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The MyD88 Pathway in Plasmacytoid and CD4+ Dendritic Cells Primarily Triggers Type I IFN Production against Measles Virus in a Mouse Infection Model

Hiromi Takaki,* Makoto Takeda,† Maino Tahara,† Masashi Shingai,*1 Hiroyuki Oshiumi,*, Misako Matsumoto,* and Tsukasa Seya*

Infection by measles virus (MV) induces type I IFN via the retinoic acid–inducible gene I/melanoma differentiation–associated gene 5/mitochondrial antiviral signaling protein (MAVS) pathway in human cells. However, the in vivo role of the MAVS pathway in host defense against MV infection remains undetermined. CD150 transgenic (Tg) mice, which express human CD150, an entry receptor for MV, with the disrupting IFNR gene (Ifnar−/−), are susceptible to MV and serve as a model for MV infection. In this study, we generated CD150Tg/Mavs−/− mice and examined MV permissiveness compared with that in CD150Tg/Ifnar−/− mice. MV replicated mostly in the spleen of i.p.-infected CD150Tg/Ifnar−/− mice. Strikingly, CD150Tg/Mavs−/− mice were not permissive to MV in vivo because of substantial type I IFN induction. MV barely replicated in any other organs tested. When T cells, B cells, and dendritic cells (DCs) isolated from CD150Tg/Mavs−/− splenocytes were cultured with MV in vitro, only the DCs produced type I IFN. In vitro infection analysis using CD150Tg/Mavs−/− DC subsets revealed that CD4+ and plasmacytoid DCs, but not CD8α+ and CD8α−CD4− double negative DCs, were exclusively involved in type I IFN production in response to MV infection. Because CD150Tg/Mavs−/− mice turned permissive to MV by anti-IFNAR Ab, type I IFN produced by CD4+ DCs and plasmacytoid DCs plays a critical role in antiviral protection for neighboring cells expressing IFNAR. Induction of type I IFN in these DC subsets was abolished by the MyD88 inhibitory peptide. Thus, production of type I IFN occurs via the MyD88-dependent and MAVS-independent signaling pathway during MV infection. The Journal of Immunology, 2013, 191: 4740–4747.

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Address correspondence and reprint requests to Prof. Tsukasa Seya and Dr. Hiromi Takaki, Department of Microbiology and Immunology, Hokkaido University Graduate School of Medicine, Kita 15, Nishi 7, Kita-ku, Sapporo 060-8638, Japan. E-mail addresses: seya-ta@pop.med.hokudai.ac.jp (T.S.) and tahirimm@sci.hokudai.ac.jp (H.T.)

*Department of Microbiology and Immunology, Graduate School of Medicine, Hokkaido University, Kita-ku, Sapporo 060-8638, Japan; and †Department of Virology 3, National Institute of Infectious Diseases, Gakuen 4-7-1, Musashimurayama, Tokyo 208-0011, Japan

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Address correspondence and reprint requests to Prof. Tsukasa Seya and Dr. Hiromi Takaki, Department of Microbiology and Immunology, Hokkaido University Graduate School of Medicine, Kita 15, Nishi 7, Kita-ku, Sapporo 060-8638, Japan. E-mail addresses: seya-ta@pop.med.hokudai.ac.jp (T.S.) and tahirimm@sci.hokudai.ac.jp (H.T.)

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and serve as a useful mouse model (16, 24). In the current study, using the CD150Tg mouse model in combination with Mavs−/−, Ifr3−/−, Ifr7−/−, and Ticam1−/− mice, we found that CD150Tg/Mavs−/− mice were not permissive to MV in vivo, whereas CD150Tg/Ifr3−/−/Ifr7−/− mice were permissive. Furthermore, CD150Tg/Mavs−/− plasmacytoid DCs (pDCs) and CD4+ DCs produced type I IFN in response to MV infection in vitro. Analysis using the myeloid differentiation factor 88 (MyD88) inhibitory peptide and MyD88−/− mice revealed that type I IFN production in these DC subsets was dependent on the MyD88 pathway. To our knowledge, this is the first study to show that type I IFN induction in MV-infected mouse DCs depends on the MyD88 pathway. The properties of the MV-permissive mouse DC subsets may be crucial for ensuring immune response, including immunosuppression during MV infection.

