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High Pathogenicity of Wild-Type Measles Virus Infection in CD150 (SLAM) Transgenic Mice

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Measles virus (MV) infection causes an acute childhood disease, associated in certain cases with infection of the central nervous system and development of a severe neurological disease. We have generated transgenic mice ubiquitously expressing the human protein SLAM (signaling lymphocytic activation molecule), or CD150, recently identified as an MV receptor. In contrast to all other MV receptor transgenic models described so far, in these mice infection with wild-type MV strains is highly pathogenic. Intranasal infection of SLAM transgenic suckling mice leads to MV spread to different organs and the development of an acute neurological syndrome, characterized by lethargy, seizures, ataxia, weight loss, and death within 3 weeks. In addition, in this model, vaccine and wild-type MV strains can be distinguished by virulence. Furthermore, intracranial MV infection of adult transgenic mice generates a subclinical infection associated with a high titer of MV-specific antibodies in the serum. Finally, to analyze new antimeasles therapeutic approaches, we created a recombinant soluble form of SLAM and demonstrated its important antiviral activity both in vitro and in vivo. Taken together, our results show the high susceptibility of SLAM transgenic mice to MV-induced neurological disease and open new perspectives for the analysis of the implication of SLAM in the neuropathogenicity of other morbilliviruses, which also use this molecule as a receptor. Moreover, this transgenic model, in allowing a simple readout of the efficacy of an antiviral treatment, provides unique experimental means to test novel anti-MV preventive and therapeutic strategies.

Measles virus (MV) infection remains one of the major causes of infant mortality in developing countries, accounting for almost 1 million deaths annually (34, 53). In addition, sporadic outbreaks of acute measles still occur in industrialized countries, provoked by low vaccination coverage, often related to parental concerns over vaccination safety (21). MV causes a highly infectious acute respiratory infection, which can be followed in certain cases by invasion of the central nervous system (CNS), the pathogenesis of which is still poorly understood (22). Acute postinfectious encephalomyelitis occurs during or shortly after acute measles and seems to be associated with an autoimmune pathogenesis. In contrast, subacute sclerosing panencephalitis (SSPE) presents a late neurodegenerative complication of measles, with an incubation period of several years, and is associated with the persistent infection of brain cells, with MV presenting numerous mutations in its genome (6). SSPE occurs in 1 in 100,000 cases of acute measles, causing progressive dementia, seizures, and ataxia. Virus spread takes place in the presence of a high titer of anti-MV antibodies, and an effective treatment for this fatal disease is still not available. The third form of MV-induced CNS disease, progressive infectious encephalitis, known as measles inclusion body encephalitis (MIBE), occurs in immunosuppressed patients 1 to 6 months following measles infection. Seizures, motor and sensory deficits, and lethargy are common, and the disease runs an acute or a subacute fatal course. Nonrestricted virus replication, due to an absent or a decreased immune response, results in cytolytic viral replication in the brain tissue (38). Although measles vaccination has significantly decreased the number of cases of the first two forms of MV-induced encephalitis, this third form remains problematic in an increasing population of immunocompromised patients (15, 32, 35) and has reemerged particularly in children infected with human immunodeficiency virus (5, 31, 46).

An appropriate small-animal model is needed to analyze MV-induced pathology and test novel preventive and therapeutic approaches. Mice are not permissive to MV infection unless neuroadapted MV strains are used (26). However, these strains have several genetic alterations, particularly in the sequence of the receptor binding hemagglutinin protein (H) (11), which is adapted for the utilization of a receptor different from the one MV uses during natural infection (thus inappropriate for the evaluation of antimeasles therapeutic approaches). Cotton rats were shown to be naturally susceptible to MV infection and development of consecutive immunosuppression (37), but, compared to mice, they are genetically and immunologically poorly characterized and their clinical signs of infection are difficult to follow. Different transgenic lines expressing the first identified MV receptor, human CD46, have been generated (20, 33, 41, 48; for a review, see reference 28);

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however, as CD46 is used mainly by vaccine MV strains, these mice were resistant to the infection by wild-type MV.

Identification of the human protein SLAM (signaling lymphocytic activation molecule), or CD150, as a receptor for both wild-type and vaccine MV strains (54) opened up new perspectives for the development of animal models to study MV pathogenesis. SLAM is a type 1 membrane glycoprotein belonging to the immunoglobulin superfamily (8). It is expressed on the surface of activated lymphocytes, macrophages, and dendritic cells and is thought to play an important role in lymphocyte signaling (52). The homology between human and murine CD150 is 57%, and murine CD150 does not serve as a receptor for MV (42). Accordingly, transgenic human SLAM (hSLAM) expression on murine immune cells allowed their permissivity to MV infection and consecutive inhibition of lymphocyte proliferation (17) and dendritic cell functions (18). In addition, when mice are crossed into a genetic background deficient in type I interferon receptor or STAT 1, the susceptibility of CD150 transgenic mice to MV infection increases and obtains a more systemic character, with the presence of transient infection in different organs (51, 56). However, MV infection was not pathogenic in any of the CD150 transgenic lines described so far. Although these different transgenic models provided new information on MV interaction with the immune system, their utilization in the study of prevention and treatment of MV infection has been limited. We have therefore generated transgenic mice ubiquitously expressing SLAM and demonstrated their high susceptibility to MV infection, presenting the first transgenic model where wild-type MV strains can induce a neurological disease. This transgenic model allowed us to compare the levels of neurovirulence of different MV strains and to test novel anti-MV therapeutic and preventive strategies and should permit further dissection of MV-induced neurological syndromes and evaluation of innovative antimeasles approaches.

MATERIALS AND METHODS

Generation of SLAM transgenic mice. The cDNA encoding hSLAM was subcloned into pHMG plasmid containing the mouse housekeeping hydroxymethylglutaryl coenzyme A reductase promoter (14), linearized, and microinjected into the pronuclei of B6DBA mice oocytes at the transgenic animal facility of ENS-Lyon (France). Two founders were identified, and one was used to generate the line. The mice were crossed in a C57BL/6 genetic background for more than 10 generations and transgenic progeny screened by PCR amplification performed on tail-derived DNA, using hSLAM-specific primers (forward, 5′-ACAGACCCCTCAGAAACAAAACCAT-3′; reverse, 5′-CGTGCAGCATGTCTGCCAGAGG-3′). For some experiments, mice were crossed with BALB/c mice and used as F_1. Mice were bred at the institute's animal facility (Plateau de Biologie Experimentale de la Souris, IFR128 BioSciences Lyon-Gerland, France), and in vivo protocols were certified by the Animal Care and Use Committee of ENS-Lyon.

