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Role of CD46 in Measles Virus Infection in CD46 Transgenic Mice

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The susceptibility of CD46 (human membrane cofactor protein) transgenic mice to measles virus (MV) infection was investigated. Cell cultures (lung and kidney) established from transgenic and control mice showed that although both could be infected only those from the CD46+ mice gave fusion. A complete round of replication with the release of infectious virus was detected exclusively in the transgenic cell cultures whose permissiveness to MV was markedly less than that of Vero cells. The ability of MV to replicate *in vivo* in mice was studied using both vaccine and laboratory-adapted wild-type strains of virus. After intraperitoneal and intranasal inoculations of transgenic mice, virus replication could not be detected. In contrast intracerebral inoculation induced infection in both transgenic and nontransgenic mice. Our results from *in vitro* infection studies support the hypothesis that CD46 is a major host cell factor involved in the MV-induced fusion process and MV entry. The studies further indicate that MV tropism is not governed solely by the expression of the CD46 gene and that the high efficiency of the replicative cycles characteristic of fully permissive host cells requires additional factors, which are lacking in both transgenic and nontransgenic mice.

INTRODUCTION

Measles is one of the primary health problems for infants in the developing world. The widespread use of live attenuated vaccines has reduced the prevalence of disease in industrialized countries (Bloom, 1989). The natural host range of measles virus (MV) is restricted to man in whom the typical clinical infection is characterized by viral replication in cells of the lymphoid system with subsequent dissemination to tissues throughout the body (Esolen *et al.,* 1993; Karp *et al.,* 1996).

The hemagglutinin (H) and fusion (F) envelope glycoproteins are involved in the receptor-mediated binding and entry of MV into cells. A host cell receptor for MV was identified as the human membrane cofactor protein, CD46 (Dörig *et al.,* 1993; Naniche *et al.,* 1993), a member of the regulator of the complement activation (RCA) family. Antibodies to CD46 inhibit MV infection in permissive host cells (Naniche *et al.,* 1993), and transfection of a cDNA coding for CD46 into murine or hamster cells nonpermissive for MV permits MV replication confirming the role of CD46 as a receptor for MV (Naniche *et al.,* 1993; Manchester *et al.,* 1994, 1995; Yanagi *et al.,* 1994). Studies *in vitro* and *in vivo* reported a restriction of MV replication in cells in both CD46 transgenic and nontransgenic murine cells (Horvat *et al.,* 1996).

In vivo animal models are essential to study the effects of the individual viral components in the context of the immune system and to evaluate the efficacy of novel vaccine and therapeutic strategies. Rodents can be infected with neuroadapted strains, but these infections are restricted to the central nervous system (Liebert and Finke, 1995). The SCID mouse with co-implants of human fetal thymus and liver cells has recently proven valuable for the study of certain aspects of MV infection (Auwaerter *et al.,* 1996), but this artificial system presents problems in studying a broader range of virus-host interactions involved in systemic MV infections.

In search of a suitable small animal model susceptible to MV, transgenic mice expressing a genomic copy of CD46 were studied for their permissivity to MV infection both *in vivo* and *in vitro.* Thus such mice can potentially express the different isoforms of CD46. We show that although cell cultures established from the CD46+ mice can be infected by MV, we were only able to detect virus infection *in vivo* after intracerebral inoculation of mice.

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TABLE 1

FACS Analysis of Expression of CD46 on Transgenic and Non-Transgenic Cell Lines of Murine Origin and on Cell Lines of Simian and Human Origin

	MAb anti-CD46, SCR 1					MAb anti-CD46, SCR 4
Cell Type	MCI20.6	29	E8/5	E12/4	13/42	10/88
Mouse transgenic[a]	+	+	+	+	+	+
Mouse non-transgenic[a]	−	−	−	−	−	−
Vero	−	+	−	−	+	+
Hela	+	+	+	+	+	+

Note. Data on monoclonal antibody reactivities; +, positive; −, negative; SCR, short consensus region.
[a] Results from kidney and lung cell cultures established from two different mice.

RESULTS

In vitro infection studies

To test the susceptibility to MV infection, cultures of lung and kidney from transgenic mice and nontransgenic controls were prepared. The transgenic mouse cells were examined with a panel of anti-CD46 MAbs, and their FACS profiles were similar to those found in cells of human origin (Table 1, Fig. 1). To test the fusability of the CD46-expressing cells, they were infected with a vaccinia recombinant expression the MV F and H proteins. This resulted in syncytia formation in the CD46+ but not in CD46− cells (Fig. 2).

Cultures from lungs and kidneys were infected with the Hallé strain of MV (m.o.i. 0.5 or 3 PFU/cell). Forty-eight hours after infection, syncytia appeared in cultures of transgenic mouse and Vero cell cultures (positive controls), whereas syncytia were not observed in cultures established from nontransgenic mouse cells (Fig. 2). Similarly syncytia developed after infection with Edmonston, Y15, and MA93F virus strains in transgenic mouse cell cultures and not in corresponding nontransgenic mouse cell cultures.

In contrast to MV infection in Vero cell cultures, the syncytia in the transgenic mouse cells did not spread to involve the entire monolayer. By indirect IF using anti-N, -M, -F, and -H MAbs intracellular viral antigen was detected in both transgenic and nontransgenic cell cultures. The majority of CD46+ cells expressed viral antigen 44 h postinfection, whereas only a few IF positive cells in nontransgenic cells were observed at this time (Fig. 3).

To test for infectious viral progeny after primary passage in mouse cells, cell culture supernatants or cell lysates (freeze-thawed cells) were passaged onto either mouse cell cultures or Vero cell cultures. Viral passage in mouse cell cultures were achieved from cell lysates of transgenic cultures but with a decreasing efficiency on further passage (Fig. 4). Cell culture supernatants of transgenic cells, but not of nontransgenic cells, contained low levels of infectious virus. When assayed in Vero cells, titers of up to 10^2 $TCID_{50}$/ml were detected in supernatants of transgenic cells but not in nontransgenic cells (data not shown).