Materials and Methods

Mice

All mice were backcrossed to C57BL/6 mice more than eight times before use. CD150Tg (16), Ticam1−/− (25), and Mavs−/− (26) mice were generated in our laboratory. Ifr3−/− and Ifr7−/− mice were provided by Dr. T. Taniguchi (University of Tokyo, Tokyo, Japan). Myd88−/− mice were provided by Drs. K. Takeda and S. Akira (Osaka University, Osaka, Japan). All mice were maintained under specific pathogen–free conditions in the Animal Facility at Hokkaido University Graduate School of Medicine (Sapporo, Japan) and used when they were between 6 and 12 wk of age. This study was carried out in strict accordance with the recommendations in the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The protocol was approved by the Committee on the Ethics of Animal Experiments in the Animal Safety Center, Hokkaido University. All mice were used according to the guidelines of the Institutional Animal Care and Use Committee of Hokkaido University, which approved this study no. E13-0024. All animal manipulations were performed with the animals under anesthesia that was made to minimize suffering. For TLR7 intracellular staining in pDCs, DCs were stained with anti-CD11b–FITC, anti-pDC Ag 1 (PDCA-1)–PE, and anti-human CD150–FITC. For T cell staining, anti-CD3–allophycocyanin and anti-CD4–PE were used. CD150Tg/Mavs−/− mice were not permissive to MV infection, as determined by the reporter Renilla luciferase from the first gene position of the MV genome, was a kind gift from Dr. Y. Yanagi (Kyushu University, Fukuoka, Japan) (29). MV-luciferase was maintained in Vero/CD150 cells (30). TLR7 reporter was determined as PFUs on Vero/CD150, and the multiplicity of infection (MOI) of each experiment was calculated based on this titer (27). Splenic CD11c+ DCs, CD4+ T cells, and CD11c+ DCs were isolated using anti-CD11b, anti-CD4, anti-CD8, and anti-CD1c MACs beads (Miltenyi Biotec). Splenic CD8α+ DCs, CD4+ DCs, and double negative (DN) DCs were isolated using CD8α+ or CD4+ DC isolation kits (Miltenyi Biotec) according to the manufacturer’s instructions. For isolation of pDCs and conventional DCs (cDCs), spleens were treated with 400 IU Mandle U/ml collagenase D (Roche) at 37°C for 30 min in HBSS (Sigma-Aldrich). EDTA was added, and the cell suspension was incubated for an additional 5 min at 37°C. After removal of RBCs with ammonium chloride–potassium lysing buffer, CD11c+ DCs were isolated using CD11c MACs beads. MACS-sorted DCs were stained with anti-CD11b–FITC, anti-pDC Ag 1 (PDCA-1–PE) (eBioscience), and anti-CD11c–allophycocyanin (BioLegend) and sorted using a FACSAria II (BD). The purity of sorted cells was >98%.

FACS analysis

For pDC staining, splenocytes were stained with anti-CD11c–allophycocyanin (BioLegend), anti–PDCA-1–PE (BioLegend), and anti-human CD150–FITC (eBiosciences). For CD4+ DCs, CD8+ DCs, and DN DC staining, splenocytes were stained with anti-CD11c–allophycocyanin (BioLegend), anti-CD4–PerCP (BioLegend), and anti-CD8–PE (BioLegend), and anti-human CD150–FITC. For B cell staining, splenocytes were stained with anti-B220–allophycocyanin (BioLegend), anti-CD19–PE (BioLegend), and anti-human CD150–FITC. For T cell staining, splenocytes were stained with anti-CD3–allophycocyanin (BioLegend), anti-CD4–PerCP, anti-CD8–PE, and anti-human CD150–FITC were used. Fluorescence intensity of CD150 was measured by flow cytometry. For TLR7 intracellular staining in pDCs, DCs were stained with anti-TLR7–FITC (IMGENEX), anti-CD11c–allophycocyanin, and anti–PDCA-1–PE using the BD Cytofix/Cytoperm Kit (BD Biosciences). For TLR7 intracellular staining in CD4+, CD8+, and DN DCs, DCs were stained with anti–TLR7–FITC (IMGENEX), anti-CD11c–allophycocyanin, anti-CD4–PerCP, and anti-CD8–PE, using the BD Cytofix/Cytoperm Kit (BD Biosciences). Stained cells were analyzed by flow cytometry.

