In Vivo Conditions Enable IFNAR-Independent Type I Interferon Production by Peritoneal CD11b+ Cells upon Thogoto Virus Infection

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ABSTRACT
Type I interferons (IFNs) crucially contribute to host survival upon viral infections. Robust expression of type I IFNs (IFN-α/β) and induction of an antiviral state critically depend on amplification of the IFN signal via the type I IFN receptor (IFNAR). A small amount of type I IFN produced early upon virus infection binds the IFNAR and activates a self-enhancing positive feedback loop, resulting in induction of large, protective amounts of IFN-α. Unexpectedly, we found robust, systemic IFN-α expression upon infection of IFNAR knockout mice with the orthomyxovirus Thogoto virus (THOV). The IFNAR-independent IFN-α production required in vivo conditions and was not achieved during in vitro infection. Using replication-incompetent THOV-derived virus-like particles, we demonstrate that IFNAR-independent type I IFN induction depends on viral polymerase activity but is largely independent of viral replication. To discover the cell type responsible for this effect, we used type I IFN reporter mice and identified CD11b+ F4/80+ myeloid cells within the peritoneal cavity of infected animals as the main source of IFNAR-independent type I IFN, corresponding to the particular tropism of THOV for this cell type.

IMPORTANCE
Type I IFNs are crucial for the survival of a host upon most viral infections, and, moreover, they shape subsequent adaptive immune responses. Production of protective amounts of type I IFN critically depends on the positive feedback amplification via the IFNAR. Unexpectedly, we observed robust IFNAR-independent type I IFN expression upon THOV infection and unraveled molecular mechanisms and determined the tissue and cell type involved. Our data indicate that the host can effectively use alternative pathways to induce type I IFN responses if the classical feedback amplification is not available. Understanding how type I IFN can be produced in large amounts independently of IFNAR-dependent enhancement will identify mechanisms which might contribute to novel therapeutic strategies to fight viral pathogens.
promotes the production of late, large-scale type I IFN produc-
tion, the second type I IFN wave (reviewed in references 13 to 15).

Collectively, it has been shown that robust type I IFN produc-
tion both in vitro and in vivo critically depends on positive feed-
back amplification via the IFNAR (16–18). As a result, mice or
isolated cells deficient for the IFNAR show substantially reduced
response (19).

Plasmacytoid dendritic cells (pDC), a rare and highly special-
ized subset of innate immune cells, are regarded as the main type
I IFN-producing cell type. Even though in vitro virtually any cell
type is able to produce type I IFNs in response to the appropriate
stimulus, pDC were shown to produce up to 100 to 1,000 times
more type I IFN than other cell types (reviewed in reference 20).
Consequently, pDC were identified as being responsible for sys-
temic type I IFN responses in vivo to a variety of infections (17, 20,
21). However, in mice depleted of pDC, considerable and protec-
tive type I levels can still be detected upon infection, indicating
that, in vivo, other cell types are capable of initiating relevant type
I IFN responses (22).

We previously showed that, in contrast to the situation in most
other viral infections, in infections with Thogoto virus (THOV),
myeloid dendritic cells (mDC) are the main type I IFN producers
in vitro while pDC hardly secrete any type I IFNs (23). THOV is a
segmented, single-strand, negative-sense RNA-encoded, tick-
transmitted orthomyxovirus which is closely related to influenza
virus (24). In contrast to studies of influenza virus isolates, THOV
replication and pathogenesis can be studied optimally in labora-
tory mice (25). Interestingly, our previous studies with THOV
confirmed a robust type I IFN response upon infection of
IFNAR-deficient mice, despite their inability to sense type I
IFNs and to initiate the enhancing positive feedback loop via
the IFNAR (23). Here, we unravel molecular mechanisms and deter-
mine the tissue and cell type capable of IFNAR-independent type
I IFN expression upon THOV infection. Our data indicate that the
host can effectively use alternative pathways to induce type I IFN
responses if the classical feedback amplification via the IFNAR is
not available. Understanding how type I IFNs can be produced by
myeloid cells in large amounts independently of IFNAR-depend-
ent enhancement might contribute to identifying mechanisms
which could lead to novel therapeutic strategies to fight viral
pathogens.

