Measles virus replication in lymphatic cells and organs of CD150 (SLAM) transgenic mice

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A transgenic mouse containing the complete human SLAM (hSLAM, CD150) gene, including its endogenous promoter for transcription, was generated by using human genomic DNA cloned into a bacterial artificial chromosome. hSLAM, the primary receptor for measles viruses (MV), was expressed on activated B, T, and dendritic cells with an expression profile equivalent to that of humans. We demonstrated that hSLAM+ cells obtained from the transgenic mouse, including activated B, T, and dendritic cells, were susceptible to MV infection in a receptor-dependent manner. Evidence was provided for transient infection in the nasal lymph nodes of hSLAM+ mice after intranasal inoculation. Virus was rapidly cleared without signs of secondary replication. To improve the efficiency of MV production, the hSLAM+ mice were bred with mice having a Stat1-deficient background. These mice were more susceptible to MV infection and produced more virus particles. After intranasal and intraperitoneal inoculation of these mice with MV, infections of the thymus, spleen, nasal, mesenteric, and leg lymph nodes were detected. Upon necropsy, enlarged lymph nodes and spleen were apparent. Flow cytometric analysis showed that abnormally large numbers of mature neutrophils and natural killer cells caused the splenomegaly. The hSLAM transgenic mouse constitutes an improved rodent model for studying the interaction of MV with immune cells that more accurately reflects the infection pattern found in humans.

Activated lymphocytes | Transgenic mouse | Dendritic cells

Measles viruses (MV) is one of the most contagious diseases known to man and is a major killer of children in the developing countries of Africa and South America. The high mortality rate associated with MV infection results from increased susceptibility to opportunistic infections. Although MV has been studied extensively, the mechanism for the immunosuppressive effects observed during MV infection remains unclear (1).

The development of a mouse model may lead to a better understanding of the immune suppression induced by MV. This was previously attempted by expressing CD46, one of the two receptors for MV, in mice (2–6). However, CD46 receptor, a ubiquitously expressed glycoprotein, is only used by vaccine or laboratory adapted strains of MV. Recently, a second receptor for MV was discovered: CD150 or signaling lymphocytic activation molecule (SLAM) (7–9). SLAM is a host cell receptor for MV was discovered: CD150 or signaling lymphocytic activation molecule (SLAM) (7–9). SLAM is a host cell receptor for MV was discovered: CD150 or signaling lymphocytic activation molecule (SLAM) (7–9). SLAM is a host cell receptor for MV was discovered: CD150 or signaling lymphocytic activation molecule (SLAM) (7–9). SLAM is a host cell receptor for MV with activated lymphocytes. CD150 mice yielded a productive infection of the lymphoid organs that resulted in enlarged lymph nodes and spleen. This mouse model should help researchers study receptor tropism and the immune dynamics of MV, and will provide a valuable tool for researchers studying the engineered vaccine and oncolytic potential of this virus (19–22).

Materials and Methods

Production of CD150 Mice. A BAC library from the Fondation Jean Dausset-Centre d’Etude Polymorphism Humaine (Paris) was screened by using primers that recognized genomic sequence 10 kb upstream of the SLAM start codon and sequence 1.5 kb downstream of the SLAM stop codon. The BAC DNA was prepared by using a bacterial artificial chromosome (BAC). We showed that our hSLAM(+/+ ) mice express hSLAM in a regulated fashion and with an expression profile identical to that in humans. hSLAM− cells obtained from the transgenic mouse, including activated B, T, and dendritic cells, were susceptible to infection by MV in a receptor-dependent manner. To improve virus infection, the hSLAM transgenic mice were bred into a stat1−/− background, which renders mice more susceptible to pathogens (15–18). Inoculation of the hSLAM(+/+ )/stat1(−/−) mice yielded a productive infection of the lymphoid organs that resulted in enlarged lymph nodes and spleen. This mouse model should help researchers study receptor tropism and the immune dynamics of MV, and will provide a valuable tool for researchers studying the engineered vaccine and oncolytic potential of this virus (19–22).