Experimental infection and luciferase assay

Mice were infected i.p. with MV-luciferase at the indicated doses. For in vivo blockade of the type I IFNAR, mice were i.p. injected with 2.5 mg MAR1-5A3, a mAb against IFNAR-1 (BioLegend), 1 d prior to infection. Tissues were collected from the mice at different time points, and the efficiency of infection was measured by luciferase assay. Cells (1 × 107) from various tissues were harvested in 100 μl lysis buffer. The amount of protein in each lysate was determined by bicinchoninic acid assay. Luciferase assay was performed using a Dual-Luciferase Reporter Assay System (Promega), and luciferase activity was read using a Luminoskan plate reader (Thermo Scientific). To measure the efficiency of in vitro infection, cells (5 × 104–4 × 105) were harvested in 25 μl lysis buffer for luciferase assay. For MyD88 inhibition assays, cells were pretreated with 50 μM MyD88 inhibitory peptide (RQIKIFNWQRKMKWKK-RDVLPQVCVNS-NH2; InvivoGen) or the control peptide (RQIKIFNWQRKMKWKK-SLHGRDPEMAFF-NH2; InvivoGen) for 6 h, and then cells were infected with MV. MyD88 inhibition experiments contained a sequence from the MV-luciferase reporter construct, including the type I IFN–responsive domain (RDVLPGT) preceded with a protein transduction sequence (RQIKIFNWQRKMKWKK) derived from antennapedia, which enables the peptide to translocate through the cell membrane. For intratracheal infection with MV, mice were anesthetized and injected with MV-luciferase (8 × 107 PFU/50 μl in PBS) intratracheally. At 3 d after inoculation, mice were sacrificed and perfused with PBS containing 10 μM EDTA from the right ventricle. Lung lobes were isolated, and collagenase buffer [150 U/ml collagenase D (Roche), 10 μg/ml DNAse 1 (Takara), and 5% FCS in RPMI 1640 medium] was injected into the lobes, using a 27-gauge needle. The lobes were then shredded into small pieces and incubated at 37°C for 45 min. During the last 5 min, EDTA was added at 10 μM. Any remaining small pieces were dispersed by passage in and out through a 20-gauge needle, and the suspension was passed through nylon mesh to remove debris. A single-cell suspension was prepared after RBC lysis. A total of 2 × 106 cells were harvested in 100 μl lysis buffer for luciferase assay.

ELISA

Culture supernatants of cells (1–5 × 108) seeded on 96-well plates were collected and analyzed for cytokine levels, using ELISA. ELISA kits for mouse IFN-α and IFN-β were from PBL Biomedical Laboratories. Assays were performed according to the manufacturer’s instructions.

RT-PCR and real-time PCR

Total RNA was prepared using TRIzol Reagent (Invitrogen) following the manufacturer’s instructions. RT-PCR was carried out using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) according to the manufacturer’s instructions. Real-time PCR was performed using a StepOne Real-Time PCR System (Applied Biosystems). The following oligonucleotides were used for β-actin: 5′-TTGCACCCTGC-3′ and 5′-TCGTCTACCTAGCCAACTC-3′; for Irf-β: 5′-CCAGCTTCAAAAGGCCAAGG-ACGA-3′ and 5′-CGGCCTGAAAGTGATGTTGATGT-3′; for Irf-α: 5′-CTGGCTGGCTGTGACAGACATCT-3′ and 5′-AGGCACAGGCGGCTGGTTTCTT-3′; for Ifn-β: 5′-TGGTGCGATGATGTGATTGG-3′ and 5′-TTCCCCTAAGCCCATCTG-3′; for Mavs: 5′-GAGGCCGCTGAGGCAGGGAGG-3′. Levels of target mRNAs were normalized to β-actin, and fold-induction of transcripts was calculated using the ddCT method relative to unstimulated cells.

Statistical analyses

Statistical significance of differences between groups was determined by the Student t test using Microsoft Excel software. The p values < 0.05 were considered significant.