MATERIALS AND METHODS

Mice. All mice were bred under specific-pathogen-free (SPF) conditions
at the Zentrale Tierhaltung of the Paul-Ehrlich-Institut. Wild-type (WT)
C57BL/6 mice were purchased from Harlan. All knockout mice were
backcrossed at least 10 times on the C57BL/6 background. Type I inter-
feron receptor-deficient (IFNAR−/−) mice have been described previ-
ously (3). MyD88-deficient (MyD88−/−) mice were provided by Shizuo
Akira (26), TRIF-deficient (TRIF−/−) mice were provided by Bruce But-
 ler (27), and mitochondrial antiviral signaling protein (MAVS)-deficient
(MAVS−/−) mice were provided by Jürg Tschopp (28). Generation of
MyD88−/−TRIF double-knockout mice was previously described (19). IFN-β
Δβ-luc/Δβ-luc knock-in reporter mice homozygously express firefly luc-
erase (FF-Luc) under the control of the endogenous IFN-β promoter
(29). IFN-βΔβ/Δβ knock-in reporter mice homozygously express an
IFN-β-IRE-YPF (where IRES is internal ribosomal entry site and YFP is
yellow fluorescent protein) bicistronic transcript under the control of
the endogenous IFN-β promoter (30).

Mouse experimental work was carried out using 8- to 12-week-old
mice in compliance with regulations of German animal welfare. For in-
fection, mice were anesthetized using isoflurane (CP-Pharma) and in-
fected by the intraperitoneal (i.p.) route with a total volume of 200 µl
of virus in phosphate-buffered saline (PBS). Animals were euthanized if se-
vere symptoms were observed. To determine cytokine levels, peripheral
blood was taken retro-orbitally upon anesthetization using isoflurane,
and serum was prepared. IFN-α levels in serum were analyzed using an
enzyme-linked immunosorbent assay (ELISA) kit (PBL Biomedical Lab-
oratories).

Virus and virus-like particles (VLP). Thogoto virus (THOV) lacking
the ML (where ML is an elongated form of the matrix protein) open
reading frame was used in all experiments (31). THOV was propagated
on BHK-21 cells and titrated on Vero cells. To analyze virus growth in vivo,
organs were homogenized in medium using Lysing Matrix Tubes D (MP
Biomedicals), and virus titers were determined by plaque assays. Vesicular
stomatitis virus (VSV) Indiana, Mudd-Summers isolate, was propagated
on BHK-21 cells and titrated on Vero cells.

For production of replication-incompetent virus-like particles
(VLP), 293T cells were transfected with expression plasmids encoding
the structural proteins of THOV and a reporter minigenome encoding
enhanced green fluorescent protein (eGFP) flanked by the noncoding
regions of THOV segment 5, as described previously (32). As a control,
the expression plasmid encoding the viral matrix protein was omitted
from the transfection mixture, resulting in a THOV-VLPΔM preparation
that does not contain functional VLP. Cell culture supernatants were
harvested at 48 h postinfection and subjected to ultracentrifuga-
tion for 2 h at 100,000 × g. The resulting pellets were resuspended in
PBS, and a titer of 1 × 109 fluorescence-forming units (FFU) per ml
was determined by infecting Vero cells with 10-fold dilutions of the
VLP preparations and counting eGFP-positive cells at 24 h postinfect-
ion.

Cell isolation and culture. Bone marrow (BM) cells were isolated by
flushing femur and tibia of mice with RPMI medium supplemented
with 10% fetal calf serum (FCS). Upon red blood cell lysis, cells were washed
and seeded at a density of 1 × 106 cells/ml or 2 × 106 cells/ml in medium
supplemented with granulocyte-macrophage colony-stimulating factor
(GM-CSF; 100 ng/ml [R&D Systems]) or Flt3-L (100 ng/ml; R&D sys-
tems), respectively. Flt3-L-supplemented cultures (pDC) were cultivated
for 8 days with one medium change at day 4, whereas medium of the
GM-CSF-supplemented cultures (mDC) was changed every 2 days,
depending on the status of cultures, by replacing half of the medium with
fresh cytokine-supplemented medium.

In vitro stimulation and quantification of cytokine production. For
stimulation, in vitro-differentiated DC were seeded at 1 × 106 cells/ml in
24-well culture plates in 1 ml of medium. Cpg-containing oligodeoxy-
nucleotide 2216 (CpG2216; ggGGGACGATCGTCgggggg [uppercase let-
ters, boldface; lowercase letters, normal]) was used at a concentration of
5 µg/ml. For UV irradiation of virus or VLP, a UV irradiation chamber (Herolab) was used. Irradiation
with 75 ml/cm² took approximately 10 s. After stimulation of cell cultures,
cell-free supernatant was collected and analyzed using an ELISA kit to
determine the amount of mouse IFN-α (PBL Biomedical Laboratories).

MACS. Mice were left untreated or were i.p. infected with 1 × 10⁷ PFU
of THOV. At 6 h postinfection, spleens and peritoneal exudate cells (PEC)
were isolated, and CD11b-positive cells were enriched using CD11b mi-
crobeads and an autoMACS Pro Separator (both from Miltenyi) accord-
ing to the manufacturer’s instructions. Purity of magnetically activated
lymphocyte (MACS) gate was determined by flow cytometry (FACS)
analyzer. The purity of MACS-enriched CD11b-positive cells was usually
between 95 and 99%.