Further attempts to isolate MV in transgenic mouse cells by five consecutive blind passages performed with the Hallé, Edmonston, and MA93F strains with 6- to 7-day intervals using trypsinized cells, culture supernatants, or cell lysates failed (data not shown).

In vivo infection studies

To study the susceptibility of transgenic mice to MV infection, mice were inoculated intraperitoneally, intranasally, or intracerebrally.

Intraperitoneal and intranasal infection experiments. Transgenic mice and their negative control litter mates (BALB/c × Black6/SJL) were inoculated intraperitoneally with the Hallé strain of MV (Table 2, Experiment 1). PBMC and cryo sections of lung, thymus, or spleen were examined for viral antigen by IF. MV antigen could not be detected in either the transgenic or control mice 3–9 days postinoculation (Table 2, Experiment 2). Similarly infectious virus was not detected and no serum neutralizing antibodies were found in the mice 3 weeks after inoculation (Table 2, Experiment 2).

Further studies were performed using a different MV strain (Ma93F), but neither viral antigen nor infectious virus could be detected (Table 2, Experiment 3). Only on Day 4 postinoculation were viral transcripts detected in two transgenic mice (Table 3).

In a further series of experiments, the amount of virus was increased (2.4×10^7 PFU/mouse, Experiment 4). Intranasal infection was performed in 16 transgenic mice of which 12 were also inoculated intraperitoneally. Mice were sacrificed on Days 2, 5, 7, 14, and 21 postinoculation and examined for the presence of viral transcripts. On Days 2, 5, and 7 postinoculation, MV-F transcripts

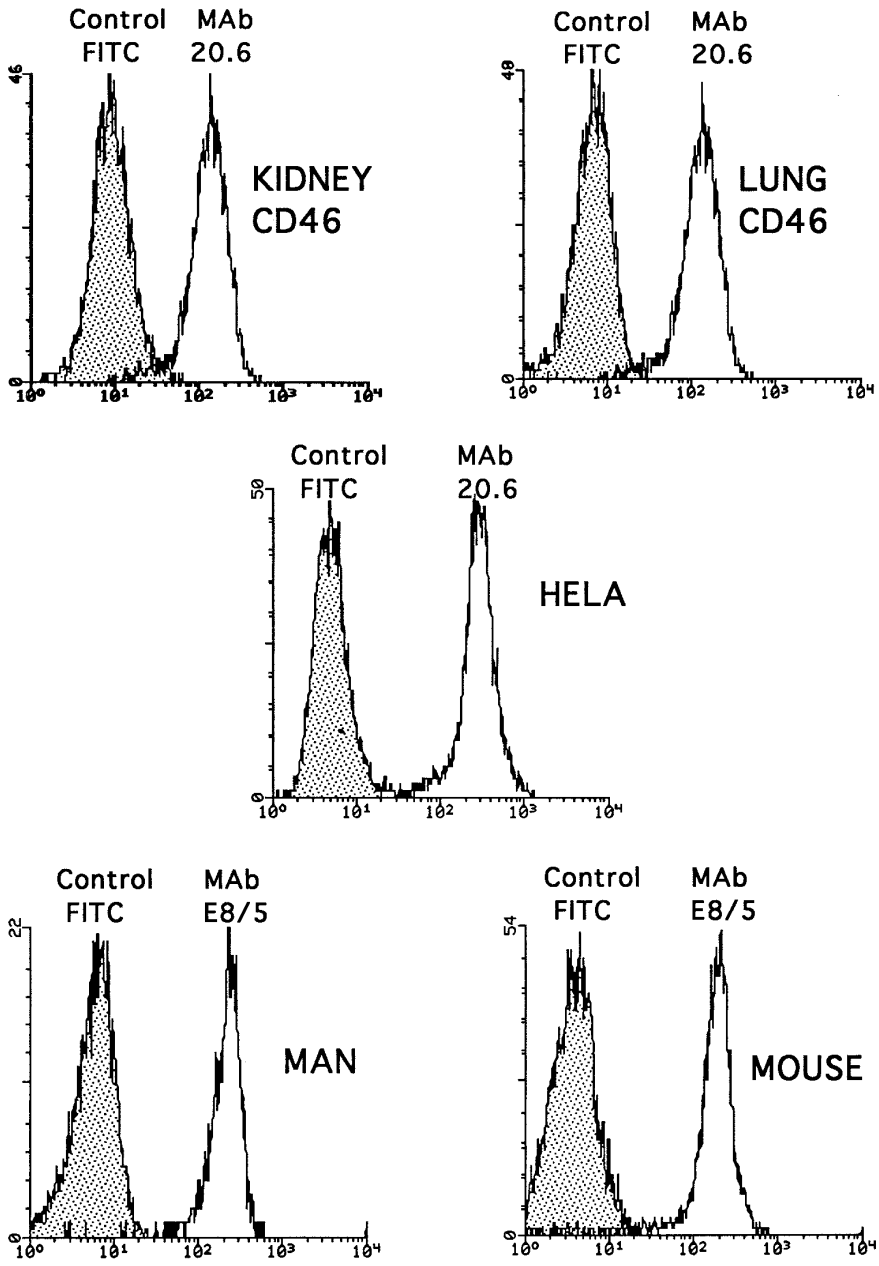

FIG. 1. Cells (5×10^5 cells) of established kidney (Kidney CD46) and lung (Lung CD46) cell cultures from CD46 transgenic mice, of HeLa cells, and of lymphocytes for man (man) and transgenic mouse (mouse) were preincubated with PBS (Control FITC) or MAb 20.6, alternatively MAb E8/5, washed, exposed to a FITC-labeled secondary antibody, and analyzed by cytofluorometry. Fluorescence histograms are shown in which fluorescence intensity (*x* axis) is plotted versus relative cell number (*y* axis).

were detected in tissue samples (lung, kidney, intestine) of five transgenic mice (Table 3). Virus isolation was attempted from lung tissue of two mice 5 days after intranasal inoculation with negative results, and no viral antibodies were detected in serum samples 4 or 20 weeks after inoculation (Table 2).

