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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

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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 rarely 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 CD8+ and CD8−/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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ype I IFNs (IFN-α/β) are crucial for protection against viral infections (1). Viral RNA is detected by cytosolic RNA sensors and induces expression of type I IFN (2). Extracellular dsRNA of a virus product is detected by the endosomal TLR3, whereas intracellular dsRNA is sensed by the retinoic acid–inducible gene I (RIG-I) and the melanoma differentiation–associated gene 5 (MDA5) (3). Upon recognizing dsRNA, TLR3 recruits the Toll/IL-1R (TIR) homology domain–containing adaptor molecule 1 (TICAM-1, also referred to as TRIF) and induces type I IFN production (4, 5). The RIG-I–like receptors (RLRs), RIG-I and MDA5, signal via the mitochondrial antiviral signaling protein (MAVS; also known as VISA, Cardif, or IPS-1) and also induce type I IFN expression (6). Knocking out these adaptor molecules results in failure to activate the transcription factors IFN regulatory factor (IRF)-3 and IRF-7, leading to an incompetence in type I IFN production and antiviral host defense (3, 7, 8). Type I IFN induction following the recognition of measles virus (MV) RNA is dependent on the RIG-I/MAVS-pathway in human epithelial cell lines (9, 10). However, the role of the RIG-I/MAVS-pathway during in vivo MV infection remains undetermined.

MV, of the genus Morbillivirus from the Paramyxoviridae family, is a highly pathogenic, nonsegmented negative single-stranded RNA virus that causes respiratory distress and immunosuppression in humans (11). Wild-type strains of MV enter cells via human CD150, which is also referred to as signaling lymphocyte activation molecule (12), and human poliovirus receptor–like protein 4 (13, 14). Expression of these receptors is restricted either to activated lymphocytes, dendritic cells (DCs), and macrophages for CD150 or to the basolateral surface of epithelial cells for PVRL4 (15). Among these cell populations, CD11c+ DCs and alveolar macrophages (AMs) are reported to be the first target cells of early-phase MV infection in the CD150 transgenic (Tg) mouse model (16, 17) and in nonhuman primates (18, 19). Moreover, DCs are found to be involved in pathogenesis and immunosuppression during and after acute MV infection (20, 21). However, it is unclear how DCs and macrophages recognize MV RNA to produce type I IFN.

Human CD150Tg mice, which are slightly permissive to MV, are used to study host responses against MV infection in vivo (16, 22–24). CD150Tg/Ifnar−/− mice, which are generated by crossing CD150Tg mice with Ifnar−/− mice, are susceptible to MV infection...
and serve as a useful mouse model (16, 24). In the current study, using the CD150Tg mouse model in combination with Mavs−/−, Irf3−/−, Irf7−/−, and Ticam1−/− mice, we found that CD150Tg/Mavs−/− mice were not permissive to MV in vivo, whereas CD150Tg/Irf3−/−/Irf7−/− 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. Irf3−/− and Irf7−/− 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 on November 13-0024. All animal manipulations were performed with the animals under anesthesia that was induced and maintained with pentobarbital sodium, and all efforts were made to minimize suffering.

Virus and cell culture
Vero/CD150 cells were maintained in DMEM supplemented with 10% heat-inactivated FBS and antibiotics. IC323, corresponding to the IC-B strain of MV (27), was recovered from the placid pIC-MV323 encoding the anti-CD150 antibody (anti-IC; IC150-150). IC323-Luci (MVF), which expresses 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). MV-luciferase was maintained in Vero/CD150 cells (30). TLR7 was generated as a plasmid containing a sequence from the MyD88 TIR domain (RDVLPGT) preceded by a protein transduction sequence (RQIKIFQQRKKMMWKKKDD) derived from antenapedia, which enables the peptide to translocate through the cell membrane. For intratracheal infection with MV, mice were anesthetized and injected with MV-luciferase (8 × 106 PFU/50 μl in PBS) intratracheally. At 3 d after inoculation, mice were sacrificed and perfused with PBS containing 10 mM EDTA from the right heart. Lung lobes were isolated, and collagenase buffer [150 U/ml collagenase D (Roche), 10 μg/ml DNase I (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 mM. Any remaining small pieces were disposed of in passage 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 × 106) 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′-TCTGCTGCTGTCGAGGTC-3′ and 5′-TCGTCATCCATGGCGAATACT-3′; for Irf3: 5′-CTGTTGAGATCATTGCCATG-3′ and 5′-TCTGTCCATGCGAACAGCTTT-3′; for Irf7: 5′-TGTGCTGAGATGGACTGTGAG-3′ and 5′-TTTTCTGAGCTCTTCCAGAAG-3′. For Irf3 expression, qRT-PCR was performed using the StepOne software. The expression levels were analyzed using the fold-change method relative to unstimulated cells.