FACS. For FACS analyses, cells were stained with the following fluo-
orchrome-labeled monoclonal antibodies and isotype controls: anti-
CD11c-allophycocyanin (APC), anti-CD69-phycocerythrin (PE)-Cy7, ant-
i-B220-PE, rat IgG2b-APC isotype control, and hamster IgG1-APC
isotype control (all from BD PharMingen); anti-CD11b-Pacific Blue and
rat IgG2b-Pacific Blue isotype control (both from CalTag/Invitrogen); anti-F4/80-APC, anti-F4/80-PE, and rat IgG2b-PE isotype control (all from AbD Serotec); and rat IgG2a-PE isotype control (from Biozol). All analyses were performed using a BD LSR II flow cytometer and BD FAC-SDiva software (BD Biosciences).

**Luciferase assay.** For measurement of luciferase activity, cells were lysed in passive lysis buffer (Promega). The lysates were centrifuged at 10,000 × g, and luciferase activity in the supernatants was determined using a luciferase assay system (Promega).

**Quantitative RT-PCR.** Cells were lysed in peqGOLD TriFast (PeqLab). RNA was isolated from the aqueous supernatant using an RNeasy mini kit (Qiagen), and 10 ng of RNA was analyzed with a one-step QuantiTect SYBR green reverse transcription-PCR (RT-PCR) kit (Qiagen). Cellular RNA was quantified with QuantiTect primers specific for mouse IFN-β (for Mm_Ifnb1_1, primer QT00249662), IFN-α2 (Mm_IFNα2, QT00253092), RIG-I (Mm_Ddx58_1_SG, QT00123515), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH; QT01658692).

To detect THOV segment 5 transcripts, a strand-specific primer pair (GenBank accession number NC_006507, positions 501 to 520 and 615 to 596) was used. Values were normalized against GAPDH using the ΔΔCt (where Ct is threshold cycle) method (33).

**RESULTS**

**IFNAR-independent type I IFN responses upon THOV infection.** We previously observed that THOV induces robust IFN-α and IFN-β secretion in mice independently of IFNAR signaling (23). As shown in Fig. 1A (left panel), intraperitoneal (i.p.) infection of mice with THOV indeed induced systemic production of IFN-α within 24 h even in the absence of type I IFN signaling via the IFNAR. However, for most viral infections, including infection with the single-stranded RNA (ssRNA)-encoded vesicular stomatitis virus (VSV), robust type I IFN induction depends on positive feedback via the IFNAR (19, 34) (Fig. 1A, right panel). How THOV infection enables efficient type I IFN production independently of this positive feedback signaling is still unclear.

In order to get a first insight into the cell type(s) responsible for IFNAR-independent IFN-α production, we generated pDC and mDC from bone marrow and isolated peritoneal exudate cells (PEC), mainly myeloid cells and macrophages, from the peritoneal cavity (35; also data not shown) of WT and IFNAR-deficient mice. Upon THOV infection, mDC and PEC from WT animals showed strong IFN-α secretion (Fig. 1B). However, IFN-α production by infected pDC was only marginal although these cells responded properly to CpgG2216 stimulation (Fig. 1B). Quantitative RT-PCR analyses revealed a more than 150-fold stronger THOV-specific signal in infected mDC than in pDC (Fig. 1C). This indicates a tropism of THOV for mDC, which most probably accounts for the reduced responsiveness of pDC toward THOV infection. Importantly, in contrast to the IFNAR-independent IFN-α production under *in vitro* conditions, cells isolated from IFNAR-deficient mice did not respond to THOV infection (Fig. 1B) although infection levels in IFNAR-deficient DC and their WT counterparts were equal (Fig. 1C).

Next, we aimed at investigating whether *in vivo* infection might be necessary for IFNAR-independent IFN-α secretion. We infected WT and IFNAR-deficient mice with THOV, isolated spleen cells and PEC at 6, 12, and 24 h postinfection, and cultivated the cells for an additional 24 h. PEC from infected WT animals secreted large amounts of IFN-α early after THOV infection (Fig. 2A). However, only minimal IFN-α could be detected in supernatants of PEC isolated from IFNAR-deficient animals at any time point investigated. Spleen cells of either WT or IFNAR-deficient animals did not produce IFN-α (Fig. 2A). Hence, only minimal IFNAR-independent IFN-α secretion can be detected in *ex vivo* cultures upon *in vivo* infection.