Virus strains. MV vaccine strain Edmonston was obtained from ATCC (VR-24), and Edmonston-Zagreb was kindly provided by D. Forcic and R. Mazuran (Immunology Institute of Zagreb, Croatia). The wild-type MV strains, G954 (genotype B3.2), CR67 (genotype B3.1), and Sm00-5 (genotype B3.1), were isolated in Gambia in 1993, Cameroon in 2001 (24), and Sudan in 2000 (12), respectively, and were propagated on activated human peripheral blood lymphocytes. Viruses were titrated by assaying PFU on Vero-SLAM cell monolayers (25). Vesicular stomatitis virus (VSV) (Indiana strain) was propagated on Vero cells.

Infection of mice and virus titration. Heterozygous suckling transgenic mice and their nontransgenic littermates were infected intranasally (i.n.) by application in both nares of 10 μl of MV (10^3 to 10^4 PFU). For intracerebral (i.c.) infection, 1- to 4-week-old animals were inoculated in the left cerebral hemisphere with the different MV strains (300 PFU) in a volume of 30 to 50 μl. Clinical signs of disease and weight of mice were assessed daily for 3 weeks after infection.

Virus titer was determined using an infectious center assay as described before (13). Briefly, murine organs were isolated and snap-frozen; after thawing and homogenization, different dilutions were cultured on Vero-SLAM cell monolayers for 4 days of culture, fixed, and then stained with methylene blue.

RT-PCR. Total RNA was isolated (RNA-NOW; Biogentex, Ozyme, France) from murine samples. For nonquantitative analysis, MV N-specific RNA was analyzed in samples prepared from the right cerebral hemispheres of MV-injected adult mice, using a Superscript One Step reverse transcription-PCR (RT-PCR) kit (Invitrogen) and MV N-specific primers (forward, 5′-ATCCGCAGGACAGTCGAAGGT-3′; reverse, 5′-AGGGTAGGCGGATGTTGTTCT-3′) as described previously (20).

For quantitative PCR, total RNA was extracted from several murine organs (brain, kidney, nasal-associated lymphoid tissue [NALT], lung, and thymus) at different days postinfection (i.n. route, 3×10^3 PFU per mouse) and treated with DNase I (Sigma). cDNA was obtained using an iScript cDNA synthesis kit (Bio-Rad) and further diluted to perform quantitative PCR using a Platinum SYBR Green qPCR super mix uracil DNA glycosylase kit (Invitrogen). The following primers were used: for the hSLAM gene, forward, 5′-GAGCAACCCTATCAGCAACAATTCC-3′, and reverse, 5′-CCCCTAACAGCCCAGCATACAC-3′, and for the N gene, forward MV G954 NP1, 5′-GCTGCCCATCCTCCAACCG-3′, and reverse MV G954 NP1, 5′-TGAGCCTTGTTCTTCCGAGATTCC-3′. In order to normalize the obtained results, one to three housekeeping genes were quantified: for ubiquitin, forward, 5′-AAGAATTCAGATCGGATGACA-3′, and reverse, 5′-GCCACTTGGAGGTTGACACTT-3′; for hypoxanthine phosphoribosyltransferase, forward, 5′-TCATTATGCCGAGGATTTGGA-3′, and reverse, 5′-CAGAGGGCCACAATGTGATG-3′; and for β-actin, forward, 5′-AAGATCTGGCACCACACCTTC-3′, and reverse, 5′-TTTTCACGGTTGGCCTTAGG-3′. For hSLAM expression analyses, the three housekeeping genes were quantified and used to calculate relative expression levels (see Fig. 1), whereas only ubiquitin was used in N expression analysis (see Fig. 3) as this gene was confirmed by Bestkeeper software to be the most accurate and stable housekeeping gene. Quantitative PCR was performed with an ABI Prism 7000 SDS, and results were analyzed using ABI Prism 7000 SDS software available in the genetic analysis platform (IFR128 BioSciences Lyon-Gerland). The level of expression of the gene of interest in an unknown sample was calculated from the real-time PCR efficiency of primers and the crossing point deviation of the unknown sample versus a standard, as described previously (43). Briefly, these standard references were included in each PCR run for every analyzed gene in order to standardize the PCR run with respect to RNA integrity, sample loading, and inter-PCR variations. The calculated relative expression represents, therefore, the ratio of the expression level of gene of interest versus the expression level of the housekeeping gene (or the geometrical mean of three housekeeping genes if necessary).

Cytofluorometric analysis. Brain structures (cortex, region of the hippocampus, and cerebellum/spinal cord) were dissociated as described before (13). Briefly, different brain structures were isolated from ice-cold phosphate-buffered saline (PBS)-perfused transgenic and nontransgenic mice and gently dissociated by pipetting. Resulting cell suspensions were passed through a 100-μm cell strainer (Becton Dickinson) to remove cell clumps, washed, and incubated with a mouse anti-hSLAM monoclonal antibody (MAb) (IPO-3; Biodesign International) and a goat anti-mouse fluorescein isothiocyanate (FITC)-conjugated antibody (Ab). Dead cells were excluded from analysis by propidium iodide staining. Cells were analyzed with a FACScan instrument (Becton Dickinson) (flow cytometry platform, IFR128 BioSciences Lyon-Gerland).

Immunochemistry. Seven-day-old SLAM transgenic mice and their nontransgenic littermates inoculated with MV i.n. (3×10^3 PFU per mouse) or i.c. (2×10^4 PFU per mouse) were sacrificed 10 days postinfection. Brains were removed and fixed in 4% paraformaldehyde in PBS overnight, transferred to a 30% sucrose solution in PBS, and frozen in Cryomount (Dako). Then, cryostat sections (10 μm) were prepared. Briefly, after being blocked and permeabilized in PBS–2% bovine serum albumin–0.1% Triton X-100, sections were sequentially incubated with primary antibody overnight at 4°C and with secondary antibody for 1 h at room temperature. The following primary antibodies were used: anti-N mouse monoclonal Cl.120 biotinylated antibody (16), anti-glial fibrillary acidic protein (GFAP) rabbit polyclonal serum (G9269; Sigma), and anti-microtubule-associated protein 2 (MAP-2) rabbit polyclonal antibody (Chemicon). These antibodies were detected using streptavidin-tetramethyl rhodamine isothiocyanate, streptavidin-FITC (Jackson Immunoresearch), and Alexa-Fluor 488 goat anti-rabbit antibody (Molecular Probes). Brain sections were analyzed using an Axioplan 2 imaging microscope (Zeiss) and a confocal Axioplan 2 LSM510

microscope (Zeiss) on the imagery platform PLATIM (IFR128 BioSciences Lyon-Gerland).

For the detection of the inflammatory process, 5-week-old mice were injected with MV and sacrificed 7 days later, brains were removed and snap-frozen, and cryostat sections (8 μm) were fixed in acetone or 4% paraformaldehyde in PBS and then analyzed. Briefly, after being blocked in PBS-4% bovine serum albumin-10% normal goat serum, sections were sequentially incubated with primary antibody overnight at 4°C (anti-CD3, -CD4, -CD8, -CD11b, and -B220 mouse monoclonal antibodies; Pharmingen), followed by incubation with secondary antibody (anti-mouse FITC) for 1 h at room temperature. Slides were analyzed with an Axioskop microscope (Zeiss).