Intracerebral challenge experiments. Intracerebral infection with the Yamagata and the Edmonston strains was performed in 4- to 5-week-old mice (Table 2, Experiments 5 and 6). Infectious virus was not recovered from the brains of the mice assayed on Days 7 and 14 postinoculation (Table 2). Cryo sections of brain tissue on Day 14 postinoculation were examined for viral antigen by IF employing the anti-N MAb 25 (Giraudon *et al.,* 1988). MV antigen was detected in the hippocampus of transgenic and nontransgenic mice inoculated with the Yamagata and Edmonston strains, respectively (Table 2, Experiments 5 and 6). The presence of viral RNA was examined in different structures of the brain 4–28 days after infection. Both in Edmonston- and Yamagata-inoculated mice,

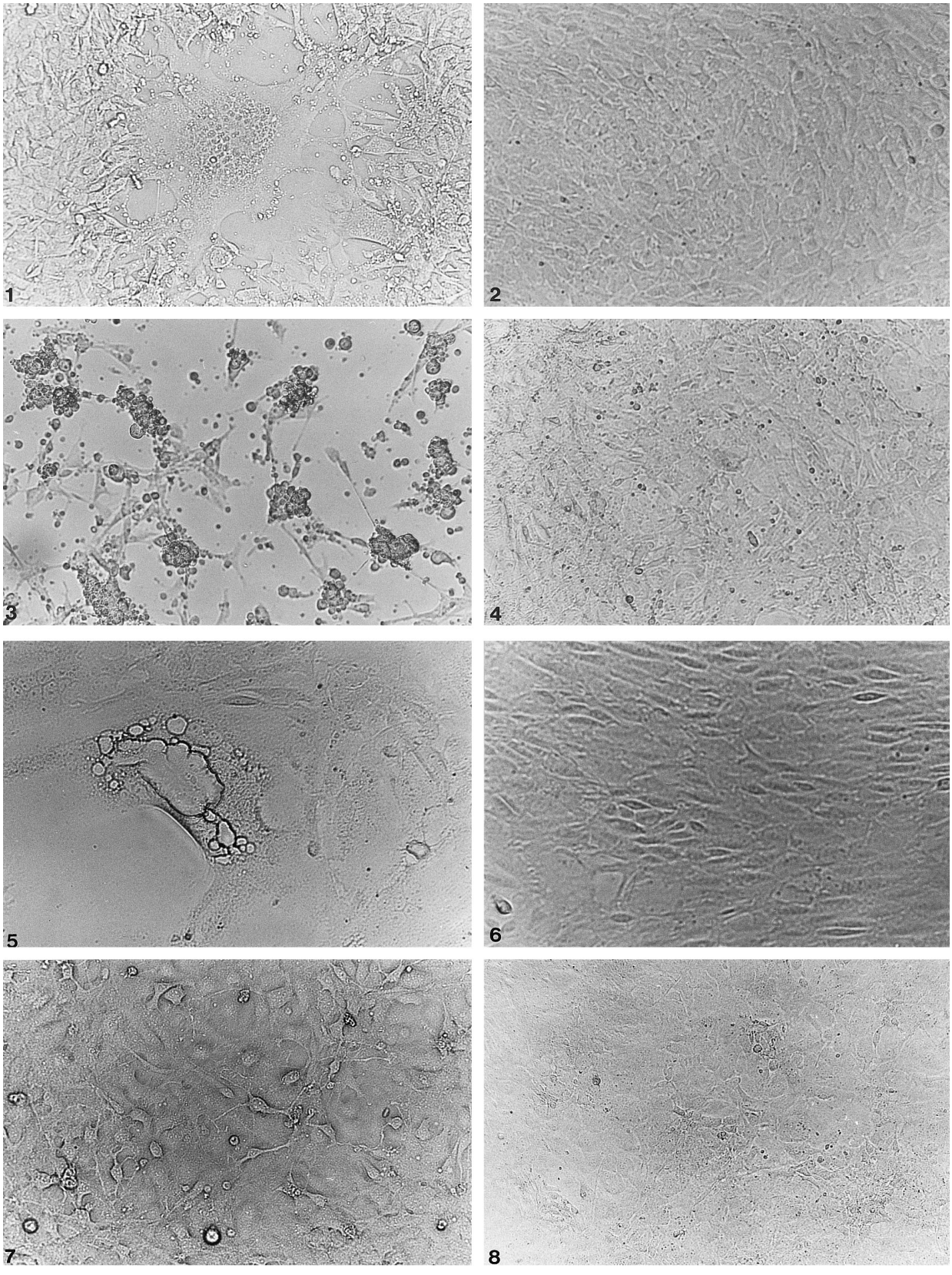

FIG. 2. Lung cell cultures of a CD46 transgenic mouse (1, 2, 5, and 6) and a normal mouse (3, 4, 7, and 8); 24 h after infection with vaccinia virus recombinant encoding the MV H and F proteins (1 and 3) and mock-infection (2 and 4); 3 days after MV infection (Hallé strain) (5 and 7) and mock-infection (6 and 8).

