Measles Virus Infection of SLAM (CD150) Knockin Mice Reproduces Tropism and Immunosuppression in Human Infection†

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The human signaling lymphocyte activation molecule (SLAM, also called CD150), a regulator of antigen-driven T-cell responses and macrophage functions, acts as a cellular receptor for measles virus (MV), and its V domain is necessary and sufficient for receptor function. We report here the generation of SLAM knockin mice in which the V domain of mouse SLAM was replaced by that of human SLAM. The chimeric SLAM had an expected distribution and normal function in the knockin mice. Splenocytes from the SLAM knockin mice permitted the in vitro growth of a virulent MV strain but not that of the Edmonston vaccine strain. Unlike in vitro infection, MV could grow only in SLAM knockin mice that also lacked the type I interferon receptor (IFNAR). After intraperitoneal or intranasal inoculation, MV was detected in the spleen and lymph nodes throughout the body but not in the thymus. Notably, the virus appeared first in the mediastinal lymph node after intranasal inoculation. Splenocytes from MV-infected IFNAR−/− SLAM knockin mice showed suppression of proliferative responses to concanavalin A. Thus, MV infection of SLAM knockin mice reproduces lymphotropism and immunosuppression in human infection, serving as a useful small animal model for measles.

Measles is a highly contagious acute childhood disease characterized by fever, cough, coryza, conjunctivitis, and a generalized maculopapular rash (for a review, see reference 15). Although effective live vaccines are available, measles still claims approximately half a million lives each year, mainly in developing countries. Measles virus (MV), the causative agent of the disease, is transmitted via aerosol droplets, and initial infection is established in the respiratory tract, though the primary target cells are not well defined. From the respiratory tract, virus enters the local lymphatics and is transported to draining lymph nodes (LNs), eventually resulting in viremia. Lymphoid tissues and organs are the principal sites of virus replication. After an incubation period of 10 to 14 days, clinical symptoms develop, accompanied by immunosuppression that often leads to secondary infections. Furthermore, MV sometimes causes neurological diseases such as postinfectious encephalitis and subacute sclerosing panencephalitis. Although numerous studies have been performed, the pathogenesis of measles is still not well understood, partly because of a paucity of convenient small animal models.

MV, a member of the genus Morbillivirus in the family Paramyxoviridae, is an enveloped virus with a nonsegmented negative-strand RNA genome (15). MV has two envelope glycoproteins, the hemagglutinin and fusion proteins. Following the binding of the hemagglutinin to a cellular receptor on a target cell, the fusion protein mediates the fusion of the viral envelope with the host cell membrane, allowing MV entry into the cell. We and others have shown that the human signaling lymphocyte activation molecule (SLAM, also called CD150) acts as a cellular receptor for MV (13, 21, 55). SLAM, a self- ligand glycoprotein, is expressed on immature thymocytes, activated T and B cells, mature dendritic cells (DCs), and macrophages in both humans and mice (4, 6, 8, 9, 27, 37, 43, 49). Recently, it was also found on platelets (34) and mouse hematopoietic stem cells (24). SLAM belongs to the immunoglobulin (Ig) superfamily and has two extracellular domains, V and C2 (9). It may be associated with the SLAM-associated protein (also known as SH2D1A or DSHP) or Ewing’s sarcoma-associated transcript 2 in its cytoplasmic tail. In CD4+ T cells, ligation of SLAM induces its binding to SLAM-associated protein, recruitment and activation of the Src-related protein tyrosine kinase FynT, and tyrosine phosphorylation of SLAM by FynT. This, combined with T-cell-receptor-mediated signals, activates downstream effectors including protein kinase C-0, leading to up-regulation of the GATA-3 transcription factor and production of Th2 cytokines such as interleukin-4 (IL-4) and IL-13 (for reviews on SLAM signaling, see references 12, 35, and 58). SLAM also controls lipopolysaccharide (LPS)-induced production of tumor necrosis factor alpha (TNF-α) and nitric oxide (NO) by macrophages (61). Mouse SLAM does not function as an MV receptor (41), and mice are not susceptible to MV.