Conflict of interest statement: No conflicts declared.

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Abbreviations: BAC, bacterial artificial chromosome; DC, dendritic cells; i.n., intranasally; MV, measles viruses; NK, natural killer; p.i., postinfection.

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**Viruses and Infections.** The GFP-expressing viruses MVedGFP, MVwtGFP, and MVedGFP-SLAMblind are described in refs. 24–26. Activated lymphocytes and DC were infected at a multiplicity of infection of 5 unless otherwise stated. Intranasal (i.n.) infections were performed similarly to methods described in ref. 6. For i.n. and i.p. infections, respectively, 2.5 × 10⁶ PFU and 1 × 10⁹ PFU were used.

**Isolation and Activation of Lymphocytes and DC.** The lymph nodes and spleens of hSLAM or C57BL/6 mice were harvested through a 0.45-μm mesh in RPMI medium 1640 containing 10% FBS and 0.1% 2-mercaptoethanol. Both T and B cells were negatively selected for; purified T cells were activated by using anti-CD3 and IL-2 (50 units/ml), and purified B cells were activated by using 20 μg/ml LPS. Cell activation status was analyzed 48 h after harvest. DC were harvested as described in ref. 27. On day 9, the cells in suspension were harvested, counted, analyzed by FACS, and replated in activation media (RPMI medium 1640 + 10% FBS/0.1% 2-mercaptoethanol/antibiotics/5 ng/ml GM-CSF/100 ng/ml LPS). Activation status was analyzed 24 h later.

**Immunoprecipitation Experiments.** Tissues from dissected mice were sonicated by a polytron homogenizer in RIPA buffer (50 mM Hepes, 150 mM NaCl, 0.1% 2-mercaptoethanol). Both T and B cells were negatively selected for; purified T cells were activated by using anti-CD3 and IL-2 (50 units/ml) and purified B cells were activated by using 20 μg/ml LPS. Cell activation status was analyzed 48 h after harvest. DC were harvested as described in ref. 27. On day 9, the cells in suspension were harvested, counted, analyzed by FACS, and replated in activation media (RPMI medium 1640 + 10% FBS/0.1% 2-mercaptoethanol/antibiotics/5 ng/ml GM-CSF/100 ng/ml LPS). Activation status was analyzed 24 h later.

**Immunohistochemistry.** Lymph nodes and spleens were snap-frozen in OCT embedding medium (EM Science) by using liquid N₂, and 10-μm sections were made by using a cryoslicer. The sections were air-dried, fixed in cold acetone for 10 min, air-dried again, and rinsed in PBS. Endogenous peroxidase was blocked by using 0.3% hydrogen peroxide for 4 min. After protein blocking, the slides were incubated with a rabbit anti-MV H antibody at a dilution of 1/100 for 1 h at room temperature, washed well in PBS, and incubated with an anti-rabbit biotinylated linking antibody for 30 min at room temperature. They were then washed well in PBS, incubated with Ultra Streptavidin-Horseradish Peroxidase Complex (ID Labs, London, ON, Canada) for 30 min, and washed again in PBS. The slides were then developed with freshly prepared chromagen, washed in running tap water, and counterstained lightly with Mayer’s hematoxylin. After another wash, they were dehydrated through alcohols, cleared in xylene, and mounted in Permount (Fisher Scientific).

**Antibodies.** Monoclonal antibodies specific for CD150 (clone A12) were purchased from BD Biosciences Pharmingen. Monoclonal antibodies recognizing H were purchased from Chemicon. A rabbit polyclonal antibody was generated against the C terminus of MV H protein. Antibodies that recognize CD11c, B220, CD4, CD8, B7.2, Iab, Gr-1, NK1.1, and CD11b were purchased from BD Biosciences.