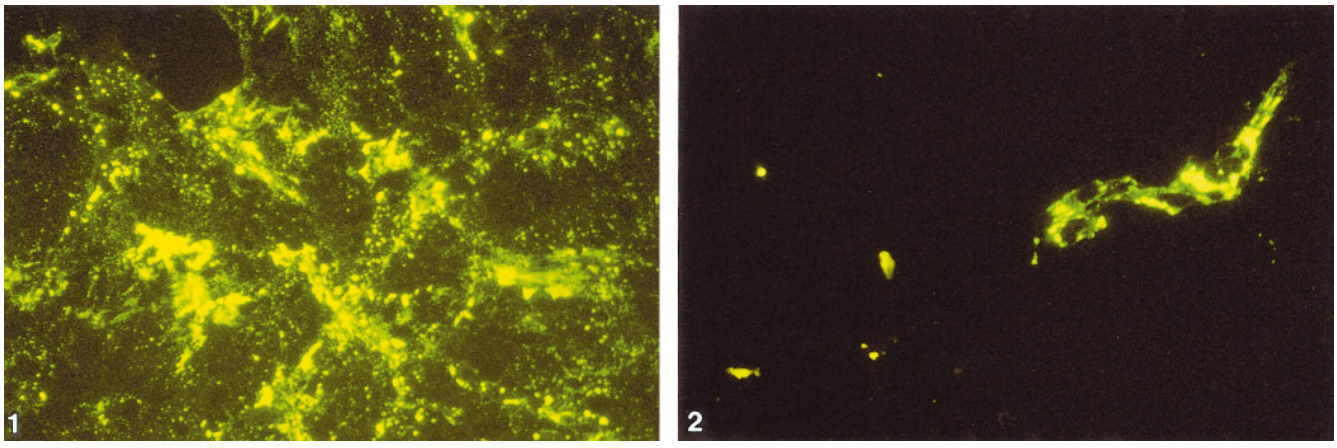

FIG 3. Lung cell cultures of a CD46 transgenic mouse (1) and a normal mouse (2) 44 h after MV inoculation (Edmonston strain). The cells were assayed in indirect immunofluorescence with a MAb against the MV N protein.

MV-F transcripts were detected from Day 4 through Day 28 (Table 4, Fig. 5). It should be noted that it can not be totally excluded that a small amount of amplicons at early time points postinoculation could derive from viral mRNA of the inoculum. Our results, however, clearly indicated that the hybridization signals of amplicons deriving from a constant amount of total RNA increased within the first week postinoculation (data not shown). This time-related kinetics of detectable amplicons provided further evidence that an active transcription/replication of viral nucleic acids has taken place in the mouse brains.

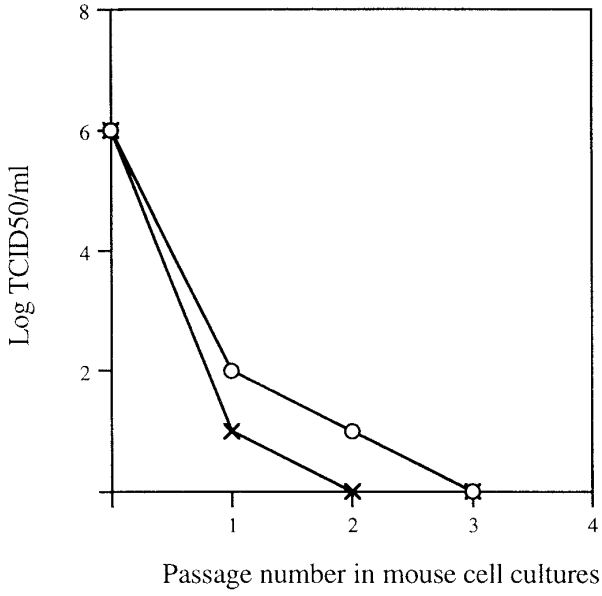

FIG. 4. Replication of MV in CD46-transgenic and nontransgenic mouse cell cultures. The Hallé and Edmonston strains (10^6 $TCID_{50}$) propagated and titrated in Vero cells were passaged in lung cell cultures of CD46-transgenic (○) and nontransgenic mice (×). Both curves represent results obtained with the Hallé and the Edmonston strains. Cell monolayers were incubated with MV (starting with a m.o.i. of 3) overnight at 37°C, then the infecting virus was washed off and replaced with fresh medium, and the cells were further incubated for 4 days. Lysates of these incubations were prepared by freeze-thawing and inoculated onto new mouse cell monolayers. Virus passaged in mouse cell cultures was titrated by determining the highest dilutions that infected 50% of the mouse cell cultures ($TCID_{50}$) as determined by IF detection of intracellular MV antigen.

There was no pronounced difference in the presence, the spread, and the persistence of detectable viral nucleic acids in the transgenic and nontransgenic mice. There was, however, a specific distribution of virus within the different structures of the brain. Thus in the cerebellum, MV appeared to have a restricted growth as compared to the frontal cortex and the hippocampus (Table 4). In 19 of 20 transgenic mice, viral RNA was detected in the hippocampus, in 15 of the 20 mice viral RNA was detected in the cortex, whereas only 7 of the 20 mice had detectable viral transcripts in the cerebellum. On Day 28 after inoculation of the Yamagata strain, viral transcripts were no longer detectable in the cerebellum in either transgenic or in nontransgenic mice (Table 4).

Overt clinical symptoms or death were observed in 3 of the 50 intracerebrally MV-inoculated mice, all of which were nontransgenic. Between Days 14 and 21, two mice inoculated with the Yamagata strain showed clinical signs of disease with retarded growth, ruffled fur, and cramps; one died the day after the clinical symptoms were observed (Day 16 postinoculation), the other was sacrificed on Day 21 (data not shown). Cerebral edema was observed (postmortem) on Days 4, 7 and 28 postinoculation in three mice (two transgenic and one nontransgenic) exclusively after inoculation with the Yamagata strain. No signs of disease or cerebral edema were observed in the sham-inoculated mice.