The type I interferons (alpha interferon [IFN-α] and IFN-β) play a major role in host defenses against viruses. Infected host cells detect virus-derived double-stranded RNAs and produce type I IFNs. Secreted IFNs bind to the type I IFN receptor...
mice, and red blood cells were removed by treatment with 1.66% NH₄Cl. The
derived from bone marrow (BM) cells cultured with granulocyte-macrophage
cultured in RPMI 1640 medium containing 10% fetal bovine serum. DCs were
have been described previously (40, 54). Splenocytes isolated from mice were
shown suppression of proliferative responses to mitogen stim-
 Splenic T cells obtained from MV-infected knockin mice
knockin mice reproduces tropism and immunosuppression in
measles patients (19, 64). Thus, MV infection of SLAM
ulation like peripheral blood mononuclear cells (PBMCs) from
inoculation in vivo, the virus replicated in the lymphoid organs
replication in vitro. After intraperitoneal (i.p.) or intranasal
mechanisms. The V, C, and phospho (P) proteins of MV have
have evolved strategies to counteract these antiviral defense
(IFNAR) on the surface of neighboring cells, activating the
Janus kinase (Jak)/signal transducer and activator of transcription
(Stat) signaling pathway. This in turn induces the tran-
scriptional activation of target genes, the products of which render the cells resistant to virus replication through various
mechanisms, including degradation of viral mRNAs, inhibition
of viral translation, and inhibition of cell growth. Many viruses
have evolved strategies to counteract these antiviral defense
mechanisms. The V, C, and phospho (P) proteins of MV have
been reported to block the Jak/Stat signaling pathway (38, 42,
47, 53, 56).

The V domain of human SLAM is necessary and sufficient
for MV receptor function (41). To develop a small animal
model for measles, we generated knockin mice in which the V
domain of mouse SLAM was replaced by that of human
SLAM. The human/mouse chimeric SLAM had an expected
distribution and normal function in the knockin mice. Splenocytes
and DCs from the SLAM knockin mice permitted MV
replication in vitro. After intraperitoneal (i.p.) or intranasal
inoculation in vivo, the virus replicated in the lymphoid organs
of knockin mice that were crossed with mice lacking IFNAR.
Splenic T cells obtained from MV-infected knockin mice
materials and methods
Cells and viruses. The derivations and culture conditions of the cell lines used have
been described previously (40, 54). Splenocytes isolated from mice were
cultured in RPMI 1640 medium containing 10% fetal bovine serum. DCs were
derived from bone marrow (BM) cells cultured with granulocyte-macrophage
 colony-stimulating factor (GM-CSF) (23). Briefly, BM cells were prepared from
mice, and red blood cells were removed by treatment with 1.66% NH₄Cl. The
BM cells were suspended with RPMI 1640 medium supplemented with 10% fetal
bovine serum, 100 μM 2-mercaptoethanol (Sigma), 10 ng/ml recombinant mouse
GM-CSF (Peprotech), and 50 μg/ml gentamicin. Then, 1 × 10⁷ BM cells were
seeded in each well of a 24-well plate. Nonadherent cells were discarded, and the
remaining cells were fed with fresh medium. At day 6, nonadherent and loosely
adherent cells were harvested and used as immature DCs. The IC-B strain of MV
(25, 26) and recombinant MVs expressing enhanced green fluorescent protein
(IC323-EGFP) (18) and red fluorescent reporter protein (IC323-DsRed) (52)
were used as immature DCs. The IC-B strain of MV

Generation of human SLAM knockin mice. A 129/Sv mouse genomic DNA
fragment encompassing the first, second, and third exons of the mouse SLAM
gene was subcloned into pBluescript II (Stratagene), and a fragment containing
the loxP sequences and the neomycin resistance gene (provided by D. E.
Rancourt) was introduced between exon 1 and exon 2. Then, exon 2 encoding the
V domain was replaced with the corresponding human sequence, using the gene
SOEing method (57). The targeting sequence was further subcloned into lambda
DASH2ETK phage (56) containing the herpes simplex virus thymidine kinase
sequence for negative selection. The resulting targeting vector was electropo-
erated into the embryonic stem (ES) cells of the 129/Sv mouse background. G418
and ganciclovir-resistant clones were screened by PCR and Southern blot anal-
ysis. The locations of the PCR primers and probes used for Southern blot
analysis are depicted in Fig. 1A (sequences of probes and primers provided on
request). In Southern blot analysis, EcoRI digestion of genomic DNA generated
a 5-kb band from the endogenous wild-type (WT) allele, whereas the 0.8-kb fragment
was generated from the locus in which the

