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*J Immunol* 2005; 175:3252-3261; doi: 10.4049/jimmunol.175.5.3252

http://www.jimmunol.org/content/175/5/3252
Wild-Type Measles Virus Infection in Human CD46/CD150-Transgenic Mice: CD11c-Positive Dendritic Cells Establish Systemic Viral Infection

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We generated transgenic (TG) mice that constitutively express human CD46 (huCD46) and/or TLR-inducible CD150 (huCD150), which serve as receptors for measles virus (MV). These mice were used to study the spreading and pathogenicity of GFP-expressing or intact laboratory-adapted Edmonston and wild-type Ichinose (IC) strains of MV. Irrespective of the route of administration, neither type of MV was pathogenic to these TG mice. However, in ex vivo, limited replication of IC was observed in the spleen lymphocytes from huCD46/huCD150 TG and huCD150 TG, but not in huCD150 TG and non-TG mice. In huCD150-positive TG mouse cells, CD11c-positive bone marrow-derived myeloid dendritic cells (mDC) participated in MV-mediated type I IFN induction. The level and induction profile of IFN-β was higher in mDC than the profile of IFN-α. Wild-type IC induced markedly high levels of IFN-β in mDC, as opposed to human dendritic cells. We then generated huCD46/huCD150 TG mice with type I IFN receptor (IFNAR1)−/− mice. MV-bearing mDCs spreading to draining lymph nodes were clearly observed in these triple mutant mice in vivo by i.p. MV infection. Infectious lymph nodes were also detected in the double TG mice into which MV-infected CD11c-positive mDCs were i.v. transferred. This finding suggests that in the double TG mouse model mDCs once infected facilitate systemic MV spreading and infection, which depend on mDC MV permissiveness determined by the level of type I IFN generated via IFNAR1. Although these results may not simply reflect human MV infection, the huCD150/huCD46 TG mice may serve as a useful model for the analysis of MV-dependent modulation of mDC response.

The Journal of Immunology, 2005, 175: 3252−3261.

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M eases virus (MV) causes severe immune suppression followed by secondary infections that result in high rates of mortality of infants particularly in developing countries. Human CD46 (huCD46) (1, 2) and TLR-inducible human CD150 (huCD150) (3, 4) have been identified as MV receptors. Although huCD150 is the primary entry receptor for MV, it is not expressed in epithelial cells of the respiratory tract through which MV infection is known to initiate. The mechanism of wild-type MV spreading to systemic organs, therefore, remains unsolved.

No entry receptor for MV has been identified in mice. MV tropism is represented by its receptors, huCD46 and huCD150. Although rodents express the orthologues of these receptors, they fail to act as entry receptors for MV (2, 5, 6). MV infection of adult rodents is restricted to the brain-adapted strains obtained by intracerebral inoculations (7, 8). Transgenic (TG) mice expressing either huCD46 (9−11) or huCD150 (12, 13) have been established as models to investigate MV pathogenicity. Initially, most of the studies were performed using huCD46 TG mice. MV entry was found to be more efficient in mouse cells that express huCD46, the receptor for the MV vaccine Edmonston (ED) strain (1), and probably for several wild-type strains as well (14). However, TG rodents expressing huCD46 are not susceptible to MV infection when inoculated by routes other than intracerebral injection (15). Depletion of type I IFN receptors in the huCD46 TG mice led to high susceptibility to ED (16−18). These earlier studies, however, were performed before huCD150 was identified as the main receptor for wild-type MV strains (4). No in vivo studies have been attempted using wild-type MV strains and huCD46/huCD150 double TG mice for understanding the mechanisms of wild-type MV infection.

Using huCD150 single TG mice, several reports (12, 13) suggested that wild-type MV strains infect cells that constitutively express huCD150 but fail to induce systemic infection. huCD150 is inducible in human T cells and is up-regulated in myeloid dendritic cells (mDCs) and B cells in response to activation signaling from TLR (19−21). Thus, TG construct with just lck or CD11c
promoter reported earlier (12, 13) may not be adequate for the study of natural huCD150 distribution, MV entry, and resultant infection via huCD150. Establishment of huCD46/huCD150 double TG mice with human-like distribution profile are necessary for the analysis of wild-type MV spreading and following host immunomodulatory events, particularly measles-mediated immune suppression (22–24).

Therefore, for this study, we generated huCD46/huCD150 double TG mice with or without type I IFN-αβ receptor (IFNR1). MV replication and spreading were surveyed by GFP-expressing wild-type Ichinose (IC) strain. In in vivo infection studies, however, neither type of MV was pathogenic to these TG mice irrespective of the route of administration. Analyses of these TG mice suggested that the critical factor in wild-type MV systemic infection appears to depend on the level of type I IFN generated by CD11c-positive dendritic cells (DCs) in this mouse model. In fact, DCs were infected with MV in the huCD150-positive TG mice with no IFNR1. In this study, we first report that huCD46/huCD150 double TG mice are sensitive to wild-type MV once MV-infecting mDCs are supplemented. Thus, infected mDCs facilitate establishing systemic MV infection in the TG mice. These double TG mice allow us to analyze immunomodulatory function of the MV receptors during MV infection (25, 26). The double TG mice with human-like huCD46/huCD150 expression profiles may be a better model for wild-type MV infection compared with huCD150 constitutively expressing mice.