Results

CD150Tg/Mavs−/− mice are not permissive to MV

To quantitate the efficiency of MV infection, we used a recombinant MV-luciferase that expresses the reporter Renilla luciferase from the insert of the MV genome (29). CD150 expression levels did
not differ among various spleen cells from CD150Tg, CD150Tg/Ifnar<sup>−/−</sup>, CD150Tg/Ifnβ<sup>−/−</sup>/Ifnγ<sup>−/−</sup>, CD150Tg/Ticam1<sup>−/−</sup> or CD150Tg/Mavs<sup>−/−</sup> mice (Supplemental Fig. 1). Each knockout mouse strain was i.p. injected with 1 × 10<sup>6</sup> PFU of MV-luciferase. In accord with previous data (16, 22), luciferase activity in various tissues derived from MV-infected CD150Tg mice was very low 2 d after inoculation (Fig. 1A). In contrast, luciferase activity was predominantly increased in spleen and lymph nodes (LNs) derived from MV-infected CD150Tg/Ifnar<sup>−/−</sup> mice, compared with other tissues (Fig. 1A). MV-P mRNA expression was also increased in MV-infected CD150Tg/Ifnar<sup>−/−</sup> spleen and LNs, similarly to luciferase activity (Fig. 1B). These results indicate that spleen and LNs are the major target tissues of i.p.-injected MV in CD150Tg/Ifnar<sup>−/−</sup> mice, as shown in a previous report (24). Luciferase activity was increased in the lung of CD150Tg/Ifnar<sup>−/−</sup>, but not CD150Tg/Mavs<sup>−/−</sup>, mice after intratracheal injection of MV.

**FIGURE 1.** CD150Tg/Mavs<sup>−/−</sup> mice were resistant to in vivo MV infection. (A) CD150Tg, CD150Tg/Ifnar<sup>−/−</sup>, CD150Tg/Ifnβ<sup>−/−</sup>/Ifnγ<sup>−/−</sup>, CD150Tg/Mavs<sup>−/−</sup>, and CD150Tg/Ticam1<sup>−/−</sup> mice were infected i.p. with 1 × 10<sup>6</sup> PFU MV-luciferase. After 2 d, cells were isolated from each organ and lysed with lysis buffer for luciferase assay. The amount of protein in each lysate was determined by bicinchoninic acid assay. Luciferase activity in each lysate was measured and normalized by the amount of protein. Data are shown as luciferase activity per 1 mg of protein and means ± SD of three independent samples. *p < 0.05. (B) CD150Tg/Ifnar<sup>−/−</sup> mice were infected with MV (1 × 10<sup>6</sup> PFU). At 2 d after inoculation, total RNA was collected from the indicated tissues, and the expression level of MV-P mRNA in each tissue was determined by real-time PCR. MV-P mRNA expression is shown as expression relative to β-actin. Data are means ± SD of three independent samples. (C) CD150Tg, CD150Tg/Ifnar<sup>−/−</sup>, and CD150Tg/Mavs<sup>−/−</sup> mice were intratracheally injected with MV-luciferase (8 × 10<sup>5</sup> PFU). At 3 d after inoculation, luciferase activity was measured in cells from lungs. Data are means ± SD of two independent samples. (D) At the indicated days post infection (d.p.i.), luciferase activity in 1 × 10<sup>7</sup> splenocytes was measured. Three mice were analyzed for each genotype. Data are representative of two independent experiments. *p < 0.05 versus CD150Tg. (E) CD150Tg, CD150Tg/Ifnar<sup>−/−</sup>, and CD150Tg/Mavs<sup>−/−</sup> mice were infected i.p. with 1 × 10<sup>6</sup> PFU MV-luciferase. At 2 d.p.i., CD19<sup>+</sup>, CD4<sup>+</sup>, CD8<sup>+</sup>, and CD11c<sup>+</sup> cells were isolated from splenocytes, using anti-CD19, anti-CD4, anti-CD8, and anti-CD11c MACS beads. Luciferase activity in CD19<sup>+</sup>, CD4<sup>+</sup>, CD8<sup>+</sup>, and CD11c<sup>+</sup> cells, as well as LNs, was measured and normalized by the total number of cells. Data are shown as the luciferase activity per 1 × 10<sup>7</sup> cells. Data are means ± SD of three independent samples. *p < 0.05. (F) CD150Tg, CD150Tg/Ifnar<sup>−/−</sup>, CD150Tg/Ifnβ<sup>−/−</sup>/Ifnγ<sup>−/−</sup>, and CD150Tg/Mavs<sup>−/−</sup> mice were infected i.p. with 1 × 10<sup>6</sup> PFU MV-luciferase. At 2 d.p.i., mRNA levels of Ifn-β, Ifi1, and Ccl10 in spleens were determined by real-time-PCR. Data are means ± SD of three independent samples. *p < 0.05.
cytes from CD150Tg/Mavs increased in CD150Tg/Mavs in vivo when type I IFN signaling was blocked by anti-IFNAR Ab. Irf7 CD150Tg/Mavs are responsible for resistance to MV infection in CD150Tg/Mavs cannot increase in CD150Tg/Mavs. In contrast, luciferase activity in splenocytes was significantly increased in CD150Tg/Ifna2−/− and CD150Tg/Irf3−/−/Irf7−/− mice compared with CD150Tg, CD150Tg/Mavs−/−, or CD150Tg/Ticam1−/− mice 2 d after inoculation (Fig. 1D). We also examined luciferase activity in LNs and CD4+ CD8+, CD19+, and CD11c+ cells isolated from splenocytes of the mice. As shown in Fig. 1E, luciferase activity in these cells from MV-infected CD150Tg/Mavs−/− mice was much lower than in those from CD150Tg/Ifna2−/− mice. To investigate the reasons why CD150Tg/Mavs−/− mice were resistant to MV infection, we examined the expression level of mRNA coding antiviral protein in splenocytes (Fig. 1F). The expression of Ifn-β and two IFN-inducible genes, Ifit1 and Cxcl10, was upregulated by MV infection in splenocytes from CD150Tg/Mavs−/− mice, but not in those from CD150Tg/Ifna2−/− and CD150Tg/Irf3−/−/Irf7−/− mice (Fig. 1F). Type I IFN levels in sera were below the detection limit throughout the time course (data not shown). These data suggest that in vivo MV infection induces the expression of type I IFN and IFN-inducible genes to elicit resistance to MV infection in CD150Tg/Mavs−/−. Moreover, type I IFN induction by in vivo MV infection occurs via the IRF3/IRF7-dependent and MAVS-independent pathway.