DISCUSSION

Our impetus of the present study is the need for a small animal model for MV infection. In the CD46 trans-

TABLE 2

Measles Virus Infection Experiments in CD46-Transgenic and Non-Transgenic Mice

Experiment	Viral challenge[a]	Experimental period (days)	Mice[b]		MV isolation	MV antigen detection[c]		Sero-conversion[d]
			n	CD46-Tg/non-Tg		PBMC	Tissue	
1	Hallé	9	2	CD46-Tg	ND	−	−	−
	5×10^6 PFU i.p.		2	non-Tg	ND	−	−	−
2	Hallé	21	4	CD46-Tg	-cocult[e]	ND	ND	−
	5×10^6PFU i.p.		1	non-Tg	-cocult[e]	ND	ND	−
3	Ma93F	10	6	CD46-Tg	ND	−	ND	−
	2×10^6PFU i.p.		3	non-Tg	ND	−	ND	−
4	Hallé	140	16	CD46-Tg	-lung[f]	ND	ND	−
	4×10^6PFU i.n.							
	2×10^7PFU i.p.							
5	Yagamata	28	12	CD46-Tg	−[g]	ND	+[h]	−
	5×10^3 PFU i.c.		13	non-Tg	−[g]	ND	ND	−
6	Edmonston	28	12	CD46-Tg	−[g]	ND	ND	−
	5×10^5 PFU i.c.		13	non-Tg	−[g]	ND	+[h]	−

Note. Data on virus isolation, antigen detection, and seroconversion; +, positive result; −, negative result; ND, not determined.

[a] The Hallé and Edmonston vaccine-related strains, the Ma93F wild-type isolate, and the Yagamata SSPE strain were used. In Experiments 1–4, the mice received intraperitoneal (i.p.) and/or intranasal (i.n.) viral challenge material purified from culture supernatants. The mice in Experiments 6 and 7 were inoculated intracerebrally (i.c.) with suspended Yagamata-infected Vero cells or purified Edmonston virus.

[b] Number (*n*) of CD46 transgenic (CD46-Tg) and nontransgenic (non-Tg) mice.

[c] Peripheral blood mononuclear cells (PBMC) were tested for intracellular MV antigen by indirect immunofluorescence test (IF) on microscope slides and for cell surface antigen by cytofluorometry. PBMC were tested on Days 3, 6, and 9 postinoculation in Experiment 1 and on each day from Day 3 to 10 postinoculation in Experiment 3. Cryo sections of lung, thymus, and spleen tissue were examined for viral antigen by IF on Days 6 and 9 postinoculation.

[d] Examination for MV antibodies in serum samples, taken the first day and at the end of the experimental period, was done by virus neutralization test.

[e] PBMC harvested from two mice at each time point was tested on Days 3, 6, 9, 11, 15, and 18 postinoculation for infectious MV by cocultivation with Vero cells.

[f] Lung tissue from two mice harvested on Day 5 postinoculation was tested for infectious MV in Vero cell cultures.

[g] Brain tissue harvested from individual mice on Days 7 and 14 was tested for infectious MV in Vero cell cultures.

[h] In cryo sections of brain tissue harvested on Day 14 MV antigen was detected in hippocampus.

genic mouse lines investigated, the CD46 gene was expressed in a number of tissues and in B and T lymphocytes at levels similar to man. An advantage to using a genomic construct to generate transgenic mice is that the tissue expression of the various CD46 isoforms appears to have the same pattern as that of corresponding human tissues (Johnstone *et al.*, 1993). It has been demonstrated that four major isoforms of glycosylated CD46 in transfected rodent cells bind MV hemagglutinin with their first and second short consensus regions (SCR) (Iwata *et al.,* 1995; Manchester *et al.,* 1995).

The *in vitro* cultured transgenic lung and kidney cells permitted MV attachment and syncytia formation. Similarly, the CD46 transgene was found to render the cells capable of syncytia formation upon infection with a vaccinia recombinant expressing the H and F proteins, whereas parallel nontransgenic mouse cells did not produce syncytia. Low amount of MV were detected in cell culture supernatants only in the transgenic mouse cells and not in corresponding nontransgenic mouse cells.

After intraperitoneal and intranasal infection, viral transcripts were detected only in tissues of transgenic mice. They were limited to the lung, intestines, and kidneys, being detectable only during the first week postinoculation. No infectious virus could be isolated from any of the animals.

Reports from *in vivo* studies in transgenic rats also reported the lack of recovery of infectious MV (Niewiesk et al., 1997). Our results confirm and extend these findings, suggesting that CD46 expression is not sufficient *in vivo* to render rodents susceptible to MV infection, although explanted cell cultures could grow virus.

A recent report indicated that CD46 influences the *in vivo* susceptibility of neurons of transgenic mice, and it was concluded that CD46 should be absolutely essential to confer susceptibility to infection and disease of the CNS of mice (Rall *et al.*, 1997). Thus Rall et al., (1997) found MV antigen in transgenic mouse brains, while no staining for MV antigen was found in brains of nontransgenic mice infected at any age. This notion was not confirmed in the present study, where MV transcripts and antigen appeared in the brain of both transgenic and nontransgenic mice. The discrepancy between our findings in adult mice and those of Rall et al., (1997) in neonates and adults may be related to the different

TABLE 3

Detection of Measles Virus F Transcripts in CD46 Transgenic and Nontransgenic Mice after Intraperitoneal and Intranasal Viral Exposure

Experiment	Viral challenge	Days postinoculation	Mice[a]		Tissue			
			n	CD46-Tg/non-Tg	Lung	Spleen	Kidney	Intestine
3	Ma93F	4	2	CD46-Tg	+/−	−/−	−/−	−/+
			1	non-Tg	−	−	−	−
		7	1	CD46-Tg	−	−	−	−
			1	non-Tg	−	−	−	−
		10	2	CD46-Tg	−/−	−/−	−/−	−/−
			1	non-Tg	−	−	−	−
4	Hallé	2	2	CD46-Tg	+/−	−/−	$+^{w}$/−	$+^{w}/+^{w}$
		5	2	CD46-Tg	$+^{w}$/−	−/−	−/−	$-/+^{w}$
		7	2	CD46-Tg	+/−	−/−	−/−	−/−
		14	2	CD46-Tg	−/−	−/−	−/−	−/−
		21	2	CD46-Tg	−/−	−/−	−/−	−/−
	Control[b]	—	2	CD46-Tg	−/−	−/−	−/−	−/−