Materials and Methods

Cell culture and reagents

Vero, Vero/huCD150 (a signaling lymphocyte activation molecule (SLAM)), which are Vero cells with stable expression of huCD150 obtained by transfection of huCD150/pCXN2 and antibiotic selection, HEK293, and 293-3-46 cells, kindly provided by M. A. Billeter (Institute of Molecular Biology, University of Zurich, Zurich, Switzerland) were maintained in DMEM supplemented with 10% heat-inactivated FCS and antibiotics. B95a cells were maintained in RPMI 1640 supplemented with 5% heat-inactivated FCS (JRH Biosciences) and antibiotics. Polymyxin B, LPS from Escherichia coli, serotype O111:B4, and Endotoxin Test Kit (Lonza) were obtained from Sigma-Aldrich. The mycoplasma lipopeptide macrophage-activating lipopeptide (MALP-2) was prepared as described (27). MALP-2 was treated with polyclonal (10 μg/ml) for 1 h at 37°C before stimulation of cells (27). Usually, 100 ng/ml LPS and 100 nM MALP-2 were used in MD0 TLR stimulation. Isotype controls for PE rat IgG2a, PE rat IgG2b, PE mouse IgG1, PE or FITC golden syrian hamster IgG, FITC mouse IgG1, and FITC mouse IgG2a, and mAb for PE anti-mouse CD8a, PE anti-mouse CD4, PE anti-mouse CD3ε, PE anti-human CD3ε, PE anti-human CD4, PE anti-human CD8a, PE anti-human CD19, and FITC anti-human CD150 were obtained from eBioscience. FITC or PE anti-mouse CD11c mAb was obtained from BD Pharmingen. FITC anti-human CD46 (MCP) mAb was obtained from Ancell. HRP-conjugated goat anti-rabbit IgG s were obtained from American Qualex. Rabbit polyclonal bodies against huCD150 or huCD46 were produced in our laboratory.

TG method

Spermatzoa were dispersed from epididymis of 12-wk-old mature B6D2F1 male mice in 400 μl of TYH medium diluted to 1 × 107/ml and frozen in liquid nitrogen immediately. To prepare artificial chromosome (BAC) DNA carrying huCD46 or huCD150 was purchased from Incyte Genomics, and purified by using Large-Construct kit (Qiagen) according to the manufacturer’s instructions. Each BAC DNA (5 μg/ml in TE buffer) was added to the thawed sperm. The mixtures were incubated for 5 min at room temperature, and mixed with one-tenth volume of 12% PVP-HCZB (polyvinyl-pyrrolidone-HEPES-Chatot, Ziamok, and Barister medium). The metaphase II oocytes for microinjection from B6D2F1 female mice were prepared as previously described (28). These oocytes were maintained in kSOM (potassium simplex optimized medium) under mineral oil equilibrated in 5% (v/v) CO2 in air at 37°C until use.

Generation of huCD46 and huCD150 TG mice

The huCD46 and huCD150 TG mice were produced by using the intracytoplasmic sperm injection transgene method (29). TG mice were made in accordance of the guidelines of the Animal Care and Use Committee of Osaka University. For microinjection, sperm heads were aspirated to a pipette attached to a piezoelectric pipette-driving unit and a sperm head was injected into each oocyte as previously described (28). After injection, the eggs were incubated in kSOM to the 2-cell stage, and were transferred to ICR pseudopregnant females. Among pups born, the huCD46 and huCD150 TG mice were detected by genomic PCR using huCD46- and huCD150-specific primers. The PCR primers used were as follows: 5'-aagcttctgaccatatcctgctctagc-3' and 5'-ggaattcgcacgatcca-ggtttcagg-3', and 5'-gatatccgtattctgtggca-3' for huCD46 and 5'-gtgatacagggaggggtttcagg-3', and 5'-gatatccgtattctgtggca-3' for huCD150.

Southern blotting was performed with PCR products from human genome using those primers and with DIG High Prime DNA Labeling and Detection starter kit II (Roche Applied Science).

The IFNR1 -/- mouse (30) was obtained from The Jackson Laboratory. All mice were backcrossed at least six times to be a C57BL/6J background.