Next, we performed in vivo infection experiments using IFNAR Ab. Type I IFNR in CD150Tg/Mavs−/− mice was blocked by injecting 2.5 mg of the MAR1-5A3 mAb against IFNAR-1, 1 d before infection. This dose of Ab is reported to block antiviral effects of type I IFN in vivo (31). In MV-infected CD150Tg/Mavs−/− mice, blocking of IFNAR increased luciferase activity in LNs up to 180-fold, and in splenocytes up to 10-fold (Fig. 2A). Similarly, luciferase activity was increased up to 6-fold in CD19+, 8-fold in CD4+, 29-fold in CD8+, and 17-fold in CD11c+ cells by IFNAR blocking (Fig. 2B). These results indicate that CD150Tg/Mavs−/− mice are permissive to MV infection once IFNAR is functionally neutralized.

CD11c+ DCs produce type I IFN via the MAVS-independent pathway during MV infection

To determine which cell types were responsible for type I IFN induction in MV-infected CD150Tg/Mavs−/− mice, we performed an in vitro infection assay using splenocytes. When infected with MV in vitro, the luciferase activity in B cells and T cells isolated from CD150Tg/Mavs−/− mice was comparable to activity in those from CD150Tg/Ifna2−/− mice (Fig. 3A). Treatment with anti-IFNAR Ab did not affect the infection efficiency in CD150Tg/Mavs−/− T and B cells (Fig. 3A). Unlike T and B cells, CD150Tg/Mavs−/− CD11c+ DCs were not permissive to MV (Fig. 3A). These data suggest that CD11c+ DCs, but not lymphocytes, are responsible for resistance to MV infection in CD150Tg/Mavs−/− mice. Because CD150Tg/Mavs−/− mice were permissive to MV in vivo when type I IFN signaling was blocked by anti-IFNAR Ab (Fig. 2A, 2B), we examined whether treatment with anti-IFNAR Ab increased the efficiency of MV infection in vitro. CD150Tg/Mavs−/− CD11c+ DCs were permissive to MV in the presence of anti-IFNAR Ab (Fig. 3A). Consistent with results in Fig. 3A, type I IFN was detectable in the culture supernatant of MV-infected CD150Tg/Mavs−/− CD11c+ DCs, but not of T or B cells (Fig. 3B and data not shown). Expression of Ifn-β and Ifna-α4 mRNAs in CD150Tg/Mavs−/− CD11c+ DCs was also upregulated within 6 h after MV infection (Fig. 3C). These data suggest that CD11c+ DCs, but neither T nor B cells, mainly produce type I IFN through the MAVS-independent pathway in response to MV infection in vitro.