FIG. 1. Targeting of mouse SLAM gene. (A) The targeting strategy. (a) The segment of the mouse SLAM gene containing exons 1 to 3 (filled boxes) is shown with the recognition sites of the restriction enzyme EcoRI. (b) The targeting vector has the human SLAM exon 2 (empty boxes) and the neomycin resistance gene (neo) with loxP sequences placed between the mouse SLAM exon 1 and the human SLAM exon 2. The loxP sequences are indicated by ovals. (c) The chromosomal locus after homolo-
gous recombination with the targeting vector is shown. (d) The chimeric SLAM allele (knockin) is produced by Cre recombinase-medi-
ated excision of the neomycin resistance gene. PCR primers (Pri.1 to
Pri.3) and a probe used for Southern blot analysis are shown. (B) South-
ern blot analysis of targeted ES cell clones. Genomic DNA isolated from the
cells was digested with EcoRI, electrophoresed on a 1% agarose gel,
transferred onto a membrane, and hybridized with the probe shown in
panel A. A 5-kb band was detected in the wild type (WT) and heterozy-
gote (H/WT). An 8-kb band resulting from insertion of the loxP sequences and neomycin resistance gene was detected in the heterozygote. (C) Germ
line transmission of the targeted allele (left) and Cre recombinase-medi-
ed excision of the neomycin resistance gene (right), as revealed by PCR
analysis. To confirm germ line transmission, PCR was performed using
species-specific primers (Pri.1, mouse; Pri.2, human) and a common
primer (Pri.3). The 0.5-kb fragment was generated from the WT allele,
whereas the 0.8-kb fragment was generated from the locus in which the
mouse SLAM exon 2 was replaced by the human SLAM exon 2. To
confirm the excision of the neomycin resistance gene, PCR was performed
using primers Pri.4 and Pri.5. No fragment was generated from the WT
allele because Pri.5 was specific for the human sequence. The 4.0-kb
fragment was amplified when the neomycin resistance gene was placed
between the regions recognized by the primers. The 1.2-kb fragment was
generated after the Cre recombinase-mediated excision.

Figure 1A shows the targeting strategy for the mouse SLAM gene. (A) The
segment of the mouse SLAM gene containing exons 1 to 3 (filled boxes) is shown
with the recognition sites of the restriction enzyme EcoRI. (B) The targeting
vector has the human SLAM exon 2 (empty boxes) and the neomycin resistance
gene (neo) with loxP sequences placed between the mouse SLAM exon 1 and the
human SLAM exon 2. The loxP sequences are indicated by ovals. (C) The chromosomal locus after homologous recombination with the targeting
vector is shown. (D) The chimeric SLAM allele (knockin) is produced by
Cre recombinase-mediated excision of the neomycin resistance gene. PCR primers (Pri.1 to Pri.3) and a probe used for Southern blot analysis are
shown. (B) Southern blot analysis of targeted ES cell clones. Genomic
DNA isolated from the cells was digested with EcoRI, electrophoresed on a 1% agarose gel, transferred onto a membrane, and hybridized with the probe shown in panel A. A 5-kb band was detected in the wild type (WT) and heterozygote (H/WT). An 8-kb band resulting from insertion of the loxP sequences and neomycin resistance gene was detected in the heterozygote. (C) Germ
line transmission of the targeted allele (left) and Cre recombinase-mediated
excision of the neomycin resistance gene (right), as revealed by PCR analysis. To confirm germ line transmission, PCR was performed using
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allele because Pri.5 was specific for the human sequence. The 4.0-kb
fragment was amplified when the neomycin resistance gene was placed
between the regions recognized by the primers. The 1.2-kb fragment was
generated after the Cre recombinase-mediated excision.
RESULTS

Generation of human SLAM exon 2 knockin mice. To generate knockin mice with human/mouse chimeric SLAM in which the V domain of mouse SLAM is replaced by that of human SLAM, a targeting vector was constructed as shown in Fig. 1A. The vector was electroporated into ES cells generated from 129/Sv mice. After drug selection, the ES cell clones were screened by PCR and Southern blot analysis, and correctly targeted ES cell clones (the targeted allele containing human SLAM exon 2 and the neomycin resistance gene [neo] cassette placed between the loxp sequences is designated H) were selected (Fig. 1B). They were transferred into C57BL/6 blastocyes to generate chimeric mice, which were then crossed with C57BL/6 mice. Transmission of the targeted allele (H) was confirmed by PCR (Fig. 1C, left). The heterozygous mice (WT/H) were crossed with mice expressing Cre recombinase to obtain human SLAM knockin mice (the allele in which the mouse SLAM exon 2 is replaced by the human SLAM exon 2 is designated h) by removing the neomycin resistance gene cassette (Fig. 1C, right). The knockin mice were backcrossed with C57BL/6 mice, and the homozygous knockin mice (h/h) were used for the following experiments.