Western blotting

Various tissues from huCD46 and huCD150 TG mice were homogenized by a Potter-type homogenizer in 1% (v/v) Triton X-100, PBS, 1 nM PMSF, 10 mM benzamidine, 1 μg/ml pepstatin, and 1 μg/ml leupeptin. The extracts were centrifuged at 15,000 × g, and protein was estimated by Bio-Rad color reagent. A total of 20 μg of protein was subjected to SDS-PAGE under nonreducing conditions and transblotted onto nylon membranes. The membranes were incubated with anti-huCD46 polyclonal Ab or anti-huCD150 polyclonal Ab for 2 h, washed with PBS containing 0.5% Tween 20 three times and incubated with HRP-conjugated goat anti-rabbit IgG for 1 h at 37°C. Following second incubation, the membranes were washed three times with PBS-Tween 20 and proteins were detected with an ECL chemiluminescence kit (Amersham Biosciences).

Plasmid construction and rescue of recombinant viruses

For the preparation of wild-type MV IC-B strain (31, 32) expressing enhanced GFP (EGFP), we constructed p(+)MV323/GFP. Fragment 1, which has BssHII site at promoter leader sequence with EcoRI site, was amplified by PCR with p(+)MV323/GFP as template using forward primer 5'-AGTCCGCGGAggeggegGTATAACGACTACA-3' (ggcgg is BssHII site and underline is T7 promoter sequence), and reverse primer 5'-gaaccgttctaatctgytctgtGCC-3' (gaaccgttctaatctgytctgt is EcoRI site and underline is a part of 5' noncoding region of the N gene). Fragment 2, which has NorI site, the N gene terminator sequence, and part of the N gene coding sequence, is amplified by PCR with p(+)MV323/GFP as template by forward primer 5'-CTggcgeggeAATGGTATATAAACTTACGGATTCAAGTCTATTA-3' (ggcgegge is NorI site and underline is the N gene terminator sequence, and the N gene initiator) and reverse primer 5'-acgggtactctacctggtctgccggcACACACTGAGAAAAG-3' (acgggtactctacctggtctgccggcACACACTGAGAAAAG is HindIII site and underline is a part of N gene coding region). The fragment 1 was digested with BssHII and EcoRI, fragment 2 was digested with NorI and HindIII, and the EGFP gene was cut from pEGFP-N1 plasmid (Clontech Laboratories) with EcoRI and NorI. These three DNA fragments were ligated and the chimeric plasmid clone containing 5' BssHII site, T7 promoter, leader initiation signal (the 5' region of the N gene) EGFP gene, and terminator sequence of the 3' region of the N gene/CTT sequence (the 5' region of the N gene containing an initiation sequence), which have EspI site 3' sequences, was generated. The accuracy of the fragment was confirmed by sequencing. The insert and p(+)MV323 were digested with BssHII and EspI, and the fragment containing an EGFP gene was inserted into p(+)MV323.

Recovery of infectious MV was performed as previously described (33, 34). MV323, MV323/GFP, and MV2A (ED strain) were recovered from p(+)MV323, p(+)MV323/GFP, and p(+)MV2A, respectively.

Immunofluorescence staining and confocal microscopy

Mock- or MV323/GFP-infected mouse splenocytes were stained with PE rat IgG2a isotype control, PE rat IgG2b isotype control, PE golden syrian hamster IgG isotype control, PE anti-mouse CD8a mAb, PE anti-mouse CD4 mAb, PE anti-mouse CD3ε mAb or PE anti-mouse CD19 mAb for 30 min at room temperature in FACS buffer. After washing, the stained cells were fixed in 0.5% formaldehyde in PBS and visualized at a magnification ×60 under a FLUOVIEW (Olympus). Images were captured using the attached computer FLUOVIEW software.
**FACS cytometric analysis of cell surface Ags**

FACS methods were previously described (35). Briefly, cells were suspended in PBS containing 0.1% sodium azide and 1% BSA (FACS buffer) and incubated for 30 min at 4°C with FITC-labeled mAbs and PE-labeled mAbs. Cells were washed and fluorescence intensity was measured by FACS.

**Preparation of MV strains and in vitro MV infection analysis**

Recovered viruses, MV2A (ED strain), MV323 (IC-B strain), and MV323GFp (IC-B strain containing EGFP gene), were passaged in B95a cells and titered on Vero/SLAM cells. Low-passaged viruses were used in this experiment. The receptor usage of the strains was confirmed on Chinese hamster ovary (CHO), CHO/huCD46, or CHO/huCD150 cells (5). For in vitro viral infection assay, single cell suspensions of spleen from TG or non-TG littermates were obtained by passage through sterile mesh. After removing erythrocytes using Lympholyte-M (Cedarlane Laboratories), purified cells were cultured in RPMI 1640 supplemented with 10% heat-inactivated FCS, 10 mM HEPES, 1 mM sodium pyruvate, 0.1 mM non-essential amino acids, 0.05 mM 2-ME, 50 U/ml mouse IL-2, 20 ng/ml PMA, and 1 μg/ml ionomycin for 24 h. FACS analysis was performed 48 h after infection.

