV NC gene by PCR using peNI as the template. This PCR fragment was digested with EcoRI and XhoI and subcloned into the same sites in the pSP71 vector (Promega) in a 3′ to 5′ orientation with respect to the T7 promoter. The template was linearized with EcoRI for generation of the antisense MV N riboprobe using T7 RNA polymerase. For detection of CD46 protein in whole-animal sections, we used anti-CD46 monoclonal antibody E4.3 or rabbit polyclonal antibody to CD46 (Manchester et al., 1994) followed by ^{125}I Staph A, and for MV proteins polyclonal SSPE antibody followed by ^{125}I Staph A or fluorochrome probes (Oldstone et al., 1986; Lipkin et al., 1989; Manchester et al., 1994; Rall et al., 1997).

A riboprobe to detect MV N using digoxigenin was also used on free-floating, vibratome-cut sections (40 μm thick) of mouse brains fixed in 4% paraformaldehyde or sorted lymphocytes as described (Tishon et al., 1988; Holz et al., 1996).

Immunocytochemical and Histological Assays

Immunocytochemistry was also done on tissues either frozen in CME cut in 8 to 10 μM sections by cryomicrotome, 40 to 75 μM sections made with a Leica 10002 Vibratome, or FACS sorted cells. Most often, sections or cells were placed on either sialin-coated superfrost-plus slides or round-bottomed 96-well plates and fixed for 1 min in 1% paraformaldehyde, washed, and stained with monoclonal antibodies to MV HA, MV F, MV N (obtained through WHO Reference Center, E. Norrby, Sweden, and R. Cattaneo, Zurich), CD46, MBP, NSE, NF, F4/80, MAC-1, CD4, CD8, B220, or polyclonal antibody to MV (SSPE sera) or GFAP as described (Manchester et al., 1994; von Herrath et al., 1994; Evans et al., 1996; Rall et al., 1997; Horwitz et al., 1999). Secondary antibody was biotinylated or fluoresceinated goat anti-rat IgG, rat anti-mouse IgG, or goat anti-human IgG, followed by avidin-POD conjugate (for biotinylated antibody) from Boehringer-Mannheim (La Jolla, CA). With biotinylated antibodies, color reactions developed with diaminobenzidine in the presence of hydrogen peroxide as described (Evans et al., 1996; Horwitz et al., 1999). Specificity of reagents and controls used have been published (von Herrath et al., 1994; Evans et al., 1996; Rall et al., 1997).

Suspensions of living cells were phenotyped by FACS analysis for expression of CD46 and MV HA, F, or N as described (McChesney and Oldstone, 1989; Oldstone et al., 1991; Manchester et al., 1994; Rall et al., 1997; Homann et al., 1998).

Histological analysis was done on tissues fixed in zinc formalin (10%), paraffin embedded, sectioned, and stained with hematoxylin and eosin.

Electron Microscopy

Infected mouse brain was divided sagitally, and one-half was processed for examination by light microscopic immunocytology to localize sites of MV expression. The other half was fixed in 2%

glutaraldehyde in phosphate-buffered saline, postfixed in 1% OSO_4, dehydrated, and embedded in Eri 812 resin. Thin sections were stained with uranyl and lead salts and examined in a JOEL 100 CX electron microscope at 80 KV.

Detection and Quantitation of Cytokine and Chemokine RNAs by RPA

Cytokine mRNAs were measured using the RiboQuant RPA assay system (Pharmingen, La Jolla, CA) as recommended by the manufacturer as described (Evans et al., 1996). Either total brain RNA (20 μg/sample) or poly(A)-enriched RNA (5 μg) was used, and bands corresponding to protected cytokine or chemokine mRNAs were quantitated on a Molecular Dynamics PhotoImager using the program ImageQuant and normalized to the amounts of L32 and GAPDH standards in each lane.

CTL Assay

The functional activity of primary (day 7) and immune (day 45–60) splenic lymphocytes was determined by in vitro ^{51}Cr release assay (Oldstone et al., 1991; von Herrath et al., 1994; Evans et al., 1996). All samples were run in triplicate, and CTL to LCMV were raised by inoculating either FVB/N, BALB/c, or C57BL/6 mice (6- to 8-weeks-old) i.p. with 1×10^5 pfu of LCMV ARM 53b. After 60 to 90 days, selected mice were given 1 to 2×10^5 pfu of MV i.v. Four days later, their spleens were harvested, and the lymphocytes recovered were cultured for 4 days by a standard protocol (Oldstone et al., 1991; von Herrath et al., 1994). Lymphocytes were then tested against MHC-restricted FVB/N ^{51}Cr-labeled fibroblasts infected 2 days earlier with LCMV ARM or MHC mismatched but similar infected targets.