Determination of MV-specific antibodies in murine serum by ELISA. Sera were taken from infected mice every 4 weeks for 16 weeks from the retroorbital vein and tested for anti-MV antibodies by enzyme-linked immunosorbent assay (ELISA). MV nucleoprotein obtained from baculovirus-infected insect cells and purified as described previously (29) was coated on 96-well ELISA plates overnight (1 μg/well). Plates were blocked and sera were tested in several dilutions by use of goat anti-mouse horseradish peroxidase-conjugated Ab (A3673; Sigma) and revealed with *o*-phenylenediamine substrate, measuring absorbance at 490 to 650 nm. The titer of N-specific antibodies in each serum sample was determined using a standard curve established with sera from mice immunized with MV in complete Freund adjuvant and expressed in relative units.

Production of recombinant human sSLAM. SLAM was rendered soluble by the removal of the protein's transmembrane region (30 amino acids) and then expressed in the baculovirus expression system. Briefly, two BspE1 sites were introduced into the cDNA encoding human SLAM, at nucleotides 830 and 920, respectively. After BspE1 digestion, the modified SLAM cDNA was then subcloned into the transfer vector pAcHLT-B (Pharmingen), which contains the polyhedrin promoter and the six-His epitope tag, via the PstI and SmaI sites, resulting in plasmid pAcHLT-B-sSLAM. *Spodoptera frugiperda* (SF9) insect cells (Invitrogen) were transfected with a mixture of plasmid pAcHLT-B-sSLAM and wild-type baculovirus DNA (*Autographa californica* multiple nucleopolyhedrosis virus; Pharmingen), and recombinant virus was recovered from the supernatant. To obtain the recombinant protein, supernatant from infected SF9 cells, grown in Grace's insect medium (Gibco BRL) containing 10% fetal calf serum and penicillin-streptomycin at 28°C, was harvested 3 days postinfection and centrifuged and soluble SLAM (sSLAM) was purified according to the QIAGEN protocol for high-level expression and purification of six-His-tagged proteins (QIAexpressionist). Purity of recombinant protein was analyzed by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis with Coomassie brilliant blue staining.

Virus neutralization assays. MV G954 (25 PFU) was incubated with different dilutions of either recombinant sSLAM protein or anti-MV hemagglutinin cl.55 neutralizing antibody (16), in a final volume of 500 μl, for 1 h at 37°C. These mixtures were then layered on Vero-SLAM cells in six-well microtiter plates, and 2 ml of DMEM-2% fetal calf serum was added to each well 2 h later. After 4 days of culture, cells were fixed in 10% formaldehyde and then stained with methylene blue for viral titration. Neutralization was expressed as a percentage of the reduction of PFU compared to levels for control wells without Ab.

For in vivo assays, MV G954 (150 PFU) was incubated with either sSLAM (225 ng) or its elution buffer (NaH_2PO_3, 50 mM; NaCl, 300 mM; imidazole, 250 mM) for 1 h at 37°C and then inoculated i.c. into 7-day-old suckling SLAM transgenic mice and nontransgenic littermates. As a control for sSLAM antiviral specificity, 150 PFU of VSV (Indiana strain) was incubated with 225 ng of sSLAM or control buffer for 1 h at 37°C and injected i.c. into 7-day-old suckling C57BL/6 mice. All animals were observed daily for 4 weeks.

Statistical analysis. Data were expressed as means ± standard deviations. Statistical analyses were performed using a standard Wilcoxon rank test. Briefly, mice were ranked depending on the duration of their survival time, number 1 being the mouse surviving for the shortest time. Mice alive at the end of the experiment were given an arbitrary survival time of 21 days (corresponding to the end of the observation period). Statistical significance between groups was then determined using appropriate tables.

RESULTS

Generation of CD150 (SLAM) transgenic mice. Transgenic mice were generated using a construct in which human SLAM cDNA was linked to the mouse hydroxymethylglutaryl coenzyme A reductase promoter region containing 1.35 kb of upstream sequence, the first (noncoding) exon, and the first intron (Fig. 1A) (14). Human SLAM-specific RNA was detected in various transgenic murine tissues by quantitative PCR using an ABI Prism 7000 SDS, as described in Materials and Methods, and was absent in control littermates and samples without RT (Fig. 1B and data not shown). As the highest expression level was observed in transgenic murine brain, this organ was then analyzed for protein expression. Cell surface expression of SLAM protein was observed in different murine brain structures: cortex, cerebellum/spinal cord, and region of the hippocampus (Fig. 1C).

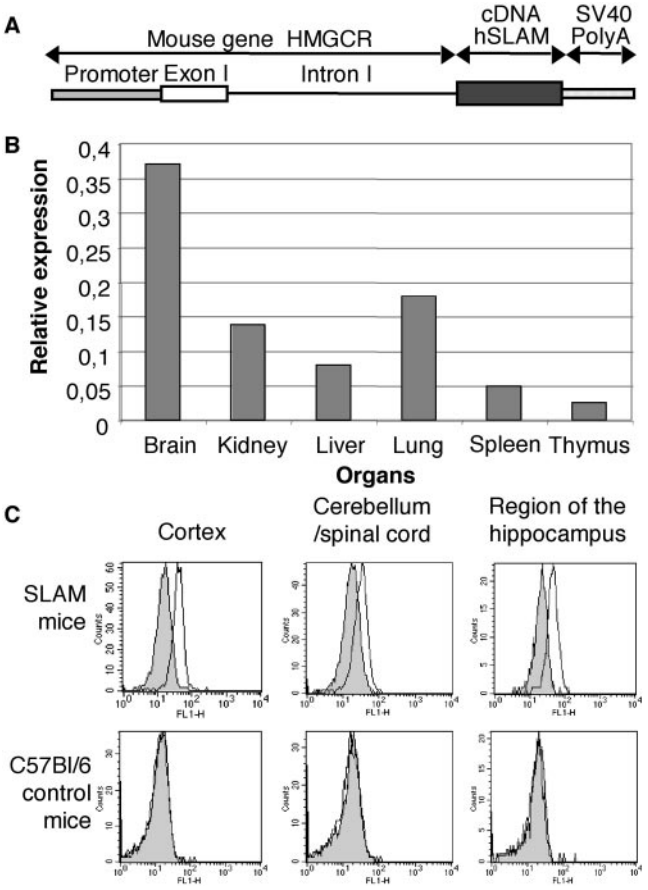

FIG. 1. Expression of SLAM in transgenic mice. (A) DNA construct used to generate transgenic mice. (B) Detection of hSLAM mRNA in SLAM transgenic mouse tissues by quantitative PCR using an ABI Prism 7000 SDS, as described in Materials and Methods. Relative expression of the SLAM transgene was calculated using three different housekeeping genes (β-actin, hypoxanthine phosphoribosyltransferase, and ubiquitin). These results are representative of results obtained from four different mice. (C) Surface expression of hSLAM protein in different brain structures of SLAM transgenic mice, determined by flow cytometry, using anti-SLAM MAb IPO3 (open histogram) followed by FITC-conjugated goat anti-mouse Ab. A negative-control experiment (filled histogram) was performed using the secondary antibody only. Flow cytometric analysis was carried out with a FACScan instrument, and results are representative of four independent experiments. HMGCR, hydroxymethylglutaryl coenzyme A reductase; SV40, simian virus 40; FL1-H, fluorescence intensity in the first laser channel.