**Preparation of DCs**

Mouse bone marrow-derived mDCs were prepared by the modified technique (36, 37) of earlier reported method (38). Briefly, 0.5–1 × 10⁶ bone marrow cells/2 ml/well in RPMI 1640 supplemented with 10% heat-inactivated FCS, 10 mM HEPES, 1 mM sodium pyruvate, 0.1 mM nonessential amino acids, and 0.05 mM 2-ME were cultured overnight in 24-well plates. Nonadherent cells were harvested, resuspended in the same medium supplemented with 10 μg/ml mouse GM-CSF, and cultured in the mouse GM-CSF-containing medium. On day 3, adherent cells were cultured in fresh medium with 10 μg/ml mouse GM-CSF. On day 6, nonadherent cells and loosely adherent cells were harvested and used for experiments as immature DCs (38). Immature DCs were resuspended and cultured in fresh RPMI 1640 medium with 10 μg/ml mouse GM-CSF. Cellular RNA was estimated 24 h postinfection.

Human PBMC were isolated from buffy coat of normal healthy donors by density gradient centrifugation followed by ficoll-hypaque (Amersham Biosciences) (27). For human immature DC preparation, CD14⁺ monocytes were obtained from human PBMC by using MACS system (Miltenyi Biotec) with anti-human CD14 mAb-conjugated microbeads and kept in RPMI 1640 (Invitrogen Life Technologies) containing 10% FCS, 500 IU/ml human GM-CSF, 100 IU/ml human IL-2, and infected by MV323. Culture media were centrifuged to remove cell debris and supernatants were stored at −80°C until the assay. The level of secreted human IFN-β in the culture medium was determined with an ELISA kit (FUJIREBIO) for human IFN-β according to the manufacturer’s protocol.

**Results**

**Human-like expression of huCD46 or huCD150 in TG mice**

Ubiquitous expression of huCD46 was not obtained in TG mice that were previously generated with huCD46 cDNA (39). A number of splicing variants in huCD46, which appeared in an organ-specific manner (40), participated in differential cell-mediated immune response (26). To generate the natural expression profile of huCD46 and its isoforms, we transferred the 194-kb BAC DNA carrying the huCD46 gene into mouse embryo and obtained TG mice with human-like CD46 expression pattern. Similarly, huCD150 TG mice were generated by transferring the huCD150 BAC DNA (185 kb) into mouse embryo. These mice have a human-like CD150 expression profile, which is inducible in T cells and up-regulated in mDCs and B cells. We examined the expression profiles of huCD46 and huCD150 in various organs of each line of our TG mice by SDS-PAGE and Western blotting (Fig. 1A). In huCD46 TG mice, huCD46 was ubiquitously expressed with the size variation of isoforms in an organ-specific fashion similar to human (41, 42). The brain of these mice notably expressed a low molecular mass moiety of huCD46 in the TG mice similar to human form. In addition, similar to human, huCD150 appeared in a tissue-specific manner in the thymus and spleen in huCD150 TG mice. In non-TG mice, no signals of huCD46 or huCD150 were detected in Western blots, suggesting no cross-reaction of the Abs with the mouse counterparts.

**Cell populations expressing huCD46 or huCD150 in TG mice**

We next analyzed cells from spleens of huCD46 TG, huCD150 TG, or non-TG mice by FACS analysis (Fig. 1B). In huCD46 TG mice, huCD46 was expressed on the surface of both CD3- or CD19-positive splenocytes. In huCD150 TG mice, huCD150 was expressed on CD19-positive cell surface, but minimal expression occurred on CD3-positive cells. Neither CD4 nor CD8 T cells expressed huCD150 (data not shown). Under similar FACS conditions, human PBMC ubiquitously expressed CD46 and partly expressed CD150 (Fig. 1B, right). When splenocytes from huCD150 TG mice were stimulated with PHA (data not shown) or PMA, ionomycin, and murine IL-2 (Fig. 1C, left), FACS profiles of the lymphocyte populations were altered. In addition, the levels of huCD150 in CD4⁺ and CD8⁺ T cell and CD19⁺ B cell populations were up-regulated (Fig. 1C). Similar results were obtained with human PBMC when the cells were similarly stimulated (Fig. 1C, right). Neither huCD46 nor huCD150 was expressed in cells from non-TG mice as expected (see Fig. 5, B and C, labeled as...
These results show that huCD150-inducible TG mice were established, which may suit the study for susceptibility of cell species to MV strains.

We then generated huCD46/huCD150 double TG mice by mating both TG lines (Fig. 2A). Gene analysis showed that the littermates of huCD46/huCD150 TG, huCD46 TG, huCD150 TG, and non-TG mice followed Mendel’s laws of inheritance (Fig. 2A), suggesting that huCD46/huCD150 transgene does not bring about any lethal defect. The presence of huCD46 and huCD150 genes in litters was monitored by PCR (data not shown) and confirmed by Southern blot analysis (Fig. 2B). Expression profiles of huCD46 and huCD150 in each litter were also confirmed by Western blot and FACS analysis (data not shown).