CD4+ DCs and pDCs are responsible for MV-induced type I IFN production in CD150Tg/Mavs−/− mice

Our data indicate that type I IFN induction following the MV recognition in mouse DCs is independent of the MAVS pathway. Various types of DCs have been identified in mouse secondary lymphoid tissues, including three CD11chigh subsets: CD8α+, CD4+, and DN CD4− CD8α− DCs (32), and one subset of CD11clow pDCs (33). To identify the type I IFN-producing subsets, we used a cell sorter to isolate CD11chigh (cDCs) and CD11clow pDCs from splenocytes, with purity > 98% (data not shown). CD150Tg/Ifna2−/− cDCs and pDCs were permissive to MV (Fig. 4A and 4C). In contrast, both CD150Tg/Mavs−/− cDCs and pDCs

FIGURE 2. CD150Tg/Mavs−/− mice were permissive to MV after treatment with anti-IFNAR Ab in vivo. CD150Tg/Mavs−/− mice were injected i.p. with 2.5 mg of anti-IFNAR Ab at 1 d before MV-luciferase infection (1 × 105 PFU per mouse). At 2 d post infection, luciferase activity in LNs, splenocytes (A), and (B) CD19+, CD4+, CD8+, and CD11c+ cells isolated from splenocytes was measured and normalized by the total number of cells. Data are shown as the luciferase activity per 1 × 106 cells. Data are means ± SD of three independent samples. * p < 0.05.
produced type I IFN (Fig. 4B, 4D) and were not permissive to MV (Fig. 4A, 4C). The resistance to MV infection was abolished by neutralizing type I IFNR with anti-IFNAR Ab (Fig. 4A, 4C). These data indicate that, like CD11c+ DCs, CD150Tg/Mavs−/− pDCs and cDCs were barely permissive to MV because of the type I IFN production.

For more detailed study on the type I IFN-producing subsets, we further separated cDCs into CD8α+ and CD4+ and DN DCs, using MACS beads. The purity of these subsets was > 90% (data not shown). Ifn-β mRNA was not induced in CD150Tg/Mavs−/− CD8α+ DCs or DN DCs in response to MV infection (Fig. 5A, 5B). However, MV infection induced expression of Ifn-β mRNA in CD4+ DCs from CD150Tg/Mavs−/− mice (Fig. 5C). Different from CD150Tg/Mavs−/− DCs, CD150Tg CD8α+ and DN DCs expressed Ifn-β mRNA in response to MV infection (Fig. 5A, 5B). These data indicate that the expression of Ifn-β mRNA induced by MV infection is MAVS dependent in CD8α+ DCs and DNs, but MAVS independent in CD4+ DCs.

The MyD88-dependent pathway is essential for MV-induced type I IFN production in pDCs and CD4+ DCs under MAVS deficiency