Distribution of chimeric SLAM is normal in SLAM knockin mice. To examine the expression of human/mouse chimeric SLAM, thymocytes from the knockin mice were stained with MAbs against CD4, CD8, and human SLAM. The anti-human SLAM MAb used (IPO-3) (49) has been shown to react with the recombinant mouse SLAM possessing the human V domain in place of the mouse V domain but not with mouse SLAM (41). CD4+ and CD8+ single-positive as well as CD4+CD8+ double-positive thymocytes from the knockin mice, but not from C57BL/6 mice, expressed the chimeric SLAM (Fig. 2A), consistent with the reported expression profiles of mouse SLAM in thymocytes (8). CD4+CD8+ double-negative thymocytes also expressed the chimeric SLAM (Fig. 2A). We also found that all of these four populations of thymocytes from C57BL/6 mice expressed mouse SLAM on their surface (unpublished data). Mouse splenic T and B cells express low levels of SLAM, which is substantially increased after stimulation (8), whereas in humans, most unstimulated T and B cells do not express SLAM (9). As expected, spleen T and B cells freshly isolated from the knockin mice showed low levels of chimeric SLAM expression (Fig. 2B), and the expression levels on T and B cells were increased after stimulation with ConA and LPS for 24 h, respectively (Fig. 2C). SLAM expression on DCs from the knockin mice was also examined. Most of the BM-derived immature DCs did not express the chimeric SLAM, but its expression was strongly induced after stimulation with LPS or zymosan A for 24 h (Fig. 2D). The results were consistent with SLAM expression profiles in human immature and mature DCs (6, 27, 37, 43). These data indicate that the distribution of chimeric SLAM was normal, and its expression was appropriately induced after stimulation in the SLAM knockin mice.

Chimeric SLAM of knockin mice has normal function. To examine whether replacement of the V domain of mouse SLAM with that of human SLAM perturbed the production of cells of the immune system, the proportions of cells constituting splenocytes were compared between the SLAM knockin and C57BL/6 mice. Splenocytes were stained with MAbs spe-
specific for T cells, macrophages, DCs, B cells, and natural killer cells and then analyzed by flow cytometry. There was no significant difference between the two groups of mice (Fig. 3A).

If the chimeric SLAM cannot function properly, the knockin mice were stained with anti-CD4, -CD8, and human SLAM MAbs. Expression of the chimeric SLAM in the indicated populations of thymocytes is shown. Empty profiles indicate cells from the knockin mice, and solid profiles show cells from C57BL/6 mice. (B) Splenocytes were stained with anti-CD3ε, -B220, and -human SLAM MAbs. Expression of the chimeric SLAM was analyzed in the indicated populations. Empty profiles indicate cells from the knockin mice, and solid profiles represent cells from C57BL/6 mice. (C) Splenocytes from the knockin mice were left unstimulated or stimulated with ConA or LPS and then stained with anti-CD3ε, -B220, and -human SLAM MAbs. Cells were gated by CD3ε or B220 expression and then examined for chimeric SLAM expression. Solid profiles indicate cells stained with the IgG1 isotype control. Thick empty profiles represent unstimulated cells, and thin empty profiles indicate stimulated cells. (D) BM cells prepared from the knockin mice were cultured in medium containing recombinant GM-CSF. The cells were left unstimulated or stimulated with LPS or zymosan A for 24 h. Chimeric SLAM expression was examined in CD11c⁺ cells. Solid profiles indicate unstimulated cells. Thick and thin empty profiles indicate LPS- and zymosan A-stimulated cells, respectively.

FIG. 2. Expression profiles of the chimeric SLAM. (A) Thymocytes prepared from mice were stained with anti-CD4, -CD8, and -human SLAM MAbs. Expression of the chimeric SLAM in the four populations of thymocytes is shown. Empty profiles indicate cells from the knockin mice, and solid profiles show cells from C57BL/6 mice. (B) Splenocytes were stained with anti-CD3ε, -B220, and -human SLAM MAbs. Expression of the chimeric SLAM was analyzed in the indicated populations. Empty profiles indicate cells from the knockin mice, and solid profiles represent cells from C57BL/6 mice. (C) Splenocytes from the knockin mice were left unstimulated or stimulated with ConA or LPS and then stained with anti-CD3ε, -B220, and -human SLAM MAbs. Cells were gated by CD3ε or B220 expression and then examined for chimeric SLAM expression. Solid profiles indicate cells stained with the IgG1 isotype control. Thick empty profiles represent unstimulated cells, and thin empty profiles indicate stimulated cells. (D) BM cells prepared from the knockin mice were cultured in medium containing recombinant GM-CSF. The cells were left unstimulated or stimulated with LPS or zymosan A for 24 h. Chimeric SLAM expression was examined in CD11c⁺ cells. Solid profiles indicate unstimulated cells. Thick and thin empty profiles indicate LPS- and zymosan A-stimulated cells, respectively.