**Preparation of EGFP-labeled MV**

To monitor MV infection, we generated EGFP-expressing recombinant MV (IC323GFP) based on the wild-type IC strain. Plasmid containing full-length cDNA of the IC strain and the EGFP gene was constructed as shown in Fig. 3A. GFP expression reflects viral replication in this construct. The rescued MV323GFP showed almost the same growth kinetics as the parental recombinant MV323. Its receptor usage was confirmed with CHO cells expressing huCD46 or huCD150 (Fig. 3B). MV2A (ED strain) rescued from p(+)/MV2A, was used as the laboratory-adapted strain. When infected with MV2A, both CHO/huCD46 and CHO/huCD150 cells produced the typical syncytia, whereas MV323 infected CHO/huCD150 cells but not CHO/huCD46 cells (data not shown). MV323GFP also infected CHO/huCD150 cells to form syncytia exhibiting green autofluorescence, but did not form any syncytium in CHO/huCD46 and intact CHO cells.

**In vitro analysis of MV infection of splenocytes from TG mice**

To examine in vitro MV infection, the splenocytes were isolated from each TG mouse and infected with MV323GFP at a multiplicity of infection (MOI) of 0.25 (Fig. 4A). When prestimulated with PMA and ionomycin, some splenocytes from the double TG or huCD150 TG mice were positive in GFP, a marker for infection. The stimulation was essential for MV permissiveness as reported before (14). However, under any infectious conditions the splenocytes from huCD46 TG and non-TG mice were GFP-negative. The results were confirmed to be reproducible by semiquantitative FACS analysis (Fig. 4A). In contrast, MV323GFP infected human PBMC even at low MOI of inoculation (Fig. 4A, bottom panels). Human PBMC appear to be more susceptible to MV323GFP than the splenocytes of the double TG and huCD150 TG mice.

To examine which populations of splenocytes of huCD46/huCD150 TG or huCD150 TG mice were infected with MV323GFP, we segregated them with PE-conjugated anti-mouse CD3, CD4, CD8, CD11c, or CD19 mAb and analyzed them by FACS and confocal microscopy (Fig. 4, B and C). MV323GFP inoculation rendered the CD3-, CD4-, CD8-, and CD19-positive cells green. But CD11c-positive cells barely turned green even at...
high MOI inoculation (Fig. 4B). Thus, the CD11c-positive splenocytes that are representative of DCs are less susceptible to wild-type MV compared with other cell types.

**Wild-type MV fails to infect the TG mice in vivo**

Next we examined in vivo MV infection. Six- to 10-wk-old double TG, huCD150 TG, huCD46 TG, or non-TG mice were injected i.p., i.v., intranasally, or s.c. with MV323GFP at a dose of $10^4$ to $10^6$ TCID$_{50}$. None of the TG mice showed any sign of illness. Two to 4 days after inoculation, the sections of brain, spleen, thymus, lymph nodes, lung, kidney, liver, intestine, and heart from each mouse were examined for GFP-positive cells to monitor infection by MV323GFP. No GFP-positive cells were detected in any tissue. Following i.p. injection of MV323GFP in newborn 2-day-old or 1-wk-old TG or normal mice, no GFP was detected. Intravenously transferred MV323GFP-infected splenocytes to double TG mouse also did not show any GFP-positive lesions (data not shown).

**MV fails to effectively replicate in mouse DC**

It was reported earlier that huCD150 were minimally expressed in human monocyte-derived DCs and up-regulated by stimulation of TLRs (20). We confirmed the expression and up-regulation of huCD150 in mDCs from huCD46/huCD150 TG mice (Fig. 5A). mDCs prepared from the TG mice by conventional method (the purity confirmed by the CD11c marker) expressed both huCD150 and huCD46 with a pattern similar to human monocyte-derived DCs. When mDCs were stimulated with LPS, a ligand of TLR4, expression of huCD150 was up-regulated. Similar huCD150 up-regulation was observed by stimulation with MALP-2, which is a ligand of TLR2. Of note, the level of huCD46 was not influenced in mDC in which TLRs were stimulated.

FIGURE 2. Scheme for generation of double TG mice and confirmation of genotypes. A, Heterogenic human CD46 and human CD150 TG mice were mated, and Mendelian inheritance of the offspring of huCD46/huCD150, huCD46, huCD150 is diagrammatically represented. B, Southern blots of gene transfer profiles of double TG mice.