Statistical analyses
Statistical significance of differences between groups was determined by the Student’s 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\(^{-/-}\), CD150Tg/If\(^{3/-}\)/If\(^{7/-}\), CD150Tg/Ticam1\(^{-/-}\) or CD150Tg/Mavs\(^{-/-}\) mice (Supplemental Fig. 1). Each knockout mouse strain was i.p. injected with \(1 \times 10^6\) 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\(^{-/-}\) mice, compared with other tissues (Fig. 1A). MV-P mRNA expression was also increased in MV-infected CD150Tg/Ifnar\(^{-/-}\) 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\(^{-/-}\) mice, as shown in a previous report (24). Luciferase activity was increased in the lung of CD150Tg/Ifnar\(^{-/-}\), but not CD150Tg/Mavs\(^{-/-}\), mice after intratracheal injection of MV.
cytes from CD150Tg/Mavs increased in CD150Tg/Mavs infection. Although luciferase activity in splenocytes was slightly increased in CD150Tg/Mavs mice 1 d after i.p. inoculation, luciferase activity in splenocytes of CD150Tg/Mavs mice was decreased to the same degree as in those of CD150Tg mice a few days after inoculation (Fig. 1A, 1D, 1E). In contrast, luciferase activity in splenocytes was significantly increased in CD150Tg/Ifnar-/- 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/Ifnar-/- 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/Ifnar-/- 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 through 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/Ifnar-/- 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 Ifn-α/β 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 CD11c+ subsets: CD8α+ CD4+, and DN CD4- CD8α- DCs (32), and one subset of CD11c- pDCs (33). To identify the type I IFN-producing subsets, we used a cell sorter to isolate CD11c+ DCs and CD11c- pDCs from splenocytes, with purity > 98% (data not shown). CD150Tg/Ifnar-/- 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 infected 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 in the presence of control peptide, 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). 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 a 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/polyctydilic 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 pre-treated 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/Myd88−/− pDCs were infected with MV in vitro. Ifn-β mRNA expression was not induced in MV-infected CD150Tg/Myd88−/− 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 blockage of type I IFN function resulted in permisiveness of CD150Tg/Mav+/− 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/Mav+/− produced type I IFN after MV infection (Figs. 3–5). Treatment with inhibitory peptide for MyD88 drastically decreased type I IFN expression in IFNAR−/− mice (Fig. 1C). Moreover, CD8α+ pDCs/CD4+ DCs but also MAVS in other cells contributes to the protection against systemic infection by MV. Because the MyD88 pathway also participates in host defense against MV (17). AMs derived from CD150Tg/Ifnar−/− mice completely lacked the ability to produce 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/Mav+/− mice. 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 (36), 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/Mav+/− 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 could 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/Ifnar2/2 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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Expression levels of human CD150 in various splenocytes derived from wild-type, CD150Tg, CD150Tg/Ifnar−/−, CD150Tg/Irf3−/−Irf7−/−, CD150Tg/Mavs−/− and CD150Tg/Ticam1−/− mice were measured by FACS. (A) Whole splenocytes, (B) CD19+B220+ B cells, (C) CD3+CD4+ T cells, (D) CD3+CD8+ T cells, (E) CD11c+ DCs, (F) CD11c+PDCA-1+ pDCs, (G) CD11c+CD4+ DCs, (H) CD11c+CD8α+ DCs and (I) CD11c−CD4+CD8α− DN DCs. Mean fluorescence intensities of CD150-FITC from each population are in the histograms. One representative experiment of two is shown.
Supplemental Figure 2. TLR7 expression in DC subsets.

(A) DC subsets were isolated from spleenocytes of wild-type mice by MACS beads or FACS sorting and levels of Tlr7 mRNA in each subset were quantified by real-time PCR. Tlr7 mRNA expression is shown as relative expression to β-actin. Data are means ± SD of three independent samples.

(B) DCs were stained with anti-TLR7 antibody (red line) or isotype control (gray line). Expression of TLR7 protein in each subset was analyzed by FACS. One representative experiment of two is shown.
Supplemental Figure 3. Effect of MyD88 inhibitory peptide on IFN-β production.

Aria-sorted WT pDCs were pretreated with the indicated concentration of control peptide or MyD88 inhibitory peptide for 6 hours following stimulated with 2 μg/ml of R837 for 24 hours. (A) IFN-β in the culture supernatants were measured by ELISA. (B) Cells were stained with trypan blue and live cells were counted. Data are shown as the percentage of cells viability and means ± SD of three independent samples. (C) CD11c+ DCs were pretreated with 50 μM of control or MyD88 inhibitory peptide for 6 hours following stimulated with 50 μg/ml of poly I:C for 24 hours. IFN-β in the culture supernatants were measured by ELISA.
Supplemental Figure 4. MV recognition in murine dendritic cells. MV RNA is recognized by cytosolic RNA sensors, RIG-I and MDA5, in human epithelia cells. In murine CD4+ CD8α+ and DN DCs, the RLR-MAVS pathway is involved in MV-induced type I IFN induction. In CD4+ DCs and pDCs, the TLR7-MyD88 pathway also participates in the type I IFN induction. The recognition pathways of MV RNA are different from each cell type and several pathways of type I IFN induction engage protection against MV infection in this mouse model.