FIG. 3. Functions of the chimeric SLAM. (A) Proportions of cell populations that constitute the spleen. Splenocytes were stained with MAb specific for the indicated cell surface molecules and analyzed by flow cytometry. Data are presented as mean percentages ± standard deviations for three mice. (B) Secretion of IL-4 (left) and IFN-γ (right) by CD4⁺ T cells after stimulation with anti-CD3 and -CD28 MAbs. (C) Production of TNF-α (upper) and NO (lower) by peritoneal macrophages after stimulation with LPS and/or IFN-γ. Filled and empty bars indicate cells from knockin and C57BL/6 mice, respectively.
mice may exhibit some of the defects observed in SLAM knockout mice. It has been reported that IL-4 secretion by SLAM/H11002/CD4/H11001 cells is down-regulated, whereas IFN-γ production is up-regulated (61). Unlike those from SLAM knockout mice, CD4/H11001 cells from the knockin mice produced both IL-4 and IFN-γ at levels similar to those from C57BL/6 mice after stimulation with anti-CD3 and anti-CD28 antibodies (Fig. 3B). Peritoneal macrophages from the knockin mice were also examined for the production of TNF-α and NO in response to LPS and/or IFN-γ. Although their production was impaired in SLAM/H11002/macrophages (61), those obtained from the knockin mice produced TNF-α and NO at levels similar to those from C57BL/6 mice (Fig. 3C). These data imply that the chimeric SLAM in the knockin mice retains the normal function of SLAM.

Splenocytes and DCs from SLAM knockin mice permit MV entry and growth in vitro. It was then examined whether cells from the knockin mice could support MV growth. Single-cell suspensions were prepared from the spleens of the knockin and C57BL/6 mice. Since MV is known to hardly replicate in unstimulated lymphocytes (22), splenocytes were stimulated with ConA and then infected with the IC323-EGFP at an MOI of 1.0. This recombinant virus is based on a clinical MV isolate (the IC-B strain) that utilizes SLAM as a cellular receptor. While splenocytes from C57BL/6 mice were hardly infected by IC323-EGFP, those from the knockin mice allowed IC323-EGFP entry and GFP expression at 48 h postinfection (Fig. 4A, upper panels). Although almost all splenocytes expressed the chimeric SLAM when freshly isolated from the knockin mice (see Fig. 2), GFP expression was not detected without ConA stimulation (unpublished data).

Next, the IC-B and Edmonston strains of MV were examined for their growth in splenocytes stimulated with ConA (Fig. 4B, upper graphs). The Edmonston strain is an attenuated vaccine strain of MV (11) and uses human CD46, besides SLAM, as a receptor (55). Stimulated splenocytes were infected with the respective strains at an MOI of 0.6 each. Neither strain grew well in splenocytes from C57BL/6 mice. In contrast, the IC-B strain, but not the Edmonston strain, replicated efficiently in splenocytes from the knockin mice. Its maximum titer was only severalfold lower than that in phytohemagglutinin-stimulated human PBMCs (unpublished data).

The type I IFNs act as essential mediators of antiviral innate immunity in the host. To examine how the type I IFN system affects MV growth, SLAM knockin mice were crossed with mice lacking IFNAR. As expected, splenocytes from the IFNAR−/− mice did not permit the growth of IC323-EGFP, whereas those from the IFNAR−/− SLAM knockin mice expressed GFP strongly at 48 h after IC323-EGFP infection (Fig. 4A, lower panels). Importantly, growth of the IC-B strain was not enhanced in splenocytes from the IFNAR−/− SLAM knockin mice, compared with those from the IFNAR−/−