Sheep Red Blood Cell Immunization and Assay

Preliminary studies indicated that YAC-CD46 mice failed to make antibodies to SRBC when inoculated with 1×10^5 SRBC but did when immunized with 5×10^5 or 1×10^6 SRBC given i.p. Low-antibody titers occurred in some mice 5 days, reaching maximal titers in all mice at 12 days after inoculation. Two- to three-month-old FVB/N YAC-CD46 mice were given 1×10^6 SRBC i.p., and 5 days later one group received 1.2×10^6 pfu of MV i.v. or similar inoculum of MV-free vehicle. Twelve days later individual mice were bled and antibodies to SRBC titered as described (Oldstone et al., 1973). Prior to initiation of SRBC immunization, all mice were bled and individual sera shown not to hemagglutinate SRBC at 1:8 dilution.

Acknowledgments

This is Publication Number 11754-NP from the Department of Neuropharmacology, The Scripps Research Institute, La Jolla, California. This work was supported in part by USPHS grant AI36222 and Training Grants MH19185 (J. P., D. N., and formerly to M. M.) and AG00080 (D. H.), a fellowship from the Swiss National Foundation to A. H., and the World Health Organization to D. N.; S. D. is grateful to Günther Blobel for making available the use of his laboratory and the EM facility. The authors thank Urs Christen for assistance using the ImageQuant program.

Received March 3, 1999; revised July 26, 1999.

References

Ashkenazi, A., and Dixit, V.M. (1998). Death receptors: signaling and modulation. Science *281*, 1310–1312.

Baskar, J.F., Smith, P.P., Nilaver, G., Jupp, R.A., Hoffman, S., Peffer, N.J., Tenney, D.J., Colberg-Poley, A.M., Ghazal, P., and Nelson, J.A. (1996). The enhancer domain of the cytomegalovirus major immediate-early promoter determines cell type specific expression in transgenic mice. J. Virol. *70*, 3207–3214.

Blixenkrone-Moller, M., Bernard, A., Bencsik, A., Sixt, N., Diamond, L.E., Logan, J.S., and Wild, T.F. (1998). Role of CD46 in measles virus infection in CD46 transgenic mice. Virology *249*, 238–248.

Dorig, R., Marcel, A., Chopra, A., and Richardson, C.D. (1993). The human CD46 molecule is a receptor for measles virus (Edmonston strain). Cell *75*, 295–305.

Eastman, C.L., Urbanska, E., Love, A., Kristensson, K., and Schwarcz, R. (1994). Increased brain quinolinic acid production in mice infected with a hamster neurotropic measles virus. Exp. Neurol. *125*, 119–124.

Evans, C.F., Horwitz, M.S., Hobbs, M.V., and Oldstone, M.B.A. (1996). Viral infection of transgenic mice expressing a viral protein in oligodendrocytes leads to chronic central nervous system autoimmune disease. J. Exp. Med. *184*, 2371–2384.

Fugier-Vivier, I., Servet-Delprat, C., Rivailler, P., Rissoan, M.C., Liu, Y.J., and Rabourdin-Combe, C. (1997). Measles virus suppresses cell-mediated immunity by interfering with the survival and functions of dendritic and T cells. J. Exp. Med. *186*, 813–823.

Games, D., Adams, D., Alessandrini, R., Barbour, R., Berthelette, P., Blackwell, C., Carr, T., Clemens, J., Donaldson, T., Gillespie, F., et al. (1995). Alzheimer-type neuropathology in transgenic mice overexpressing V717F β-amyloid precursor protein. Nature *373*, 523–527.

Griffin, D.E., and Bellini, W.J. (1996). Measles virus. In Fields Virology, B. Fields et al., eds. (Philadelphia: Lippincott-Raven), pp. 1267–1312.

Griffin, D.E., and Hardwick, J.M. (1999). Virus infections and the death of neurons. Trends Microbiol. *7*, 155–160.

Grosjean, I., Caux, C., Bella, C., Berger, I., Wild, F., Banchereau, J., and Kaiserlian, D. (1997). Measles virus infects human dendritic cells and blocks their allostimulatory properties for $CD4^+$ T cells. J. Exp. Med. *186*, 801–812.

Holz, A., Schaeren-Wiemers, N., Schaefer, C., Pott, U., Colello, R., and Schwab, M. (1996). Molecular and developmental characterization of novel cDNAs of the myelin associated/oligodendrocyte basic protein. J. Neurosci. *16*, 467–477.

Homann, D., Tishon, A., Berger, D.P., Weigle, W.O., von Herrath, M.G., and Oldstone, M.B.A. (1998). Evidence for an underlying CD4 helper and CD8 T cell defect in B cell-deficient mice: failure to clear persistent virus infection after adoptive immunotherapy with virus-specific memory cells from μMT/μMT mice. J. Virol. *72*, 9208–9216.