SLAM transgenic mice are highly sensitive to MV infection. SLAM transgenic mice and nontransgenic littermates were infected by either the i.n. or the i.c. route with various wild-type or vaccine MV strains (Table 1). The occurrences of clinical

TABLE 1. Sensitivities of 7-day-old mice to infections with different MV strains

Inoculation method	Viral strain (PFU)	Mouse type (no. of mice)	Clinical signs (%)	Mortality (%)	Mean (±SD) survival time (days)[a]
Intranasal	G954 (1.5×10^3–3×10^3)	Control (10)	0	0	
	G954 (1.5×10^3–3×10^3)	SLAM (12)	100	100	13.5 ± 3
	Edmonston (10^4)	Control (8)	0	0	
	Edmonston (10^4)	SLAM (5)	20	20	21 ± 0
Intracranial	G954 (3×10^2)	Control (3)	0	0	
	G954 (3×10^2)	SLAM (6)	100	100	7.7 ± 1
	CR67 (6×10^2)	Control (5)	0	0	
	CR67 (6×10^2)	SLAM (10)	100	100	8.6 ± 1.6
	SM00-5 (3×10^2)	Control (4)	0	0	
	SM00-5 (3×10^2)	SLAM (6)	100	100	9.2 ± 1.5
	Edmonston (3×10^2)	Control (10)	0	0	
	Edmonston (3×10^2)	SLAM (5)	80	60	9.3 ± 2.1*
	Edmonston-Zagreb (3×10^2)	Control (5)	0	0	
	Edmonston-Zagreb (3×10^2)	SLAM (7)	57	43	12.7 ± 2.1**

[a] *, mean survival time was significantly different from those of the groups of SLAM transgenic mice injected with strains G954 and CR67 (Wilcoxon test with a risk factor of 5%). **, mean survival time was significantly different from those of all other groups of SLAM transgenic mice i.c. injected with other MV strains (Wilcoxon test with a risk factor of 1%).

signs and the survival rates of infected mice were evaluated on a daily basis. Suckling infected transgenic mice, in contrast to littermate controls, developed an acute neurological syndrome characterized by seizures, ataxia, lethargy, and weight loss, leading to death within 3 weeks after infection. Both i.n. and i.c. infections induced similar symptoms, although after i.n. inoculation, infection took longer to develop. However, mice were not sensitive to the intraperitoneal route of infection, even when a dose 10 times higher than the one used for i.n. infection was applied, probably due to the lack of permissive environment necessary for initial virus replication.

Similar sensitivities to the infection were observed with three different wild-type strains: G954, CR67, and Sm00-5. However, vaccine strains were significantly less virulent after both i.n. and i.c. infection, resulting in clinical signs of infection in only 20% of mice infected i.n. with the Edmonston strain and in 80% and 57% of transgenic mice infected i.c. with the Edmonston and Edmonston-Zagreb strains, respectively (Table 1). A significantly prolonged survival time (10 ± 1.5 days) was also observed with an increased dose (×20) of Edmonston-Zagreb virus by the i.c. route (Table 1 and data not shown). As all of the MV strains studied are known to use SLAM as a receptor, these data suggest an attenuated neuropathogenic capacity for the vaccine strains in this transgenic model.

The genetic background of the transgenic mice did not seem to play a role, as mice crossed to the C57BL/6 background and those crossed with BALB/c mice showed similar susceptibilities to infection (data not shown). Finally, the severity of the infection declined with the age of the mice: in 2-week-old transgenic mice, i.n. infection with the wild-type virus induced clinical signs and mortality in only 20% of mice. After i.c. infection, 2-week-old transgenic mice were still very sensitive, while infection of 3-week-old mice induced clinical signs and limited mortality. At last, in 4-week-old mice, MV infection was no longer lethal (Fig. 2).

MV spread in suckling transgenic mice. We next analyzed MV propagation in different organs after i.n. infection with MV strain G954, each day up to day 10, when clinical signs were present in the majority of infected transgenic mice. Quantitative PCR was used to measure the appearance of MV nucleoprotein (N) transcripts in different organs (Fig. 3). MV replication was first detected at a low level in the lungs of transgenic mice starting from day 3 postinfection and was continually expressed at a high level from day 8 postinfection (Fig. 3A). MV N was detected in NALT starting from day 6 (Fig. 3B) and in kidneys and thymus from day 8 postinfection (Fig. 3D and E). The highest relative expression of N was observed in the brains of infected mice, starting from day 6 up to the sacrifice of animals (Fig. 3C). A parallel study of i.n.

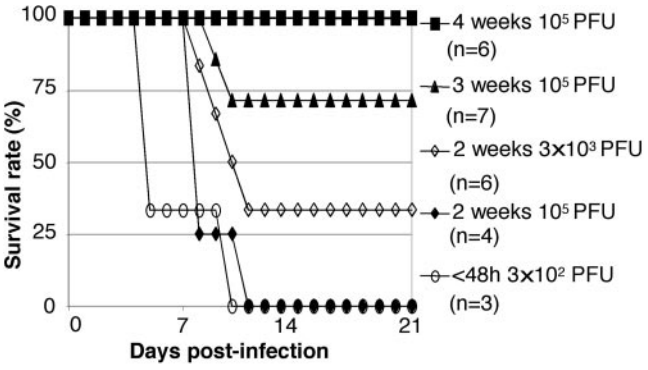

FIG. 2. Survival rates of mice transgenic injected i.c. with indicated doses of MV G954 at different ages (*n*, number of injected mice in the group).