FIG. 4. MV infection in vitro. (A) MV infection of activated splenocytes. Splenocytes were prepared from the knockin mice (h/h) or C57BL/6 mice (WT) and stimulated with ConA. The cells were then infected with IC323-EGFP at an MOI of 1.0. Photographs were taken at 48 h postinfection. (B) Growth curves of the virulent IC-B (left) and Edmonston vaccine (right) strains of MV in activated splenocytes from mice. Activated splenocytes were infected with each strain at an MOI of 0.6. At the indicated times, cells and culture medium were collected, and the viruses in them were titrated on Vero/hSLAM cells. Black and red symbols indicate the titers of viruses produced in activated splenocytes from C57BL/6 and from knockin mice, respectively. Data are presented as mean titers ± standard deviations for triplicate assays. (C) MV infection of DCs. BM-derived DCs were treated with LPS (right) or left untreated (left). GFP expression was examined at 48 h postinfection. The upper and lower panels of panels A to C show the results for mice with intact IFNAR (IFNAR+/+) and deficient IFNAR (IFNAR−/−), respectively.
FIG. 5. MV infection in vivo. (A) Syncytia observed in the mediastinal LN. Two days after IC323-EGFP inoculation i.p., a mediastinal LN taken from the IFNAR−/− SLAM knockin mice was examined under a light and a fluorescence microscope. The microscopic images obtained were merged. Syncytia are indicated by white arrows. (B) MV spread in lymphoid organs. IFNAR−/− SLAM knockin mice were inoculated i.p. with IC323-EGFP. At day 5, the thymus, spleen, and LNs were taken from the mice and examined for GFP expression under a fluorescence microscope. (C) Analysis of MV-infected lymphocytes. IFNAR−/− SLAM knockin mice were inoculated i.p. or mock infected with IC323-DsRed. Lymphocytes from several mediastinal LNs were pooled, stained with FITC-labeled MAb against B220 or CD3, and analyzed by flow cytometry. The ordinate and abscissa show fluorescence for FITC and DsRed, respectively. (D) Virus titers in lymphoid organs of mice. IFNAR−/− SLAM knockin mice were inoculated intranasally with the IC-B strain of MV. At the indicated days after inoculation, lymphoid organs were taken from the mice, and virus titers were determined. The virus titers are expressed as PFU/g of organ. Each symbol indicates the sample from a single mouse. The sample designated “Mesenteric” contained pooled mesenteric and lumbar LNs, whereas that designated “Others” contained pooled cervical, axillary, and inguinal LNs.
SLAM knockin mice (Fig. 4B, lower left). The data indicate that the type I IFN system does not apparently restrict replication of the IC-B strain in this in vitro condition. The growth of the Edmonston strain was still very low in splenocytes prepared from the IFNAR−/− SLAM knockin mice (Fig. 4B, lower right). Since the peak titer of the Edmonston strain was about 100-fold lower than that of the IC-B strain on the IFNAR−/− background, the growth of the Edmonston strain must be also restricted in mouse splenocytes by some mechanism other than the type I IFN system.

BM-derived DCs from the knockin mice were infected with IC323-EGFP before or after stimulation with LPS (Fig. 4C). A small number of immature DCs expressed GFP at 48 h postinfection, regardless of whether they were prepared from the IFNAR+/+ or IFNAR−/− knockin mice. When maturation was induced by stimulation with LPS, a higher number of IFNAR−/− DCs expressed GFP, presumably because LPS induced SLAM expression on most DCs (Fig. 2D). In contrast, stimulated IFNAR+/+ DCs did not express GFP at all, presumably because LPS induced type I IFNs in BM-derived DCs via Toll-like receptor 4 (1).

SLAM knockin mice lacking IFNAR support the in vivo growth of MV after i.p. or intranasal inoculation. To assess MV infection in vivo, mice were inoculated i.p. with 5 × 10^5 PFU of IC323-EGFP. When C57BL/6 or IFNAR+/+ SLAM knockin mice were inoculated, no GFP signals were detected in the lymphoid organs (unpublished data). Furthermore, when the IC-B strain, rather than the recombinant IC323-EGFP, was used to infect the IFNAR+/+ SLAM knockin mice, no infectious virus could be isolated from the lymphoid organs (unpublished data). Previous studies have shown that type I IFNs act as a barrier to efficient MV replication in mice (32, 33, unpublished data). Previous studies have shown that type I IFNs act as a barrier to efficient MV replication in mice (32, 33, unpublished data). In contrast, infectious viruses were recovered from the minor DCs expressed GFP at 48 h postinfection, regardless of whether they were prepared from the IFNAR+/+ or IFNAR−/− knockin mice. In contrast, infected DCs expressed GFP, presumably because LPS induced SLAM expression on most DCs (Fig. 2D). In contrast, stimulated IFNAR+/+ DCs did not express GFP at all, presumably because LPS induced type I IFNs in BM-derived DCs via Toll-like receptor 4 (1).