FIGURE 3. Generation of EGFP-labeled wild-type MV (MV323GFP). A, Scheme for GFP-labeled MV323 (MV323GFP) construction shows the p(+)MV323 plasmid (upper) coding wild-type MV IC strain antigenome and a construct of EGFP. The EGFP construct was placed upstream of the N gene of IC. The construct of p(+)MV323GFP that expresses GFP and viral proteins is also shown (lower). B, Receptor usage of MV323GFP. Rescued virus MV323GFP from p(+)MV323GFP was inoculated on CHO/huCD150, CHO/huCD46, and intact CHO cells, and 2 days later receptor tropism of MV323GFP was confirmed by syncytium formation and GFP fluorescence. MV2A (ED strain) rescued from p(+)MV2A was used as a control that can use both receptors. Magnification, $\times 100$.
TG and huCD150 TG, the mRNA levels of type-I IFN (IFN-α and IFN-β) were markedly increased in the mDCs (Fig. 6, B and C). MV2A induced IFN-α and IFN-β in the mDCs from double TG, huCD150 TG, and huCD46 TG (Fig. 6, B and C). Induction of IFN-α was less compared with IFN-β. In mouse blood cells, however, neither IFN-β nor IFN-α message was virtually detected: only a trace amount of IFN-γ message was measurable (data not shown). Local, rather than systemic, induction of type I IFN-β would occur by mDCs in response to wild-type MV. The levels of total IFN-β production depend on the combined actions of MV strain types and IFN inducibility of mDCs. Because IL-12 p40 and IL-10 were induced from human DCs as a consequence of MV infection, we also measured the mRNA level of murine IL-12 p40 and murine IL-10 of mDCs (Fig. 6, D and E). Results showed that the mRNAs of these cytokines were also induced by MV infection via the MV receptors. MV infection was confirmed by determination of the mRNA level of the H protein gene of MV (Fig. 6, F).

Although the same lots of virus stocks were used for infection, MV323 replicated better in mDCs from huCD150 TG than huCD46/huCD150 TG mouse lines. MV2A replication was high in mDCs from huCD150 TG and low in mDCs from the double TG and huCD46 TG mouse lines. The coexpressed huCD46 would probably inhibit viral replication in mouse mDCs.

**DC have critical role in the establishment of MV infection in vivo**

Differential susceptibility to MV of mDCs prepared from huCD46/huCD150 TG mouse vs human DCs earlier described, allowed us to speculate that DCs play a critical role in the development of systemic MV infection in vivo. Because type I IFN induced by
wild-type MV acted on huCD46/huCD150-positive mDCs in a positive feedback manner, we generated huCD150/huCD46/IFNAR1/-/-/- triple mutant mice, by mating the double TG mice with the IFNAR1 knockout mouse. First, we examined whether mDCs from huCD46/huCD150 TG/IFNAR1/-/-/- mice were susceptible to wild-type MV infection in vitro. When the mDCs were inoculated with MV323GFP at MOI = 0.25, many GFP-positive mDCs were detected by fluorescence microscopy (Fig. 7A) and FACS analysis (Fig. 7B). Moreover, robust type IFN production was detected in mDCs (Fig. 7C). The mRNA levels of mouse IFN-β and IFN-α were measured using quantitative PCR. As viral replication increased, IFN-β production also increased (Fig. 7, B and C). IFN-α production was largely suppressed because of the lack of the secondary signal via IFNAR1 (Fig. 7B, right). Because mDCs in triple mutant mice became susceptible to wild-type MV, we next analyzed the results of in vivo MV infection. For this, the triple mutant mice were inoculated i.p. with MV323GFP at a dose of $5 \times 10^5$ TCID$_{50}$. Two days later, some of the tissues were collected and their sections were directly observed by inverted fluorescence microscope (Fig. 8A). GFP-positive cells were detected in mesenteric lymph nodes, inguinal lymph nodes, parathymic lymph nodes, and spleen. Yet,
infection visualized by transfer of MV-infected mDCs into IFNAR1−/− mice. Merging profiles are shown to the right. Magnification, ×100. B, CD11c-positive cells reserve MV323GFP. Mesenteric lymph nodes were fixed with 4% paraformaldehyde and the sections were stained with PE-conjugated anti-mouse CD11c mAb (red). EGFP (green) and CD11c (red) were observed by confocal microscopy. Merging profiles are shown to the right. Magnification, ×400. C, MV infection visualized by transfer of MV-infected mDCs into IFNAR1−/− TG mice. mDCs prepared from huCD46/huCD150 TG/IFNAR1−/− mice were infected with MV323GFP at MOI = 1. At 24 h later, MV323GFP-infected mDCs (1 × 106) were i.v. injected into huCD46/huCD150 TG, huCD46 TG, huCD150 TG, and non-TG mice (IFNAR1 is normal in all TG mice). Two days later, each mouse was dissected and tissues were directly examined under an inverted fluorescence microscope. Magnification, ×100. The results were confirmed by two additional experiments.