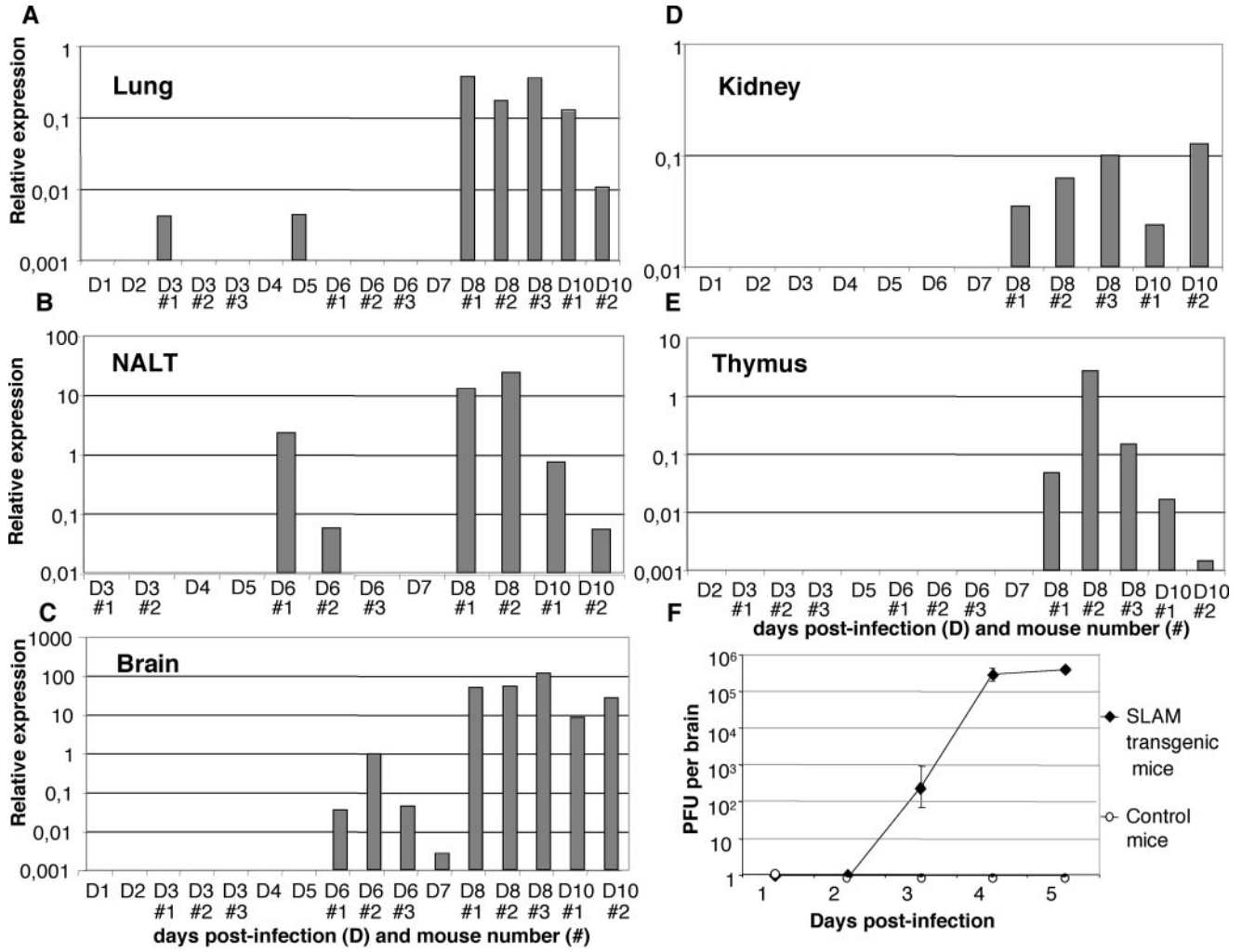

FIG. 3. Kinetics of MV replication in suckling mice. (A to E) Seven-day-old transgenic mice were infected by the i.n. route with 3,000 PFU of MV (G954 strain), and every day after infection (D1, D2, etc.), one to three mice (designated #1, #2, and #3) were sacrificed and analyzed. When no number is given, only one mouse was analyzed at that time point. The following organs were sampled: lungs (A), kidneys (B), NALT (C), thymus (D), and brain (E). RNA was extracted and analyzed by quantitative PCR for the presence of MV N, and relative expression of N was calculated as the ratio of N expression level versus ubiquitin expression level, as described in Materials and Methods. (F) Isolation of infectious virus particles from brains of i.c. infected neonatal mice. SLAM transgenic mice and control nontransgenic littermates were inoculated i.c. 2 days after birth with the MV G954 strain (2×10^4 PFU/mouse). At the indicated times, animals were sacrificed, brains were removed, and viral titers were evaluated on Vero-SLAM monolayers. Data are presented as means of PFU/brain ± standard deviations for groups of two to four mice for each point of titration.

inoculated nontransgenic littermates occasionally showed only transient low levels of N mRNA in the brain and NALT at day 8 postinfection, but it failed to demonstrate any important viral replication in the other organs at any of the time points analyzed (data not shown). Although a high quantity of N mRNA was measured in i.n. infected transgenic mice, no infectious particles could be detected until day 10. Nevertheless, productive MV replication in the brain was observed in i.c. infected transgenic mice early after infection. Thus, to analyze the extension of viral replication in mice, we measured viral titers in the brains of i.c. infected transgenic mice and their control littermates at different time points after infection with the wild-type MV strain G954. Infectious virus particles were detected after coculturing Vero-SLAM cells with brain tissue from infected suckling transgenic mice, starting 72 h after i.c. infection, and the titer reached a maximum of 5×10^5 PFU per brain 4 to 5 days after infection (Fig. 3F). Moreover, virus was also isolated from the spinal cords of the i.c. infected transgenic mice (data not shown). Taken together, these results suggest an active viral replication and propagation in the MV-infected suckling SLAM transgenic mice, particularly in the CNS of infected animals.

MV replication in adult transgenic mice. We next analyzed whether MV replication could be detected in the brains of MV-inoculated adult mice, as this organ seems to be the most susceptible to MV infection in this model. MV N-specific RNA was readily detected in the brains of transgenic, but not nontransgenic, mice up to 3 months after i.c. MV injection (Fig. 4A), demonstrating the persistence of MV infection in the brain after virus inoculation. However, in contrast to results with brains of