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Since the respiratory tract is the natural route of MV infection in humans, mice were inoculated intranasally with 4 × 10^4 PFU of the IC-B strain. No virus was recovered from the lymphoid organs of any IFNAR−/− C57BL/6 mouse during the period examined (2 to 11 days postinfection) (unpublished data). In contrast, infectious viruses were recovered from the mediastinal LNs and spleens of the IFNAR−/− SLAM knockin mice as early as 2 days postinfection (Fig. 5D). At 5 to 9 days postinfection, considerable titers of MV were detected in spleens and LNs from ~50% of the infected IFNAR−/− SLAM knockin mice. No infectious virus was detected in the thymus at any of the time points examined. At 11 days postinfection, the infectious virus was no longer detected in any organs. Virus titers reached a peak at 2 to 5 days postinfection in the mediastinal LN and at 7 days postinfection in other LNs (cervical, axillary, inguinal, mesenteric, and lumbar) and the spleen. These results suggest that, after intranasal inoculation, the virus first reached and replicated in the mediastinal LN, from which it spread to other lymphoid organs throughout the body.

LYMPHOCYTES OBTAINED FROM MV-INFECTED SLAM KNOCKIN MICE SHOW PROLIFERATION INHIBITION WHEN STIMULATED WITH CONA. MV has been known to cause immunosuppression. When stimulated in vitro with a mitogen, PBMCs from MV-infected patients show reduced proliferation (19, 64). To examine whether inhibition of lymphocyte proliferation also occurs in SLAM knockin mice, splenocytes were obtained from IFNAR−/− SLAM knockin mice at 4 days after i.p. inoculation with 5 × 10^5 PFU of the IC-B strain of MV and stained with CFSE. The stained splenocytes were stimulated with ConA for 4 days, stained with PE-conjugated anti-CD3 antibody, and analyzed by flow cytometry and ModFit software. When the splenocytes were obtained from uninfected mice, most of the stimulated T cells divided at least a few times, and undivided cells accounted for only ~25% (data from three mice: 25, 27, and 23%) of total T cells (Fig. 6). In contrast, ~58% (70, 57, and 45%) of splenic T cells did not divide, when obtained from infected mice. In these experiments, only 0.10 to 0.40% of splenocytes were infected by MV at 4 days postinfection, as determined by infectious center assay.

DISCUSSION

Several types of transgenic mice expressing human CD46 or SLAM, cellular receptors for MV (65), have been generated as small animal models for measles. CD46 is a complement regulatory molecule and is expressed ubiquitously in humans (29). CD46 transgenic mice (7, 20, 30, 39, 44), however, may not be
suitable for the analysis of MV pathogenesis because CD46 acts as a receptor only for MV vaccine strains and not for most viruses in measles patients (40). Two groups produced SLAM transgenic mice using human SLAM cDNA driven by nonauthentic promoters and showed that MV could infect these mice (16, 17, 46). However, the expression profiles of SLAM are not natural in these mice. Recently, SLAM transgenic mice were generated using human genomic DNA with its endogenous promoter for transcription (48, 62). The mice showed an expression profile of the human (transgenic) SLAM equivalent to that in humans and therefore represented the most natural animal models for measles. Yet, these mice have both human and mouse SLAM proteins, which may affect the functions of SLAM-expressing cells and modify the pathogenesis of MV infection. In this study, we generated knockin mice in which the V domain of mouse SLAM was replaced by that of human SLAM, preserving the rest of the protein (mouse SLAM) and, thus, the associated signal transduction pathway. The human/mouse chimeric SLAM had an expected distribution and normal function in the SLAM knockin mice, which also allowed the in vitro and in vivo growth of MV. Indeed, this is the first mouse model for measles in which infectious virus particles (up to 10^6 PFU/g) were demonstrated in vivo outside the brain.

Previous studies have shown that type I IFNs act as a barrier to efficient MV replication in mice (32, 33, 48, 62). In our study, splenocytes from the SLAM knockin mice allowed in vitro replication of MV, but mature DCs did not. When IFNAR was knocked out, mature DCs could support MV growth in vitro. In in vivo experiments, SLAM knockin mice did not allow MV replication unless they were crossed with mice lacking IFNAR. The V, C, and P proteins of MV have been reported to block the Jak/Stat signaling pathway in response to IFN-α/β (38, 42, 47, 53, 66). Furthermore, the V proteins of paramyxoviruses, including MV, have been shown to bind the RNA helicase MDA5, thereby inhibiting its activation of the IFN-β promoter (2; unpublished data). TLR7- and 9-mediated IFN-α/β production in human plasmacytoid DCs is also inhibited by MV (45). Some of these IFN antagonist activities of MV may not function properly in mouse cells, resulting in inefficient MV replication. In addition, Yoshikawa et al. have shown, using poliovirus receptor transgenic mice, that the IFN inducibility of cells after viral infection is weaker in vitro than in vivo, accounting for the acquisition of permissiveness to poliovirus by some cultured cells (67). These mechanisms may at least partly explain our observations that MV replicates only in SLAM knockin mice that lack IFNAR, while splenocytes from the knockin mice allow efficient in vitro replication of MV regardless of the presence or absence of IFNAR.