FIGURE 8. mDCs participate in systemic viral spreading. A, MV infection visualized in the triple mutant mice by in vivo inoculation of MV323GFP. Representative photographic images of GFP-positive organs in huCD46/huCD150 TG/IFNAR1−/− mice are shown. At 48 h after i.p. injection of 103 TCID50 of MV323GFP, the mice were dissected and tissues were directly examined under an inverted fluorescence microscope. Magnification, ×100. B, CD11c-positive cells reserve MV323GFP. Mesenteric lymph nodes were fixed with 4% paraformaldehyde and the sections were stained with PE-conjugated anti-mouse CD11c mAb (red). EGFP (green) and CD11c (red) were observed by confocal microscopy. Merging profiles are shown to the right. Magnification, ×400. C, MV infection visualized by transfer of MV-infected mDCs into IFNAR1−/− TG mice. mDCs prepared from huCD46/huCD150 TG/IFNAR1−/− mice were infected with MV323GFP at MOI = 1. At 24 h later, MV323GFP-infected mDCs (1 × 106) were i.v. injected into huCD46/huCD150 TG, huCD46 TG, huCD150 TG, and non-TG mice (IFNAR1 is normal in all TG mice). Two days later, each mouse was dissected and tissues were directly examined under an inverted fluorescence microscope. Magnification, ×100. The results were confirmed by two additional experiments.

no positive cells were found in brain, thymus, lung, heart, liver, kidney or intestine.

We prepared sections from mesenteric lymph nodes, and stained with PE-conjugated anti-mouse CD11c mAb. As shown in Fig. 8B, GFP-positive and CD11c-positive cells reserve MV323GFP. Mesenteric lymph nodes were fixed with 4% paraformaldehyde and the sections were stained with PE-conjugated anti-mouse CD11c mAb (red). EGFP (green) and CD11c (red) were observed by confocal microscopy. Merging profiles are shown to the right. Magnification, ×400. C, MV infection visualized by transfer of MV-infected mDCs into IFNAR1−/− TG mice. mDCs prepared from huCD46/huCD150 TG/IFNAR1−/− mice were infected with MV323GFP at MOI = 1. At 24 h later, MV323GFP-infected mDCs (1 × 106) were i.v. injected into huCD46/huCD150 TG, huCD46 TG, huCD150 TG, and non-TG mice (IFNAR1 is normal in all TG mice). Two days later, each mouse was dissected and tissues were directly examined under an inverted fluorescence microscope. Magnification, ×100. The results were confirmed by two additional experiments.

Discussion

We generated TG mice that express MV receptors huCD46 and huCD150. The expression profiles and the distribution of these receptors in these mice were similar to those in humans. Mouse splenocytes from the double TG mice became susceptible to wild-type MV strains in vitro. Nevertheless, despite the fact that the TG mice expressed both huCD46/huCD150, they were highly resistant to MV infection in vivo irrespective of the route of MV administration. huCD150 but not huCD46 was up-regulated in response to TLR stimulators and conferred high susceptibility to wild-type MV on mDCs. In vivo, normal CD11c-positive DCs were usually insensitive to wild-type MV infection, whereas these DCs turned susceptible to MV if their amplifiable IFN-inducing ability in response to MV infection was spoiled. Systemic MV infection was observed in the huCD46/huCD150 double TG mice with no IFNAR1 by direct MV injection or in the double TG mice with normal IFNAR1 by transfer of MV-infected mDC to them. Thus, in vivo MV permissiveness was accomplished when the IFNAR1 gene was simultaneously disrupted in DCs.

A crucial question remains elusive that this TG mouse model with natural expression profile of huCD46 and huCD150 serve as a model for the investigation of human wild-type MV infection. Different from human, IFNAR1−/− mice induce basal production of IFN-β in response to IFN regulatory factor (IRF)-3 activation, but secondary amplifiable response is severely impaired (45, 46). Opposing to the results on human DC, mouse mDC more efficiently induce type I IFN in response to wild-type MV than to infection by attenuated MV strains (Fig. 6). Wild-type MV strains barely induce amplified type I IFN production in human DCs, whereas attenuated strains robustly induce it (M. Taniguchi, N. Begum, and T. Seya, unpublished observation). Thus, pathogenicity of wild-type MV strains may not be reflected in our mouse model. MV strains, either wild-type or laboratory-adapted, appear to replicate well in human DCs if they induce only minute degrees of type I IFN (IFNAR1 (Figs. 7A and 8A)). This is true in human in which wild-type MV infections in vivo are usually mild and resolve in weeks (47). In mice, wild-type MV strains efficiently replicate in DCs and barely induce type I IFN (20, 43). Recent reports after the submission of our study clearly revealed that the MyD88-IRF-7 pathway in pDC is the main route of IFN-α induction in mice in several virus species (48, 49). Perhaps, mDC induces IFN-β via Toll-IL-1R homology domain-containing adapter molecule 1-mediated or RNA helicase RIG-I-mediated IRF-3 activation pathways, which is independent of MyD88-dependent IRF-7 activation pathway. We could test this issue regarding MV if the mouse IFN-α and IFN-β are separately determined by ELISA. At least blood cells barely induce the messages of type I IFN in response to MV infection (data not shown), and the role of pDC in our mouse MV infection model remain to
be settled. Further MV infection studies using mice disrupting either of the IFN-inducing pathways will be required to clarify the source of type I IFN in MV-infected TG mice.