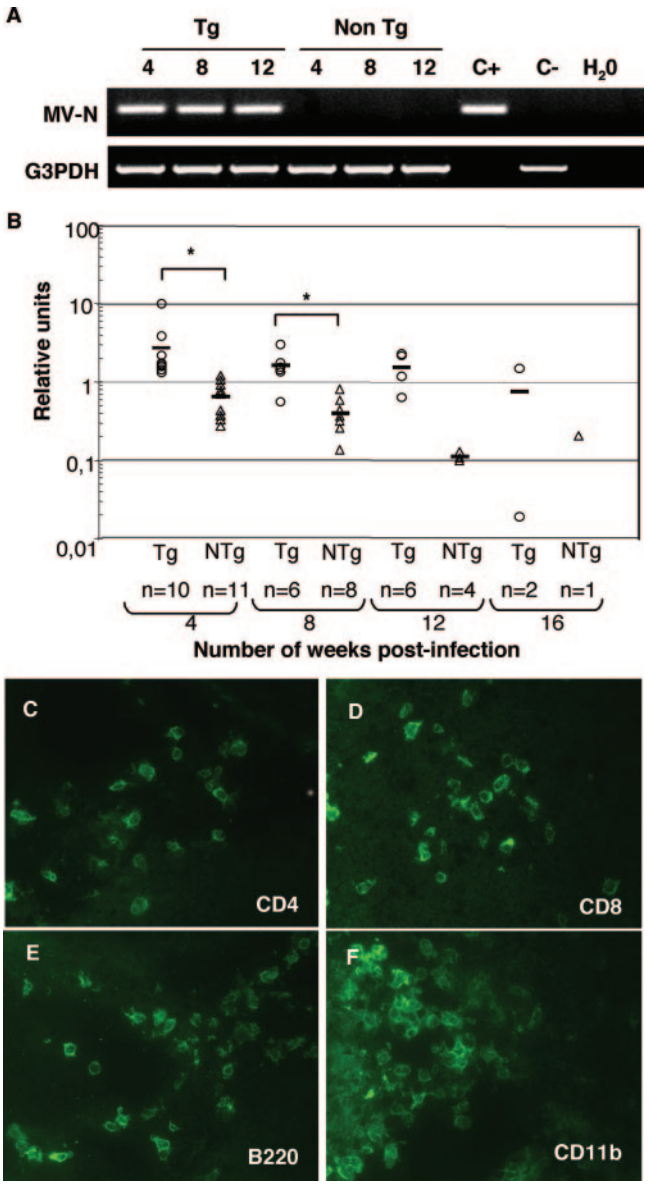

FIG. 4. MV replication in adult mice. (A) RT-PCR analysis of total RNA isolated from adult mouse right brain hemispheres 4, 8, and 12 weeks after i.c. infection with strain G954 at the age of 4 weeks (10^5 PFU/mouse) by use of N-specific and glyceraldehyde-3-phosphate dehydrogenase (G3PDH)-specific primers. (Tg, transgenic; Non Tg, nontransgenic littermate; C+, positive control, MV RNA [CR67 strain]; C−, negative control [RNA from Vero cells]). Data are representative of seven Tg and nine Non Tg mice analyzed 4 weeks after infection, five out of six Tg and eight Non Tg mice analyzed 8 weeks after infection, and three Tg and three Non Tg mice analyzed 12 weeks after infection. (B) Production of anti-N antibodies in the sera of SLAM transgenic mice and littermate controls, injected i.c. at the age of 4 weeks with 0.5×10^5 to 1×10^5 PFU of G954 MV and bled at different time points afterwards. Titers are expressed as relative units, and horizontal bars correspond to the mean of each presented group (*, mean values were significantly different using Wilcoxon test with a risk factor of 1%). NTg, nontransgenic littermates. (C to F) Leukocyte infiltrates in the brains of mice infected i.c. with MV G954 (2×10^4 PFU/mouse). Brain tissue sections were prepared from 5-week-old SLAM transgenic mice 7 days after infection and immunostained with anti-CD4 (C) or anti-CD8 (D) for detection of T cells, anti-B220 (E) for detection of B cells, and anti-CD11b (F) for the detection of microglia and monocytes/macrophages. Magnification, ×400.

infected suckling transgenic mice, infectious viral particles could not be recovered from brains of infected adult transgenic mice (data not shown).

MV infection may induce an immune response in adult mice and therefore may be used as a model in different vaccination approaches. We therefore analyzed whether MV infection generated specific anti-MV antibodies in mice. We show that MV infection of adult transgenic mice induced N-specific antibodies in the serum detectable up to 4 months after infection (Fig. 4B).

We next analyzed the presence of inflammatory cell infiltrates in the brains of MV-infected adult transgenic mice (Fig. 4C to F). Lymphocytes and $CD11b^+$ monocytes/macrophages were observed in the meninges in and around parenchymal blood vessels and the choroid plexus. Leukocyte infiltrates consisted mostly of $CD3^+$ T cells, comprising $CD4^+$ and $CD8^+$ cells (Fig. 4C and D) and to a lesser extent B lymphocytes (Fig. 4E). $CD11b^+$ cells were essentially composed of round cells, infiltrating the perivascular spaces and probably corresponding to monocytes/macrophages (Fig. 4F). These results additionally confirm the generation of a virus-specific immune response in MV-infected adult mice, which may be associated with the lower susceptibility of adult mice to MV infection. Furthermore, leukocyte infiltrates were absent in MV-inoculated nontransgenic and MV-infected suckling mice (data not shown). Finally, only a very few cells stained for N antigen in the brains of infected adult transgenic mice (data not shown), indicating that in adults, in contrast to suckling mice, the immune response may efficiently limit virus replication.

Cellular targets of MV infection in the brain. As the brain seems to be the most affected organ during MV infection in SLAM transgenic mice, we analyzed the regional and cellular sites of MV replication in this organ after i.n. and i.c. infection. Specific MV N granular staining was detected in several regions of the brains of SLAM transgenic suckling infected mice but not in nontransgenic mice inoculated with the same dose of MV (Fig. 5A and I). The most important infection was detected in the piriform cortex (Fig. 5B) and the spinal cord (Fig. 5E) as well as in the hypothalamus (Fig. 5D), the pituitary gland, and the tissues surrounding ventricles (third [Fig. 5C], fourth, and lateral ventricles). This staining was present in all analyzed sections throughout the brain. In i.n. infected transgenic mice as well as in i.c. infected mice, N-positive cells presented an unambiguous neuronal morphology (Fig. 5F and G), which prompted us to determine whether N-expressing cells were positive for the neuron-specific marker MAP-2. Indeed, we found that N was colocalized with MAP-2 (Fig. 5H), suggesting that MV replicates in neurons. However, the astrocyte marker GFAP was not expressed by N-positive cells, although an extensive astrogliosis was observed in various brain areas (Fig. 5J). These results suggest that, similarly to MV-induced encephalitis in humans (2), neurons are main MV targets in the brains of infected SLAM transgenic mice.