**Results**

**Generation of CD150 (SLAM) Transgenic Mice.** To generate transgenic mice that express human SLAM (hSLAM), a BAC containing the hSLAM gene was used. Three of four BAC plasmids isolated from this library, #2–4, had the expected BamHI and XhoI DNA restriction digestion patterns in Southern blots hybridized to a radioactive hSLAM cDNA probe (Fig. 1B). By using BAC-hSLAM#2 as a probe, FISH was performed to confirm that the BAC genomic clone mapped to the expected CD150 gene location on chromosome #1 (1q22) (Fig. 1B). Because the restriction map analysis of BAC-hSLAM#2 was not conducted, we pursued other approaches for verifying the exact location of the BAC-hSLAM#2 clone on chromosome #1. Since the hSLAM cDNA was cloned from a CD150-expressing BAC library, we expected that the BAC-hSLAM#2 clone would map to the CD150 gene location on chromosome #1. To test this possibility, we sequenced the 5′-end of the BAC-hSLAM#2 clone and found that it contained the hSLAM cDNA sequence. Furthermore, we found that the BAC-hSLAM#2 clone mapped to the CD150 gene location on chromosome #1 by using BAC-hSLAM#2 as a probe in Southern blots hybridized to human genomic DNA (Fig. 1C). These results confirm that the BAC-hSLAM#2 clone is a transgenic clone that expresses the hSLAM gene on chromosome #1.
consistent with complete coverage of the hSLAM genomic sequence, it was used to generate homozygous hSLAM transgenic mice. Three founder mice, two males and one female, were obtained, but only two mice were fertile: hSLAM#3 and hSLAM#13. hSLAM#13 has yielded homozygotes for the hSLAM transgene, whereas, to date, only hemizygotes have been obtained for hSLAM#3. Analysis of the homozygous hSLAM#13 transgenic mouse is presented in this article, but equivalent results with heterozygous hSLAM#3 mice have been obtained (data not shown). FISH analysis of mouse chromosomes from this line showed that the BAC integrated only at one locus (Fig. 1).

Profile of hSLAM Protein Expression in hSLAM Transgenic Mice. To determine the protein expression pattern of hSLAM in our hSLAM transgenic mice, RT-PCR (see the supporting information, which is published on the PNAS web site) and FACS analysis were performed. RT-PCR analysis of organs from the transgenic mice indicated that mRNA for hSLAM was synthesized in the thymus and in activated B and T cells (see the supporting information). Thymocytes were harvested from both C57BL/6 (WT) and hSLAM mice and stained with antibodies that recognize CD4, CD8, and hSLAM (Fig. 2A). In the hSLAM mice, hSLAM was strongly expressed only on CD4+ and CD8+ double-positive thymocytes. B and T cells harvested from the spleen or lymph nodes were stained for CD4, CD8, and hSLAM or B220 and hSLAM. As expected, expression of hSLAM on CD4+, CD8+, and B220+ cells was low (10%) (Fig. 2B).

To monitor hSLAM expression after activation, B and T cells were purified from the spleen and subsequently activated. FACS analysis indicated that 20% and 17% of B and T cells, respectively, could be activated to express hSLAM (Fig. 2C). Levels of hSLAM expression were comparable to those on activated PBMCs from humans (8).

In Vitro Infection of hSLAM+ Cells from hSLAM Transgenic Mice. To determine whether hSLAM expression on T cells allowed for MV infection, activated T cells were infected with three different GFP-expressing MV: GFPedMV, GFPwtfMV, and MVedGFP-SLAMblind. All of these viruses are described in refs. 24–26. GFPedMV and GFPwtfMV can use hSLAM as a receptor, whereas the MVedGFP-SLAMblind cannot use hSLAM for infection and serves as a negative control.

Inoculation of activated T cells from hSLAM#13 transgenic mice can be infected in a receptor-dependent manner by MV. Activated T cells were infected with recombinant MV (multiplicities of infection of 5) that express either the Edmonston hemagglutinin (Ed MV), the WTF hemagglutinin (WTF), or a hemagglutinin that cannot use hSLAM for viral entry (SLAMblind), in addition to the GFP reporter gene. All experiments were performed 48 h p.i. (A) GFP expression in infected T cells detected by fluorescence microscope. (B) GFP expression in infected T cells determined by FACS analysis. (C) FACS analysis showing down-regulation of SLAM on activated T cells infected with Ed MV and WTF MV.
T cells. Infection experiments were also performed with B cells and showed similar results (data not shown).