Wild-type MV-H protein acts as a ligand for TLR2 (2) and up-regulates CD150 in human monocytes (50). Although in vitro analysis speculated that DC matures into a stage vulnerable to wild-type MV through TLR2 signaling (51), whether wild-type MV actually infects mDC in vivo remains undetermined. Our model offers the possibility that the matured DC is the primary target of wild-type MV strains. Testing the role of TLR2 by MV-H protein stimulation in immature DC maturation is feasible in our model. Dysfunction of Ag-presenting capacity of DC through MV infection may be associated with modulation of TLR signaling by MV.

Previous reports suggested that MV efficiently infected human DC and suppressed DC-mediated allograft lymphocyte proliferating (MLR) (52–54). Both wild-type and vaccine strains induce defective MLR response irrespective of the induction levels of type I IFNs. Testing MLR in intact vs MV-infected mDCs prepared from these TG mice will give us a hint to resolve DC-based immune suppression. The model may enable us to test the role of huCD150 and/or huCD46 on lymphocytes in association with infected mDCs.

A comparison of the double TG mice with huCD150 TG mice in their ability to produce differential cytokine levels suggests that huCD150 TG mice more efficiently induce all four cytokines tested than do huCD46/huCD150 TG mice (Fig. 6, B–E). The replication level of MV-H was high in huCD150 TG mice and relatively low in huCD46/huCD150 TG mice (Fig. 6F). The results suggest that CD46 expressed on mDCs acts as a negative regulator for wild-type MV replication. According to previous reports, mouse macrophages expressing human CD46 significantly resist MV infection (55). These cells produced high levels of NO and IFN-αβ upon infection by MV (55, 56), suggesting that huCD46 enhances IFN-αβ production in mouse macrophages. Thus, it is possible that simultaneous expression of huCD46 and huCD150 in mDC down-regulates MV replication. Alternatively, huCD46 in lymphocytes deliver negative or positive signal in lymphoproliferation depending on the type of tail sequences as reported previously (25, 26). There are two major isosforms of huCD46 with differential cytoplasmic tails (CYTs), CYT1 and CYT2 (25, 57). NO is induced in macrophages in a CYT1>CYT2 fashion (55, 58). In mouse lymphocytes from TG mice with either CYT1 or CYT2, differential functions of these isosforms were clearly observed (26). Relationship between the huCD46 isoforms and IFN-β production will be an issue to be tested in our model.

In human myeloid DCs, TLR3 and TLR8 participate in type I IFN production (59). Mouse mDCs lack TLR8 (60, 61). RIG-I participates in sensing viral infection inside the cells to activate a signaling pathway for induction of type I IFN (62). Yet, TLR3 may sense viral infection, as in West Nile virus entry into the brain (63). Human monocytes may express CD150 in the adherent tissue to catch up inspired MV (50). Therefore, interpretation of differential responses between mouse mDC and human immature DC in wild vs vaccine strains need to take these recent findings into consideration. It is important to further analyze the natural intranasal infection of MV and its relationship to the properties of wild-type MV-infected CD11c-positive mDCs.

Our findings can be summarized as follows: 1) mouse CD11c-positive mDCs in huCD46/huCD150 double TG mice are barely susceptible to wild-type MV until IFNAR1 is depleted; 2) in the double TG mice with no IFNAR1, only CD11c-positive mDCs are vulnerable to MV at an early phase of infection in vivo; 3) robust IFN type I induction due to IFNAR1 by mDCs confers natural resistance to wild-type MV on mice; and 4) the huCD46/huCD150 double TG mouse when injected with MV-infected mDCs i.v. permits systemic infection by MV. Therefore, in this mouse model mDC carries MV to draining lymph nodes. Although the results were deduced from an artificial model case, the TG mice with or without IFNAR1 would be applicable for the study of MV-mediated initial IFN-β induction in mDC. With these mice, virus-mediated initial IFN-β inducing response and its related pathways involving IRF-3 and IRF-7 in mDCs may be critically analyzed. This is the first report on the generation of wild-type MV-sensitive mice.

Acknowledgments
We are grateful to Drs. K. Toyoshima, N. Inoue, M. Tanabe K. Funami, T. Tsujita (Osaka Medical Center for Cancer, Osaka, Japan), M. Ikawa (Osaka University), Y. Seto (Osaka City University), M. Kohanawa (Hokkaido University, Sapporo, Japan), and A. Takaoka (University of Tokyo, Tokyo, Japan) for helpful discussions. Thanks are also due to K. Shida, Y. Okuda, M. Sasaki, A. Matsuo, and H. Masuda (Osaka Medical Center for Cancer) for helpful technical support. We thank Dr. M. A. Billeter (University of Zurich) for kindly providing p(MV2a plasmid and MV rescue system from cDNA and Dr. K. Takeuchi (Tsukuba University, Ibaragi, Japan) for kindly providing p(MV323 plasmid.

Disclosures
The authors have no financial conflict of interest.

References