Soluble SLAM is a potent inhibitor of MV infection. We next generated a recombinant soluble form of SLAM by using a baculovirus expression system (Fig. 5A) and tested its capacity to inhibit MV infection in vitro and in vivo (Fig. 6B to D). Soluble SLAM exhibited a potent inhibitory activity in vitro, with 50% neutralization obtained with 50 ng of protein, which

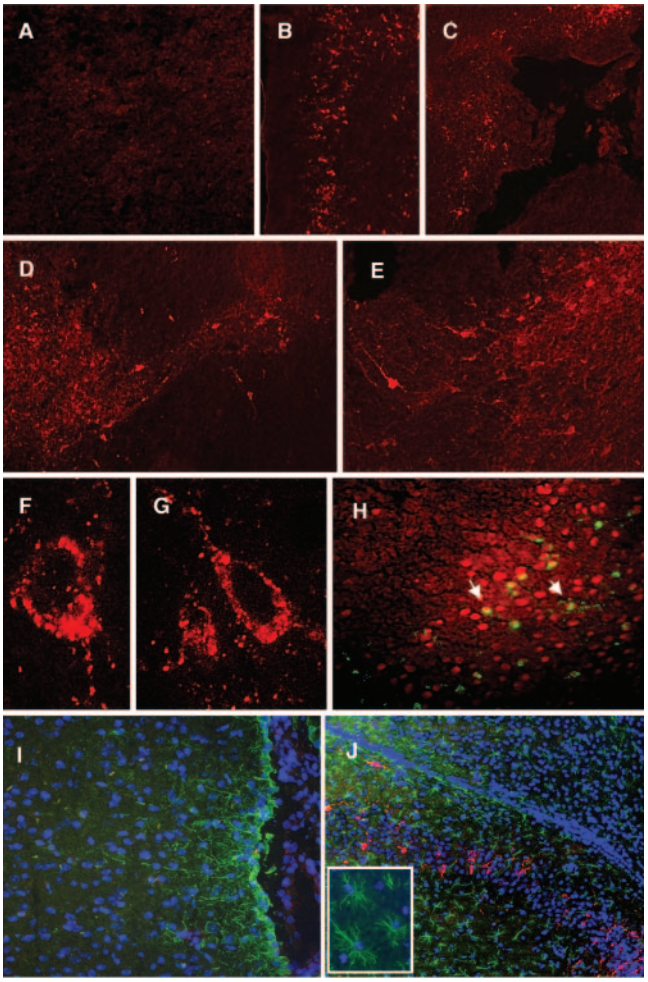

FIG. 5. Immunohistochemical detection of MV nucleoprotein (N). Brain tissue sections from i.n. (A to G) or i.c. (H to J) MV-infected suckling mice were prepared 10 days postinfection. Brain sections were observed using a classical microscope (A to E and H to J) or a confocal microscope (F and G). (A to E) Regional localization of MV N antigen. N (in red) was specifically localized in the piriform cortical region (B) and tissues surrounding the third ventricle (C), hypothalamus (D), and spinal cord (E) for transgenic mice, but not in nontransgenic mice (spinal cord) (A). Magnification, ×50. (F and G) Characteristic neuronal morphology of infected cells. Magnification, ×3,000. (H) N neuronal expression. Shown is the codistribution of N (green) with the neuronal marker MAP-2 (red) in the brains of SLAM transgenic infected mice. Arrows indicate colocalized staining. Magnification, ×400. (I and J) Brain tissue sections were prepared from MV-infected (i.c.) suckling nontransgenic (I) or SLAM transgenic (J) mice and stained for N (in red), GFAP (in green), and cell nuclei, colored with DAPI (4′,6′-diamidino-2-phenylindole) (in blue). N distribution in transgenic mice was localized in the neuronal layer of the hippocampus and was associated with astrogliosis (enlargement in panel J). Magnification for panel I, ×200; magnification for panel J, ×400.

is in the same range as the MV-neutralizing activity of anti-H MAb cl.55 (Fig. 6B). Furthermore, intracerebral coinjection of sSLAM together with MV G954 completely protected transgenic mice from lethal infection (Fig. 6C). In contrast, sSLAM did not protect mice after i.c. injection of VSV (Fig. 6D), demonstrating the MV specificity of sSLAM's antiviral activity and the suitability of the SLAM transgenic model for the study of antimeasles therapeutic approaches.

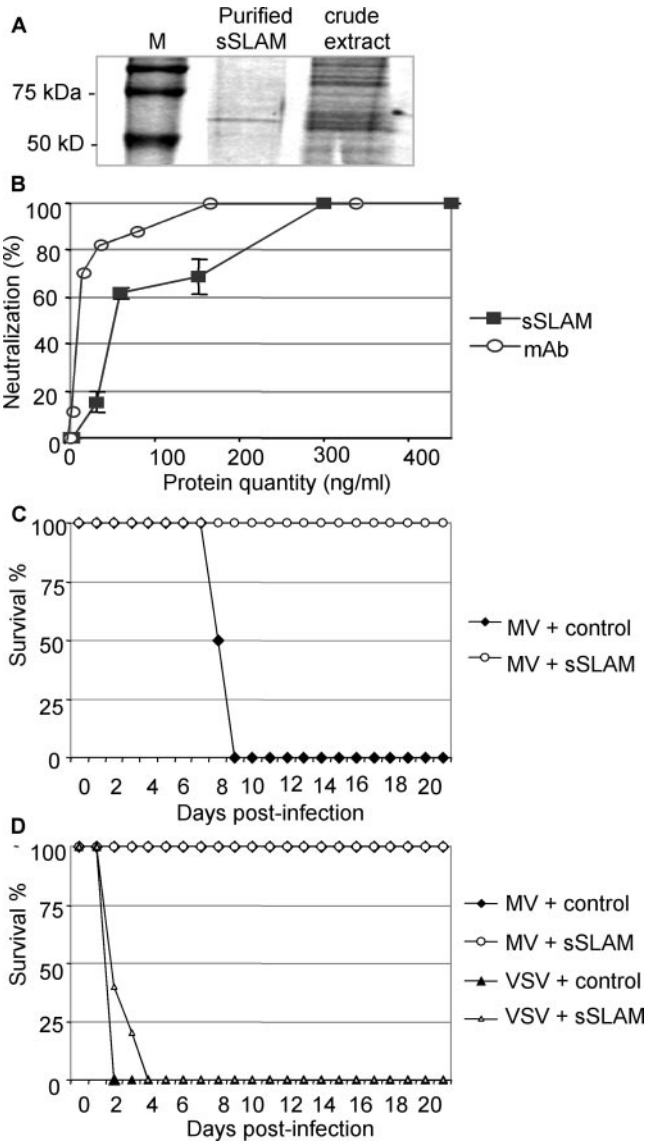

FIG. 6. Antiviral activity of the recombinant sSLAM molecule. (A) Analysis of sSLAM purification from recombinant baculovirus-infected insect SF9 cell supernatant by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. First column, molecular size marker (M); second column, purified recombinant sSLAM; and third column, crude extract before purification of infected SF9 cell supernatant. (B) In vitro neutralization of MV by sSLAM, compared with a neutralizing MAb, cl.55. MV G954 (25 PFU) was incubated with either sSLAM protein or cl.55 for 1 h at 37°C prior to infection of Vero-SLAM cells. Results are expressed as percentages of neutralization as a function of protein quantity. (C) Percentages of survival of SLAM transgenic mice following MV i.c. inoculation, 7 days after birth, in the presence ($n = 5$) or absence ($n = 4$) of 225 ng of sSLAM per mouse. (D) Percentages of survival of C57BL/6 mice after MV or VSV i.c. inoculation in the presence ($n = 1$ or $n = 5$, respectively) or absence ($n = 5$) of 225 ng of sSLAM.