To analyze hSLAM expression on DC, bone marrow from the femurs of hSLAM transgenic mice was harvested and cultivated in the presence of GM-CSF for 9 days. At day 9, the cells were activated by using LPS for 24 h. FACS analysis demonstrated that hSLAM was expressed on LPS-activated DC but not on naïve DC (Fig. 4A). Inoculation of activated DC from hSLAM mice with GFPedMV, GFPwtMV, or MVedGFP-SLAMblind resulted in receptor-dependent infection as determined by GFP expression (Fig. 4B).

hSLAM Mice Are Transiently Infected by MV. To determine whether hSLAM mice can be infected in vivo, i.n. and i.p. inoculations of hSLAM mice were performed by using Montefiore MV, a WT isolate propagated in a primate B cell line (B95-8). Before infection, mice were primed by i.p. injection with 10 μg of LPS, which induces hSLAM expression in circulating lymphocytes, thereby increasing the pool of potential MV target cells (data not shown). RT-PCR for MV N mRNA with RNA derived from the lung, nasopharyngeal lymph nodes, mesenteric lymph nodes, thymus, and spleen from infected mice was performed to monitor levels of infection at 4 or 6 days p.i. (Fig. 5A and C). After i.n. infection, MV N mRNA transcripts were observed in the nasopharyngeal lymph nodes of the hSLAM mice but not in other more distal nodes, thymus, spleen, or other organs of C57BL/6 mice. No N mRNA transcripts were detected after i.p. infections.

STAT1 Deficiency Confers a Permissive Environment for Efficient Infection of the hSLAM Mice. Previous work with CD46 transgenic mice identified the innate immune response as a barrier to efficient MV replication in mice (6, 29). Therefore, the hSLAM mice were bred into a Stat1-deficient background, which renders mice extremely susceptible to pathogenic infections (15–18). To determine whether Stat1 deficiency could generate a permissive environment for MV infection, hSLAM(+/+)stat1(−/−) mice
were observed in the local lymph nodes, but the immune response was expected because studies in CD46 transgenic mice indicated that MV is incapable of evading a strong innate immune response in mice. Because of the critical role of STAT1 in cleared the virus before it could spread further. This clearance was expected because studies in CD46 transgenic mice indicated that MV is incapable of evading a strong innate immune response in mice. Because of the critical role of STAT1 in mice were inoculated i.n. and i.p. by using Montefiore WT MV. In these mice, mRNA for MV N was detected in the lymph nodes, thymus, and spleen (Fig. 5B and D). No signs of MV infection were observed in stat1-deficient mice inoculated with the virus (Fig. 5B) indicating that expression of hSLAM was required for MV infection. Immunoprecipitation of MV H from the cell lysates of infected organs and immunohistochemistry of infected lymph nodes and spleens with an anti-MV H antibody confirmed the RT-PCR results (Fig. 5E and F).

A striking effect of MV infection in the hSLAM(+/+)/stat1(−/−) mice was the presence of enlarged lymph nodes (not shown) and splenomegaly (Fig. 6A). The onset of these symptoms corresponded to the peak of viral infection and was consistently observed only in the hSLAM(+/+)/stat1(−/−) mice. To identify the cause for the enlarged spleens, FACS analysis of the splenic cell populations was performed at multiple time points p.i. This analysis revealed a difference in the numbers of mature neutrophils and natural killer (NK) cells in infected hSLAM(+/+)/stat1(−/−) mice (Fig. 6B). In the spleens with an enlarged phenotype, almost 20% of the splenic population consisted of Gr-1 and CD11b (MAC-1) double-positive cells, which are usually mature neutrophils (30). In infected C57BL/6, stat1(−/−) and hSLAM(+/+)/stat1(−/−) mice, these cells comprise only 4–8% of the cellular profile in the spleen. In addition to the increased levels of activated neutrophils, there was a substantial rise in the number of natural killer NK1.1+ cells (19%). Again, this was much higher than that seen in infected C57BL/6, stat1(−/−), and hSLAM mice. Combined, these two cell types make up almost 50% of the cellular compartment of the enlarged spleen in the infected hSLAM(+/+)/stat1(−/−) mice. Although their role in viral clearance remains to be explored, the increased level of these two cell types is the source of the observed splenomegaly after MV infection.