Note. Data on RNA extractions examined for F transcripts by RT–PCR and Southern blot; +, positive; $+^{w}$, weak positive; −, negative.
[a] Number (*n*) of CD46 transgenic (CD46-Tg) and nontransgenic (non-Tg) mice.
[b] Two transgenic noninoculated mice served as negative controls.

mouse lines investigated. In the present studies, intracerebral inoculation of either the Edmonston (vaccine) or the Yamagata (SSPE) strains induced MV RNA transcripts during the first 4 weeks postinoculation in the same areas in the transgenic and nontransgenic mice, including the frontal cortex, hippocampus, and cerebellum. The cerebellum seemed to permit a more restrictive viral multiplication compared to the other brain structures examined. Determinants for the restricted viral multiplication in cerebellum might include susceptibility of the specific cell types to infection or physical restrictions to virus spread in rodents. A similar distribution of viral antigen has been observed in mice after intracerebral inoculation of the closely related canine distemper virus (CDV) (Bernard *et al.*, 1993), while in dogs, a natural CDV host, the virus infection induces a panecephalitis with cerebellar sites consistently involved (Summers and Appel, 1994). Interestingly, in cases of SSPE in humans the

TABLE 4

Detection of Measles Virus F Transcripts in CD46 Transgenic and Nontransgenic Mice after Intracerebral Viral Exposure

Mice group	Viral challenge	Mice (*n*)[a]	Brain tissue[b]	Days postinoculation				
				4	7	14	21	28
CD46-Tg[c]	YAG[d]	2	frontal cortex	+	−	+	++	+
			hippocampus	++	++	+	++	++
			cerebellum	+	−	−	+	−
	EDM[e]	2	frontal cortex	++	++	++	++	++
			hippocampus	++	++	++	++	++
			cerebellum	++	++	−	+	−
non-Tg[c]	YAG	2	frontal cortex	+	++	+	+	++
			hippocampus	++	++	++	++	++
			cerebellum	+	++	+	+	−
	EDM	2	frontal cortex	++	++	−	−	+
			hippocampus	++	++	+	+	+
			cerebellum	++	++	−	−	+

Note. Data on RNA extraction of brain tissue specimens examined for F transcripts by RT–PCR and Southern blot; ++, positive in both mice tested; +, positive in one of the two mice tested; −, negative in both mice tested.
[a] Number (*n*) of mice.
[b] Brain tissue of four mice (two transgenic and two nontransgenic mice) Day 14 post mock-inoculation served as negative controls.
[c] Tg, transgenic.
[d] Yagamata SSPE strain (see Table 2, Experiment 6).
[e] Edmonston vaccine strain (see Table 2, Experiment 7).

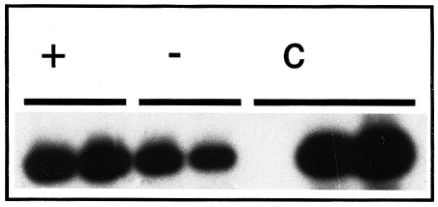

FIG. 5. Southern blot hybridization. A fragment (452 bp) of the fusion protein gene of MV was amplified by RT-PCR on polyadenylated RNA extracted from mouse brain tissue (see text). The PCR products were electrophoresed, transferred to nylon membranes, and probed with a ^{32}P-labeled internal 30 mers oligonucleotide probe (see text). Transgenic mice (+) and nontransgenic mice (−), controls (c). Lane 1, Day 14 after Yamagata inoculation; lane 2, Day 14 after Edmonston inoculation; lanes 3 and 4, Days 7 and 28, respectively, after Edmonston inoculation; lane 5, negative control (rat glioma cell line, C6); lanes 6 and 7, positive PCR controls, (MV-infected Vero cells, Hallé and Yamagata, respectively).

MV infection has also been described to involve the cerebellum (Allen *et al.*, 1996; Baczko *et al.*, 1993).

Our results of intracerebral viral challenge clearly indicate that factors other than CD46 govern the uptake of MV in cells of neural tissue of mice. Based on the described inhibitory effect on virus-cell fusion induced by the substance P, this neuropeptide receptor has been proposed to play a role in MV entry (Schroeder, 1986; Harrowe *et al.,* 1990, 1992). It is of interest to further pursue the possibility of neuroreceptors being alternative receptors for uptake, replication, and spread of MV in tissues of the central nervous system in rodents and man.

The results presented here support the idea that efficient MV replicative cycles characteristic of fully permissive host cells require other factors in addition to CD46, that are lacking in nonsusceptible tissues of transgenic mice.

There are several possible explanations for the restricted MV replication in transgenic mouse cells that express CD46 such as the lack of coreceptor factors and/or additional factors required for later stages in viral replication. Only recently has evidence for another unidentified receptor for MV on marmoset B cells appeared (Hsu *et al.,* 1998).

From studies on CD46 transgenic murine macrophages, a block of MV replication between virus transcription and protein synthesis has previously been reported (Horvat *et al.,* 1996).

Our studies on transgenic murine lung and kidney cell cultures did not reveal a total block of MV replication; however, replication with release of infectious extracellular virus was highly inefficient compared to that of primate cell cultures.