DISCUSSION

As measles virus still infects approximately 40 million people every year (53), the development of novel antimeasles reagents is highly desirable and a number of studies have developed potential antimeasles compounds (44, 45). However, a suitable

small-animal experimental model to test their efficiency, particularly against wild-type MV, was not available. We have therefore generated transgenic mice expressing human CD150 (SLAM), the receptor for both wild-type and vaccine MV strains. We show here that these transgenic mice are highly susceptible to both intranasal and intracerebral MV infection: they develop a systemic infection and a severe neurological syndrome, with a high mortality, depending on the MV strain used. After intranasal infection, MV replication was first detected in lungs and then spread to all organs, being the most intensive in the brain, where it resulted in lethal encephalitis. This is therefore the first SLAM transgenic model where MV infection induces a severe pathogenic effect. The observed pathology is most likely linked to the ubiquitous expression of human SLAM in these transgenic mice, while SLAM expression was restricted to lymphoid tissue in the other recently described murine transgenic models (17, 18, 51, 56). Most of the transgenic animal models reproduce only some of the aspects of a human disease: although the different transgenic mice described so far express SLAM similarly to what has been demonstrated with humans, they do not seem to be susceptible to the development of MV-induced encephalitis, and systemic MV infection is observed only when they are crossed in a type I interferon receptor- or STAT 1-deficient background (51, 56). However, the function of type I interferon signaling is important in the generation of the immune response to MV in mice (19). Therefore, those models will be rather difficult to use when a large-scale screening of antimeasles reagents is required or when preserved interferon signaling is desired. In contrast, the transgenic model described here allows a simple readout of the efficacy of an antiviral treatment and therefore provides a unique experimental means to test novel anti-MV preventive and therapeutic strategies. In addition, as these mice produce antimeasles antibodies in the presence of a competent immune system, they constitute a new animal model to study the efficiency of engineered vaccines based on measles virus (49).

During MV-induced encephalitis, SSPE, and MIBE, the mode of MV entry into brain cells remains obscure. CD46, expressed in the human brain, is not used by wild-type MV strains, and SLAM is considered to be restricted to the cells of the immune system; therefore, potential expression of some other receptor may be involved in MV entry into the brain. Expression of SLAM in the transgenic model described in this study may play the role of that yet-unidentified receptor and allow infection and propagation of MV in the CNS, thus permitting analysis of some aspects of MV neuropathogenesis. In human MIBE, followed by a lethal neurologic syndrome, the absence of an efficient immune response seems to allow productive MV replication in the brain so that infectious virus can be recovered from brain tissue in some cases (1, 38, 40). With infected suckling transgenic mice, the histopathological picture appears to be comparable to that found with MIBE: virus replicates in the CNS, resulting in granular staining in brain parenchyma characteristic of MV nucleocapsid and resembling the measles inclusion bodies, leading finally to the development of the lethal neurologic disease.

Although some of the neuropathology observed with this SLAM transgenic model resembles that previously described for the MV vaccine strain-infected CD46 transgenic mice (13, 33, 48), the major differences of this SLAM transgenic model are its sensitivity to the intranasal route of infection and permissivity to both vaccine and wild-type MV strains. In addition, suckling SLAM transgenic mice remain sensitive to the development of a lethal neurological syndrome up to the age of 3 weeks, while adult mice develop a subclinical infection not reported for CD46 transgenic mice. These differences are probably associated with the higher pathogenicity of wild-type MV strains, as vaccine strains tested in these study showed an attenuated pathology. This SLAM transgenic model should therefore provide the means to directly test the contribution of viral variants and the host immune response in the pathogenesis of this MV-induced fatal neurological disease. This may be especially important in the case of progressive measles encephalitis seen with immunosuppressed patients, particularly with the increasing number of human immunodeficiency virus-infected children (5, 31, 46).

SLAM has also been reported to function as a receptor for two other members of the genus *Morbillivirus* in the *Paramyxovirus* family, canine distemper virus (CDV) and rinderpest virus (RPV) (55), which infect dogs and cattle, respectively, and induce severe disease with high morbidity and mortality (4). Although human, canine, and bovine SLAMs appear to act most efficiently as receptors for MV, CDV, and RPV, respectively, all three morbilliviruses can also utilize human but not murine SLAM to infect cells in vitro (55). Thus, our human SLAM transgenic mice may be used as a convenient small-animal model for the analysis of the role of the SLAM receptor in CDV and RPV pathogenesis, in addition to that of MV. Whether emerging morbilliviruses of aquatic mammals (phocine, dolphin, and porpoise distemper viruses), exhibiting a high level of CNS infection in their natural hosts (23), may use SLAM as a receptor as well and infect SLAM transgenic mice remains to be determined.

Finally, to analyze the suitability of the SLAM transgenic model for studies of novel antimeasles therapeutic approaches, we generated recombinant soluble SLAM and analyzed its antiviral activity in the transgenic model. In contrast to the soluble CD46 molecule, which had a poor antimeasles activity as a recombinant monomeric form (10, 50) and needed octamerization to obtain the potent neutralization capacity of vaccine MV strains (7), our sSLAM exhibited strong antiviral properties against wild-type MV both in vitro and in vivo. As SLAM was proposed to be a homophilic molecule (47), its self-association and formation of SLAM homodimers may account for its potent neutralization activity. Interaction with measles hemagglutinin is localized in the extracellular V domain of SLAM at amino acid positions 60, 61, and 63 (39). The region of SLAM important for its self-association is not known, and homophilic SLAM-SLAM interaction has been suggested to play a role in the regulation of the immune response (47). However, the very low affinity of this association, being considerably weaker than most other well-characterized interactions on the cell surface, has raised the question of the physiological role of this interaction (30). Whether this region needs to be eliminated to avoid side effects sSLAM may have on the stimulation of the immune system and to allow an efficient antimeasles activity of sSLAM in vivo remains to be analyzed. Thus, sSLAM may present a basis for the development of an important candidate in the control of MV infection in immu-

nocompromised patients (1, 3) or in patients suffering from lethal forms of measles encephalitis, for which efficient therapy is still not available, and local distribution of soluble SLAM into cerebrospinal liquid may present a possible approach. Furthermore, sSLAM may be used to control local measles outbreaks and protect infants during the period when immunization is not efficient due to the presence of maternal antibodies.

In summary, these data present the development of a novel small-animal transgenic model highly susceptible to wild-type MV infection. These mice should provide a valuable tool to test innovative therapeutic strategies against measles or infection with other morbilliviruses by use of SLAM as a receptor. In addition, they should facilitate studies of oncolytic properties of MV (36) and the development of recombinant MV-based vaccines (9, 27, 49).

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