**Discussion**

The development of a small animal model that permits host cell entry and replication of MV should allow the assessment of the relevance of receptor usage by this virus. It should also have applications in the development of recombinant MV vaccines (19, 20, 31) and studying the oncolytic properties of this virus (21, 22, 32). Other groups have generated mice that express human CD46, which is the receptor used by vaccine and laboratory adapted strains of MV for virus entry (2–6, 33). These labs reported transient MV infections in these mice with limited virus production due to the induction of innate immune responses. Breeding of the CD46 mice into IFN-defective genetic backgrounds was used to overcome these difficulties. In a mouse strain deficient for IFN-α/β receptor, dissemination of MV to several tissues was observed (6, 29). Studies in mice lacking B6.CG2, which is a gene critical for the proper development of T and B cells, also resulted in more efficient spread of MV (34). However, it was not possible to analyze the role of T and B cells in MV infection in these experiments. Others studied the effect of MV infection in the brains of suckling mice, which lack innate immunity (4, 34). These studies did not recapitulate a normal course of infection or lead to the development of a neurological condition known as subacute sclerosing panencephalitis.

The most important limitation to the CD46 transgenic mouse models is the fact that CD46 supports efficient infection of only vaccine- or laboratory-adapted MV strains. Recently, however, the receptor for lymphocytic or WT strains of MV was determined to be hSLAM (8, 9, 35). Since this discovery, two transgenic mouse models, one that expresses hSLAM on T cells and one that expresses hSLAM on CD11c+ DC, were generated (36, 37). However, the utility of these mice as a small animal model for MV infection was hampered by the use of specific promoters that limited the expression of hSLAM to only certain types of cells. In contrast, we generated hSLAM transgenic mice that have a human-like expression profile through use of the human gene’s endogenous promoter.

When hSLAM mice were inoculated with MV, infection was observed in the local lymph nodes, but the immune response cleared the virus before it could spread further. This clearance was expected because studies in CD46 transgenic mice indicated that MV is incapable of evading a strong innate immune response in mice. Because of the critical role of STAT1 in
signaling downstream of types I and II IFN receptors, Stat1(−/−) mice are more susceptible to bacterial and viral infections (15–18). In stat1(−/−) mice, there is a reduction or absence of IFN-α and IFN-γ-induced expression of major histocompatibility complex (MHC) class II protein, IFN regulatory factor 1 (IRF-1), guanylate-binding protein 1 (GBP-1), the MHC class II transactivating protein (CIITA), and the complement protein, C3.

When hSLAM(+/+)/stat1(−/−) mice were inoculated with MV, infected spleens were infiltrated by mature neutrophils and NK cells. The direct involvement of these two cell types in the clearance of the MV infection was not assessed, but it has been shown that NK cells do play a primary and immediate role in antiviral responses (38–40). In addition, the neutrophil response was shown to play a significant role in the oncolytic effect of replicating MV (41).

In conclusion, the present study reports the development of a small animal model for MV infection through transgenic expression of the primary receptor of this virus, hSLAM(CD150). This model will enable studies that relate immune responses to MV pathology but may also facilitate the assessment of recombinant MV-based vaccines and oncolytic vectors.

We thank the veterinarians and technicians of the University Health Network Toronto animal facility for housing and manipulating our mice. The Amgen Transgenic Mouse Facility (Thousand Oaks, CA) injected the hSLAM gene into oocytes for generation of the heterozygous mice. We thank the veterinarians and technicians of the University Health Network Toronto animal facility for housing and manipulating our mice.

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