The creation of a suitable small animal model for MV infection by use of transgene technology awaits further insights into the crucial factors necessary for MV replication *in vivo* and *in vitro.*

MATERIALS AND METHODS

CD46-transgenic mice

Transgenic mice were generated by microinjection of an 80-kb human CD46 genomic construct into fertilized Black6/SJL mouse oocytes. Thirteen founder mice were obtained, and seven were bred for analysis of G1 offspring. Mice from all seven lines expressed the full-length 3.2-kb human CD46 message in various tissues at levels comparable to that seen in human tissues (data not shown). Nontransgenic mouse RNA did not hybridize to the human CD46 probe. RT–PCR analysis of RNA from transgenic mouse tissues (liver, heart, kidney, and spleen) to identify expression of various CD46 isoforms (Johnstone *et al.,* 1993) demonstrated the same pattern as that seen in corresponding human tissues (data not shown). Nontransgenic BALB/c and F1 generation of Black6/SJL were purchased from IFFA-CREDO, France.

A colony of mice based on the offspring of the original Black6/SJL (H-2^b) transgenic mouse line 29–3 (which carries 1 copy of the CD46 transgene) and the F1, F5, and F6 generations of crossings with BALB/c (H-2^d) were used in infection studies with MV. The mice were screened for the expression of the transgene on peripheral blood mononuclear cells (PBMC) by flow cytometric analysis as described below, and the transgene was found to be expressed in all cells of the B and T subset. CD46 was detected in lung and thymus of transgenic mice by immunofluorescence, while CD46-negative littermates were used as controls. CD46 transcripts were detected in various organs including lung, thymus, kidney, liver, intestine, and brain by RT–PCR on total RNA extractions and subsequent hybridization of Southern blots as described below.

Preliminary results suggested that CD46 is functional in the transgenic mice as assessed by the ability to protect white blood cells from complement-mediated cytotoxicity (data not shown).

MV inoculations in vivo. Inoculation in mice (4–12 weeks old) was performed intranasally (40 μl, 4×10^6 plaque-forming units (PFU)) after ether anesthesia, intraperitoneally in a 200 μl volume (2×10^6 to 2×10^7 PFU or 10^6 infected cells) or by the intracerebral route with a 20- to 30-μl volume (5×10^3 PFU and 5×10^6 PFU).

Uninoculated mice, sham-infected mice inoculated with uninfected cell suspensions or MEM alone served as controls. The inoculated mice were checked for clinical symptoms daily.

Virus and cells

Monolayer cell cultures were established from lung and kidney tissue of CD46-transgenic and nontransgenic mice (Black6/SJL) essentially as described previously (Blixenkrone-Møller *et al.,* 1992).

Vero cells, HeLa cells, and the established murine lung and kidney cultures were grown in DMEM supplemented with 10 mM HEPES, 2 mM L-glutamine, fetal calf serum (2–20%), and antibiotics.

Experiments were carried out with the following MV strains/isolates propagated in Vero cells: (i) Edmonston vaccine (Rouvax) strain originally obtained from Institut Mérieux; (ii) Hallé, a vaccine-like strain (Horta-Barbosa *et al.,* 1971); (iii) Y15 a wild-type isolate adapted to Vero cells (Giraudon *et al.,* 1988); (iv) MA93F, a recent Spanish wild-type isolate (Rima *et al.,* 1995) adapted to Vero cells; and (v) Yamagata, an SSPE strain (Homma *et al.,* 1982). Except for the latter, virus was purified from cell culture supernatants by ultracentrifugation (90 min at 110,000 *g*) in a 30–50% (w/v) discontinuous sucrose gradient; the virus band in the interphase was collected and pelleted by centrifugation in PBS containing 1 mM-EDTA. The pellet was resuspended in PBS and stored at −80°C.

Virus isolation from PBMC and from fresh post-mortem tissue was as previously described (Blixenkrone-Møller *et al.,* 1992). Briefly, subconfluent Vero cell cultures were overlaid with 10^5–10^6 PBMC with a ratio of PBMC to Vero cells from 1:1 to 1:10. Homogenized lung and brain tissue 10% w/v serially diluted were inoculated on subconfluent Vero cells. On Day 4–5 post-cocultivation the Vero cells were trypsinized and passaged. The cultures were monitored for viral antigen by immunofluorescence (IF) after a further 10–12 days.

Stocks of the vaccinia recombinant virus encoding the MV H and F proteins (VVR'H+F) (Drillien *et al.,* 1988; Wild *et al.,* 1992) were propagated as reported previously (Wild *et al.,* 1992).

Murine specimens from *in vivo* experiments

Blood was collected from the tails of the mice. PBMC were isolated from EDTA-stabilized samples by centrifugation (500 g, 20 min) on a Ficoll density gradient (Diatrizoate–Ficoll, 1.077 g/ml, Eurobio, France) and washed twice in RPMI. For intracellular IF, approximately 50,000 cells were air-dried on microscope slides prior to fixation in acetone at −20°C for 5 min.

Mouse tissue specimens from lung, thymus, liver, spleen, small intestine, and kidney were collected, snap-frozen in liquid nitrogen, and stored at −80°C. For IF cryostat sections were mounted on glass slides and fixed in acetone. Brain specimens were collected from mice, which had been perfused under deep anesthesia with cold 0.1 M phosphate-buffered saline (PBS), pH 7.4, to remove blood components. Specimens for RT–PCR from the frontal cortex, hippocampus, and cerebellum were microdissected from the mouse brains prior to storage at −80°C.

RNA extractions, RT–PCR, and Southern blotting

Total RNAs were extracted from 10 to 100 mg of tissue or 5–10 × 10^6 cultured cells according to the methods of Chomzynski and Sacchi (1987). First-strand cDNAs were synthesized from 3 μg of total RNA using reverse transcriptase (Promega, Madison, WI) under conditions described previously (Bencsik *et al.,* 1996). For MV F amplifications, oligo(dT)-primed cDNAs were used, while CD46 amplifications were primed with the CD46-specific antisense primer (see below) using AmpliTaq DNA polymerase (Perkin-Elmer, Cetus, France) (Bencsik *et al.,* 1996). Primers with expected amplicons of 451 and 381 bp for MV F and CD46, respectively, were purchased from Genosys (Cambridge, UK). For amplification of MV-specific gene sequences, a primer pair (5′GGCAATTGAGGCAATCAGACA3′ and 5′CTTGAGAGCCTATGTTGTACG3′) corresponding to mRNA sense and antisense of the MV F gene sequence was used. The DNA was PCR-amplified 1 min at 95°C, 1 min at 55°C, 15 min at 72°C during 40 cycles, followed by 15 min at 72°C.