Although they have to be crossed with mice lacking IFNAR, SLAM knockin mice otherwise serve as a good animal model for measles, reproducing many aspects of human infection.

First, the lymphotropism of MV was nicely reproduced in vitro and in vivo in the SLAM knockin mice. That the presence of a functional SLAM receptor on cells of the immune system allowed successful infection after intranasal inoculation in these mice suggests that the first targets of MV in vivo are SLAM-positive cells of the immune system in the respiratory tract, rather than epithelial cells. This is consistent with the recent report that canine distemper virus, another member of Morbillivirus, causes disease in experimentally infected ferrets strictly in a SLAM-dependent manner (60).

Second, virulent and attenuated phenotypes of MV were maintained in this animal model. The virulent clinical isolate of MV (the IC-B strain), but not the Edmonston vaccine strain, replicated well in ConA-stimulated splenocytes from the knockin mice. The Edmonston strain, which replicates efficiently in most cultured primate cell lines (54), hardly grew in cells from the knockin mice in vitro or in vivo (unpublished data), even when IFNAR was knocked out. It has been reported that lymphocytes from SLAM transgenic mice are efficiently infected by the Edmonston strain (16, 62). However, these studies either detected viral proteins with antibodies or used a GFP-expressing virus but did not examine the production of infectious virus particles. It is thus possible that the Edmonston strain of MV has growth defects in mice after the protein synthesis step. Recently, we have shown that the matrix (M) and large (L) proteins of the Edmonston strain contribute to its efficient growth in Vero cells by acting at postentry steps (51). However, recombinant MVs (based on the IC-B strain) possessing the Edmonston M or L gene grew less efficiently in SLAM-positive lymphoid cells than the parental virus (51). The same mechanisms may at least partly account for the attenuation of the Edmonston strain in humans and SLAM knockin mice.

Third, MV spread in the knockin mice reproduced probable early phases of MV infection in humans. After intranasal infection, the virus was first detected in the draining LN of the respiratory tract (mediastinal LN), followed by its spread to lymphoid organs throughout the body. This spread is also consistent with the data from MV infection of nonhuman primates (31, 36) as well as those from canine distemper virus infection of dogs (3) and ferrets (59).

Fourth, inhibition of lymphocyte proliferation was observed in the knockin mice, as in measles patients (19, 64). Although less than 1% of PBMCs are infected during the course of acute measles (14), immune responses are suppressed in measles patients for a long period of time (15). Avota et al. showed that MV envelope proteins inhibit the CD3/CD28-induced proliferation of T cells by affecting the phosphatidylinositol 3-/Akt kinase pathway (5). The nucleocapsid (N) protein of MV was shown to exhibit immunosuppressive activities by binding the Fcγ receptor type II on antigen-presenting cells (30) or the N protein receptor expressed on a large variety of cell types (28). These studies were performed in vitro. As infection of the SLAM knockin mice with the virulent IC-B strain of MV caused reduced T-cell responses to mitogen stimulation, the mice may prove useful in elucidating the mechanisms of MV-induced immunosuppression. In this regard, it should be noted that these mice retain the functional SLAM signaling pathway.

An unexpected observation in MV infection of these knockin mice was that MV failed to grow in thymocytes, despite their high expression levels of the chimeric SLAM. Furthermore, the thymus is one of the organs most severely affected in measles patients and experimentally infected primates (26, 63). Another group also found no MV-infected cells in the thymus of SLAM transgenic mice (48), but others detected MV infection of thymocytes in their mouse models (16, 62). The reason for this discrepancy is currently unknown. Since MV did not replicate in mouse thymocytes from the
IFNAR−/− SLAM knockin mice, host factors other than the type I IFN system must be involved in this block of MV growth. One possible mechanism is that MV cannot effectively prevent infection-induced apoptosis of mouse thymocytes. MV V protein was reported to inhibit p53 family member p73, thereby blocking apoptosis (10). Even under physiological conditions, thymocytes undergo extensive apoptosis during positive and negative selection (50). MV V protein may not function properly in inhibiting apoptosis of thymocytes in our mouse model. Thus, infected thymocytes may not survive long enough to support virus growth. We are currently examining this and other possible mechanisms accounting for the absence of MV infection of thymocytes in SLAM knockin mice.

In summary, we have produced a novel small animal model for MV infection that reproduces many aspects of human infection.

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REFERENCES