Horvat, B., Rivailler, P., Varior-Krishnan, G., Cardoso, A., Gerlier, D., and Rabourdin-Combe, C. (1997). Transgenic mice expressing human measles virus (MV) receptor CD46 provide cells exhibiting different permissivities to MV infection. J. Virol. *70*, 6673–6681.

Horwitz, M.S., Evans, C.F., Klier, F.G., and Oldstone, M.B.A. (1999). Detailed in vivo analysis of interferon-γ induced MHC expression in the CNS: astrocytes fail to express MHC class I and II molecules. Lab. Invest. *79*, 235–242.

Johnson, R.T., McFarland, H.F., and Levy, S.E. (1972). Age-dependent resistance to viral encephalitis: studies of infections due to Sindbis virus in mice. J. Infect. Dis. *125*, 257–262.

Lawrence, D.M.P., Vaughn, M.M., Belman, A.R., Cole, J.S., and Rall, G.F. (1999). Immune-mediated protection of adult but not neonatal mice from neuron-restricted measles virus infection and CNS disease. J. Virol. *73*, 1795–1801.

Liebert, U.G., and Finke, D. (1995). Measles virus infections in rodents. Curr. Topics Microbiol. Immunol. *191*, 149–166.

Lipkin, W.I., Villarreal, L.P., and Oldstone, M.B.A. (1989). Whole animal section in situ hybridization and protein blotting: new tools in molecular analysis of animal models for human disease. Curr. Topics Microbiol. Immunol. *143*, 33–54.

Liszewski, M.K., Post, T.W., and Atkinson, J.P. (1991). Membrane cofactor protein (MCP or CD46): newest member of the regulators of complement activation gene cluster. Annu. Rev. Immunol. *9*, 431–455.

Lomeli, H., Mosbacher, J., Melcher, T., Hoger, T., Geiger, J.R.P., Kuner, T., Monyer, H., Higuchi, M., Bach, A., and Seeburg, P.H. (1994). Control of kinetic properties of AMPA receptor channels by nuclear RNA editing. Science *266*, 1709–1712.

Long, E.O., and Jacobson, S. (1989). Pathways of viral antigen processing and presentation to CTL: defined by the mode of entry? Immunol. Today *10*, 45–48.

Manchester, M., Liszewski, M.K., Atkinson, J., and Oldstone, M.B.A. (1994). Multiple isoforms of CD46 (membrane cofactor protein) serve as receptors for measles virus. Proc. Natl. Acad. Sci. USA *91*, 2161–2165.

Markowitz, L., and Katz, S. (1994). In Vaccines, S. Plotkin and B. Mortimer, eds. (Philadelphia: Saunders), pp. 229–276.

McChesney, M.B., and Oldstone, M.B.A. (1989). Virus-induced immunosuppression: infections with measles virus and human immunodeficiency virus. Adv. Immunol. *45*, 335–380.

Mosier, D.E. (1974). Ontogeny of mouse lymphocyte function. I. Paradoxical elevation of reactivity to allogeneic cells and phytohemagglutinin in Balb-c fetal thymocytes. J. Immunol. *112*, 305–310.

Mrkic, B., Pavlovic, J., Rulicke, T., Volpe, P., Buchholz, C.J., Hourcade, D., Atkinson, J.P., Aguzzi, A., and Cattaneo, R. (1998). Measles virus spread and pathogenesis in genetically modified mice. J. Virol. *72*, 7420–7427.

Naniche, D., Varior-Krishnan, G., Cervoni, F., Wild, T.F., Rossi, B., Rabourdin-Combe, C., and Gerlier, D. (1993). Human membrane cofactor protein (CD46) acts as a cellular receptor for measles virus. J. Virol. *67*, 6025–6032.

Oldstone, M.B.A. (1997). How viruses escape from cytotoxic T lymphocytes: molecular parameters and players. Virology *234*, 179–185.

Oldstone, M.B.A. (1998). Measles virus. In Viruses, Plagues and History, M.B.A. Oldstone, ed. (New York: Oxford Press), pp. 73–89.

Oldstone, M.B.A., Tishon, A., Chiller, J., Weigle, W., and Dixon, F.J. (1973). Effect of chronic viral infection on the immune system. I. Comparison of the immune responsiveness of mice chronically infected with LCM virus with that of noninfected mice. J. Immunol. *110*, 1268–1278.

Oldstone, M.B.A., Blount, P., Southern, P.J., and Lampert, P.W. (1986). Cytoimmunotherapy for persistent virus infection: unique clearance pattern from the central nervous system. Nature *321*, 239–243.

Oldstone, M.B.A., Nerenberg, M., Southern, P., Price, J., and Lewicki, H. (1991). Virus infection triggers insulin dependent diabetes mellitus in a transgenic model: role of anti-self (virus) immune response. Cell *65*, 319–331.