The selected CD46 primers [5′-ATGGCTACCTGTCTCAGATGACGC-3′, which corresponds to mRNA sense nucleotide (nt) 298–321 and 5′-GTCACCACAATAAATCGTGC-3′ antisense nt 679–660, EMBL/GenBank database Accession No. Y00561 (Lublin *et al.,* 1988)] were run in PCR under the same conditions as given above. The quality and quantity of the RNA and the cDNA were monitored by PCR-amplification of the mouse glyceraldehyde phosphate dehydrogenase gene as detailed previously (Bencsik *et al.,* 1996). The PCR products were analyzed by electrophoresis on a 1% agarose gel, and amplicons of expected sizes (452 bp for MV-F and 381 bp for CD46) were visualized by ethidium bromide staining.

The PCR products of the MV F gene and CD46 protein gene were further analyzed by transfer to a positively charged nylon membrane (Hybond N+, Amersham, UK) and probed with 5′-end labeled [γ-^{32}P]ATP internal oligonucleotides, 5′-CCCGGATAACTCACGTCGACACAGAGTCCT-3× (Esolen *et al.,* 1993) and 5′-AAATGGCCAAGCAGTCCCTGC-3′, respectively.

All preparations from uninoculated or sham-inoculated mice and total RNA of the established murine lung cell cultures and of a rat glioma cell line (C6) were examined by RT and PCR with the MV F primer pairs as negative controls. Similarly, first-strand products of nontransgenic mice were run with the CD46 primer pair as negative controls. Preparations from HeLa cells and from Vero cells infected with the various MV strains, and isolates were used in RT–PCR as positive controls for CD46 and MV F, respectively. "Negative" control samples lacking cDNAs did not produce any amplification bands.

Antibodies, immunofluorescence, flow cytometry, and serology

Mouse monoclonal antibodies (MAbs) against the MV structural proteins, anti-nucleoprotein (N) protein MAbs (125, 25), anti-matrix protein (M) MAbs (A2123, B117, gift of V. ter Meulen), anti-F MAb (F3) (de Vries *et al.,* 1988), anti-H 55 (Giraudon *et al.,* 1988), and the following anti-CD46 MAbs: 29, E8/5, E12/4 (E. Malvoisin and J. Fayolle, unpublished results), MC120.6 (Naniche *et al.,* 1993), and 13/42, 10/88 (gift of J. Schneider-Schaulies, Würzburg, Germany) (Schneider-Schaulies *et al.,* 1995) were employed.

Tissue sections and PBMC preparations on microscope slides were examined for intracellular MV antigen in an indirect IF test employing a human hyperimmune serum as primary antibody (dilution 1/500) and a 1/40 dilution of rabbit anti-human IgG F $(ab')_2$ fragments conjugated to fluorescein isothiocyanate (FITC) (Dako, Glostrup, Denmark). Tissue sections were blocked with 5% normal rabbit serum in PBS before incubation with specific antibodies diluted in PBS containing 2% normal rabbit serum.

The cultured cell preparations and tissue sections from brain were reacted with anti-MV MAbs in an indirect IF technique essentially as previously described (Blixenkrone-Møller *et al.,* 1992). Specific MAb ascites fluids diluted 1/200 or 1/500 served as the primary antibody, FITC-conjugated rabbit anti-mouse IgG F$(ab')_2$ fragments diluted 1/100 (Dako) or 1/200 (Boehringer) were used as second layer. Cell preparations from infected and uninfected Vero cells served as controls in all IF testings.

Flow cytometric analyses. For detection of cell membrane-associated CD46, the MAbs E8/5, MCI20.6, or an unrelated mouse MAb diluted 1/500 in FACS buffer (PBS containing 4% fetal calf serum, 30 mM HEPES, and 0.1% NaN_3), were incubated with cells (5×10^5) on ice for 30 min in a 50 μl volume. After two washes, cells were incubated on ice for a further 30 min with secondary antibody at a 1/100 dilution in FACS buffer. FITC-labeled rat anti-mouse IgG1, heavy-chain specific (Serotec, clone LO-MG1–2, Oxford, U.K.) were used for PBMC, while cultured cells were incubated with FITC-labeled rabbit anti-mouse IgG F$(ab')_2$ fragments (Dako). The washed (twice in FACS buffer) cells were analyzed on a FACScan analyzer (Becton Dickinson, San Jose, CA).

To screen mice for the expression of the CD46 transgene, PBMC were incubated for 30 min on ice with a 1/100 dilution of FITC-labeled MAb E8/5, designated FI-CD46, or an unrelated FITC-labeled mouse antibody in 50 μl FACS buffer. For identification of the T and B cell populations, FITC-conjugated MAb anti-mouse Thy 1,2 (Becton Dickinson), biotinylated MAb anti-mouse CD311 (originally obtained from A. Glasebrook, Lilly Research Laboratories, Indianapolis, IN), and avidin-phycoerythrin (Sigma, St. Louis, MO) were used, and for investigation of their association with CD46, double staining with FI-CD46 and biotinylated MAb anti-Thy 1,2 or anti-CD311 and avidin-phycoerythrin were employed.

Serology. Virus neutralization tests in Vero cells were performed according to the method described previously (Blixenkrone-Møller *et al.,* 1991). Serial twofold dilutions of sera starting at 1/20 were incubated with 25 PFU of MV All samples were tested in duplicate. The highest dilution of the serum sample neutralizing MV was given as the titer.

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