Next, we tried to identify the signaling pathway involved in type I IFN induction during MV infection. pDCs and CD4+ DCs express TLR7 as a sensor for single-stranded RNA (34, 35). Recognition of single-stranded RNA through the TLR7-MyD88 pathway induces type I IFN expression in human and mouse pDCs (36–38). Therefore, we examined whether the TLR7-MyD88 pathway participated in the host defense during MV infection in mouse DCs. First, we analyzed the expression of TLR7 in pDCs and CD4+ DCs. As reported in previous studies (34, 35), we were able to detect the expression of TLR7 at both mRNA and protein levels (Supplemental Fig. 2). RNA interference cannot be used in primary pDCs because the RNA molecules induce type I IFN expression (39); therefore, we used a peptide that is fused to an antennapedia-derived cell permeant motif and inhibits MyD88 homodimerization by binding to the MyD88 TIR domain (40). pDCs from wild-type mice were cultured for 6 h in the presence of the MyD88 inhibitory peptide or the control peptide and activated with R837, a TLR7 ligand, for 24 h. The control peptide marginally affected IFN-β secretion induced by R837, and the MyD88 inhibitory peptide at 50 μM significantly reduced IFN-β production induced by R837 (Supplemental Fig. 3A). To evaluate the effects of the inhibitory peptide on cell viability, we counted the number of live cells using trypan blue staining (Supplemental Fig. 3B). Although stimulation with R837 partially induced cell death (41.3% ± 5.4%), cell viability was not changed by the addition of the inhibitory peptide at 50 μM (Supplemental Fig. 3B). MyD88 inhibitory peptide has no effects on polyinosinic/polycytidylic acid–induced IFN-β production in CD11c+ DCs (Supplemental Fig. 3C). Next, we tested the effect of MyD88 inhibitory peptide on permissiveness and type I IFN production in MV-infected pDCs from CD150Tg/Mavs−/− mice. Although CD150Tg/Mavs−/− pDCs were not permissive to MV in the presence of control peptide, infection efficiency was increased 4-fold by treatment with MyD88 inhibitory peptide (Fig. 6A). As with pDCs, the infection efficiency of CD150Tg/Mavs−/− CD11c+ DCs increased 3-fold by MyD88 inhibitory peptide treatment (Fig. 6B). When CD150Tg/Mavs−/− CD11c+ DCs, CD4+ DCs, and pDCs were pretreated with MyD88 inhibitory peptide, they did not induce type I IFN mRNA after MV infection (Fig. 6C–E). These data suggest that the MyD88 pathway is responsible for MV-induced type I IFN production in DCs under MAVS deficiency. To confirm that the MyD88 pathway is involved in type I IFN induction in MV-infected DCs, CD150Tg/Mavs−/− pDCs were infected with MV in vitro. Ifn-β mRNA expression was not induced in MV-infected CD150Tg/Mavs−/− pDCs (Fig. 6F).

Discussion

This study demonstrated that CD150Tg/Mavs−/− mice were not susceptible to MV infection in vivo (Fig. 1). This is an unexpected result because many reports have highlighted the important role of the RIG-I/MDA5-MAVS pathway in type I IFN production in MV-infected cells as a means to suppress MV replication. Ultimately, the MyD88 signal, rather than the MAVS signal, serves as a critical inducer of primary type I IFN for cell protection against...
MV replication in a mouse model. This finding would be a novel feature of MV, a negative single-stranded RNA virus, and has reminded us that cell-level studies on host innate immunity in coping with infection cannot always predict critical virus-sensing factors in whole-animal studies.

Because blockade of type I IFN function resulted in permissiveness of CD150Tg/Mavs−/− mice to MV, MAVS-independent type I IFN induction protects neighboring cells (that express IFNAR) from MV infection (Fig. 2). Among the cell subsets tested, only pDCs and CD4+ DCs from CD150Tg/Mavs−/− produced type I IFN after MV infection (Figs. 3–5). Treatment with inhibitory peptide for MyD88 drastically decreased type I IFN expression in CD150Tg/Mavs−/− DCs and rendered these cells MV permissive to MV (Fig. 6). These data indicate that the MyD88 pathway is involved in type I IFN induction in MV-infected pDCs and CD4+ DCs under a MAVS-deficient state. The results were confirmed with CD150/Myl88−/− mice (Fig. 6F). In this context, the MyD88 pathway plays a primary role in the initial phase of MV protection in vivo. However, CD150Tg/Myl88−/− mice were not permissive to MV in vivo (data not shown), suggesting that not only MyD88 in pDCs/CD4+ DCs but also MAVS in other cells contributes to the protection against systemic infection by MV. Because the MyD88 pathway participates in the initial type I IFN induction in pDCs, only a very weak but significant luciferase activity was detected in the spleen of CD150Tg/Mavs−/− mice at day 1 after inoculation (Fig. 1C). Moreover, CD8α− DCs and DN DCs from CD150Tg/Mavs−/− mice could not induce Ifn-β mRNA expression in response to MV infection in vitro (Fig. 5). These results suggest that the MAVS pathway also participates in host defense against MV infection as an alternative pathway in splenic DCs, at least in these experimental conditions.