Patterson, J.B., Thomis, D.C., Hans, S.L., and Samuel, C.E. (1995). Mechanism of interferon action: double-stranded RNA-specific adenosine deaminase from human cells is inducible by alpha and gamma interferons. Virology *210*, 508–511.

Radecke, F., Spielhofer, P., Schneider, H., Kaelin, K., Huber, M., Dotsch, C., Christiansen, G., and Billeter, M.A. (1995). Rescue of measles viruses from cloned DNA. EMBO J. *14*, 5773–5784.

Rall, G.F., Manchester, M., Daniels, L.R., Callahan, E.R., Belman, A.R., and Oldstone, M.B.A. (1997). A transgenic mouse model for measles virus infection of the brain. Proc. Natl. Acad. Sci. USA *94*, 4659–4663.

Rima, B.K., Earle, J.A.P., Baczko, K., Rota, P.A., and Bellini, W.J. (1995). Measles virus strain variations. Curr. Topics Microbiol. Immunol. *191*, 65–84.

Rockenstein, E., McConlogue, L., Tan, H., Gordon, M., Power, M., Masliah, E., and Mucke, L. (1995). Levels and alternative splicing of amyloid β protein precursor (APP) transcripts in brains of transgenic mice and humans with Alzheimer's disease. J. Biol. Chem. *270*, 28257–28267.

Rolling, B.J. (1997). Chemokines. Blood *90*, 909–928.

Rozenblatt, S., Koch, T., Pinhasi, O., and Bratosin, S. (1979). Infective substructures of measles virus from acutely and persistently infected cells. J. Virol. *32*, 329–333.

Russell, S.M., Sparrow, R.L., McKenzie, I.F.C., and Purcell, D.F.J. (1992). Tissue-specific and allelic expression of the complement regulator CD46 is controlled by alternative splicing. Eur. J. Immunol. *22*, 1513–1518.

Schnorr, J.-J., Xanthakos, S., Keikavoussi, P., Kampgen, E., ter Meulen, V., and Schneider-Schaulies, S. (1997). Induction of maturation of human blood dendritic cell precursors by measles virus is associated with immunosuppression. Proc. Natl. Acad. Sci. USA *94*, 5326–5331.

Tanaka, N., Sato, M., Lamphier, M.S., Nozawa, H., Noguchi, S., Schreiber, R.D., Tsuyimoto, Y., and Taniguich, I. (1998). Type I interferons are essential mediators of apoptosis in virally infected cells: genes to cells. Cell *3*, 29–37.

ter Meulen, V., Stephenson, J.R., and Kreth, H.W. (1983). Subacute sclerosing panencephalitis. In The Viruses, Vol. *18*, H. Fraenkel-Conrat and R.R. Wagner, eds. (New York: Plenum Press), pp. 105–159.

Thorley, B.R., Milland, J., Christiansen, D., Lanteri, M.B., McInnes, B., Moeller, I., Rivailler, P., Horvat, B., Rabourdin-Combe, C., Gerlier, D., McKenzie, I.F., and Loveland, B.E. (1997). Transgenic expression of a CD46 (membrane cofactor protein) minigene: studies of xenotransplantation and measles virus infection. Eur. J. Immunol. *27*, 726–734.

Thornberry, N.A., and Lazebnik, Y. (1998). Caspases: enemies within. Science *281*, 1312–1315.

Tishon, A., Southern, P.J., and Oldstone, M.B.A. (1988). Virus-lymphocyte interactions. II. Expression of viral sequences during the course of persistent lymphocytic choriomeningitis virus infection and their localization to the L3T4 lymphocyte subset. J. Immunol. *140*, 1280–1284.

Trgovcich, J., Ryman, K., Extrom, P., Eldridge, J.C., Aronson, J.F., and Johnston, R.E. (1997). Sindbis virus infection of neonatal mice results in a severe stress response. Virology *227*, 234–238.

von Herrath, M., Dockter, J., and Oldstone, M.B.A. (1994). How virus induces a rapid or slow onset insulin-dependent diabetes mellitus in a transgenic model. Immunity *1*, 231–242.

Yannoutsos, N., Ijzermans, J.N., Harkes, C., Bonthuis, F., Zhou, C.Y., White, D., Marquet, R.L., and Grosveld, F. (1996). A membrane cofactor protein transgenic mouse model for the study of discordant xenograft rejection. Genes Cells *1*, 409–419.

Zingoni, A., Soto, H., Hedrick, J.A., Stoppacciaro, A., Storlazzi, C.T., Sinigaglia, F., D'Ambrosio, D., O'Gara, A., Robinson, D., Rocchi, M., et al. (1998). Cutting edge: the chemokine receptor CCR8 is preferentially expressed in Th2 but not Th1 cells. J. Immunol. *161*, 547–551.