Another target cell of MV infection in nonhuman primates is the AM (18). AMs in the CD150Tg/Iifnar−/− mouse model are also permissive to MV infection (17). AMs derived from CD150Tg/Mavs−/− mice were permissive to MV infection in vitro (data not shown). This result is consistent with a previous report indicating that the RLR-MAVS pathway is predominantly used for type I IFN induction in virus-infected AMs (41). However, intratracheal inoculation with MV in CD150Tg/Mavs−/− mice resulted in a lower luciferase activity in AMs from CD150Tg/Mavs−/− mice than in those from CD150Tg/Iifnar−/− mice (data not shown), similar to the results from i.p. inoculation. These data indicate that a cell population other than AMs produces type I IFN in MV-infected lungs. In this case, pDCs in lung might act as IFN-producing cells, because pDCs in lung are reported to produce type I IFN and act as immune system defenders against infection (41). Taken together, the recognition pathways for MV to induce type I IFN differ among cell types. Existence of the several alternative pathways might engage the protection from systemic MV infection (Supplemental Fig. 4). In CD8α− DCs and pDCs, the TLR-MyD88 pathway is preferentially used to induce type I IFN in response to infections. TLR7, a sensor for viral RNA, is known to be expressed specifically in these DC subsets (34, 35) (Supplemental Fig. 2). The restricted expression pattern of TLR7 seems to reflect the ability of CD4+ DCs and pDCs to produce type I IFN in MV-infected CD150Tg/Mavs−/−. Therefore, TLR7 may be one of the candidate receptors for recognition of MV RNA. Because viral RNA is recognized mainly through the RLR-MAVS pathway in CD4+ DCs at steady state (35), it would be difficult to examine the role of the TLR7-MyD88 pathway in CD4+ DCs during viral infection in WT mice. Thus, our assay system using CD150Tg mice crossed with given knockout mice is a powerful tool to investigate the signaling pathway for host defense against MV infection.

Previous studies were mainly done using bone marrow–derived DCs (BMDCs) to analyze the immunosuppressive effects of MV in a mouse model (16, 42, 43). However, BMDCs from CD150Tg/Mavs−/− mice completely lacked the ability to produce type I IFN in response to MV infection. In contrast to the results in splenic DCs (44). Because the expression profile of pattern-recognition receptors in BMDCs was different from the profile in splenic DCs (34, 35, 45), it is reasonable to think that each cell type has its own unique type I IFN induction pathway in response to viral infection. Therefore, further investigations on immunosuppression and immunopromotion of MV in infected DCs should be performed using splenic DCs, taking into consideration the properties of resident DCs.

In this study, we used recombinant MV-luciferase that expresses the reporter Renilla luciferase from the first gene position of MV genome (29). The MV-luciferase is suitable to evaluate the efficiency of infection in vivo and in vitro correctly because the assay
Luciferase activity and MV-P mRNA in CD150Tg/Ifnar^2/2 was significantly increased compared with CD150Tg mice during infection (Fig. 1A, 1B and data not shown). Luciferase activity obtained from MV-infected Vero/CD150 cells was correlated with viral titer in culture supernatant from MV-infected cells (data not shown). Recombinant MV strains expressing a foreign gene from a first gene position of the MV genome were used in several groups for in vivo infection assay without any problem (17, 18, 24). However, the infection efficiency of recombinant Morbillivirus expressing a foreign gene from position one is reported to be attenuated in vivo (46), which is well reflected in the relatively low virus titer observed in the spleen from CD150Tg mice infected with MV-luciferase.

In mouse models, the route of MV inoculation does not always reflect the natural infection route in human MV infection. Our results obtained from experiments using the i.p. infection model also may not faithfully reflect the process of natural MV infection involving epithelial cells in humans. However, as described above, intratracheal and i.p. administration gave rise to similar results in MV infection. This issue may reflect the viral strategy of targeting myeloid cells for initial propagation. Injected MV reaches DCs or macrophages without affecting other bystander cells in any route of MV administration. However, this is not the case in other virus species with different target cells for initial infection.

We conclude that MyD88-dependent type I IFN production in CD4^+ DCs and pDCs results in initial protection against MV; thereafter, the produced type I IFN induces an antiviral state in the neighboring cells, expressing IFNAR in the mouse model.

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Disclosures
The authors have no financial conflicts of interest.

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