In order to analyze *in vivo* IFN-α production directly in certain organs, we infected WT and IFNAR-deficient animals with THOV for 6, 12, and 24 h and determined IFN-α production in lysates of spleen, liver, and lung and in the fluid within the peritoneal cavity (peritoneal exudate [PE]). Of note, we detected early (at 6 and 12 h postinfection) IFN-α secretion only in the peritoneal cavity, whereas secreted IFN-α was not detectable in the liver and lung after 24 h postinfection (Fig. 2B). At this time point, some IFN-α was also detected in livers and lungs of IFNAR−/− mice, possibly due to enhanced virus replication. Later time points of analysis are not feasible for IFNAR-deficient mice as they succumb to THOV infection around 30 h postinfection (23). Other tissues or organs,
remaining CD11b⁺ F4/80⁺ cells showed an activated phenotype upon infection, as indicated by CD69 upregulation. This activation was dramatically reduced in IFNAR-deficient mice (Fig. 2C), which is in line with previous data showing that CD69 upregulation is, at least in part, type I IFN dependent (36).

Collectively, these data suggest that 6 h of in vivo infection are sufficient to induce secretion of IFN-α within the next 24 h of in vitro culture. After 24 h of in vivo infection, cells are no longer able to produce IFN-α within the next 24 h in in vitro culture, most probably because they do not survive infection that long. This suggestion is supported by the FACS data showing that the CD11b⁺ F4/80⁺ cell population within the peritoneal cavity gets activated between 6 and 12 h postinfection and then fades. In line with this observation, no IFN-α can be detected at 6 h postinfection without subsequent in vitro culture, but IFN-α accumulates within the peritoneal cavity at 24 h postinfection.

Thus, THOV infection enables robust IFN-α production independently of the positive feedback loop via the IFNAR. However, this requires in vivo conditions which are not present during in vitro infection and ex vivo cultivation.

IFNAR-independent type I IFN production upon THOV-VLP infection does require viral polymerase activity but not viral replication. It is generally accepted that viral replication is enhanced in the absence of a functional type I IFN system. This holds true for THOV infections as well (23). In order to investigate the IFNAR dependence of IFN-α production in the absence of excessive THOV replication, we generated replication-incompetent but transcriptionally active THOV-derived virus-like particles (THOV-VLP) carrying a minigenome encoding eGFP.

Intrapерitoneal infection of WT and IFNAR-deficient mice with 10⁶ FFU of eGFP-encoding THOV-VLP induced systemic IFN-α secretion, indicating robust activity of the VLP-encapsidated nucleocapsids in the absence of viral replication (Fig. 3A). Accordingly, UV irradiation abolished the IFN-α-inducing capacity of THOV-VLP, indicating the necessity for THOV-VLP transcriptional activity (Fig. 3B). FACS analyses for eGFP-positive cells, a measure for viral polymerase activity, demonstrated high fluorescence signals in PEC isolated from THOV-VLP-infected WT and IFNAR-deficient animals (Fig. 3C), suggesting that cells in the peritoneal cavity contribute to IFN-α levels detected in the serum. Moreover, as observed for THOV (23), THOV-VLP infection was sensed via RLH (MAVS-dependent) but not via TLR (MyD88- and TRIF-dependent) to induce IFN-α secretion (Fig. 3D).

We additionally used THOV-VLP for in vitro infection of differentiated PDC and mDC and isolated PEC. As a control, cells were incubated with a preparation that lacked M, the viral matrix protein, resulting in a THOV-VLPΔM preparation that does not contain functional VLP. As observed for THOV-infected cells (Fig. 1B), THOV-VLP infection but not the THOV-VLPΔM preparation strongly induced IFN-α secretion by WT mDC and, to a lesser extent, by WT pDC and PEC (Fig. 3E). Accordingly, FACS analysis showed a clear tropism of THOV-VLP for mDC and PEC (Fig. 3F). However, cells from IFNAR-deficient animals were unresponsive to THOV-VLP treatment (Fig. 3E) even though they were infected comparably (Fig. 3F), again indicating a dominant role of myeloid cells in type I IFN production upon THOV infection and the need of in vivo conditions for IFNAR-independent IFN-α induction.

CD11b⁺ F4/80⁺ cells from the peritoneal cavity account for IFNAR-independent type I IFN production. In order to identify
the cell type in spleen and the peritoneal cavity responsible for IFNAR-independent type I IFN production, we used a reporter mouse system. We previously showed that IFN-α and IFN-β are coregulated upon THOV infection (25), and in contrast to the

the situation for IFN-α, well-established reporter mice for IFN-β expression are available. Hence, we used IFN-β (iβmob/mob) mice that express YFP along with IFN-β under the control of the endogenous IFN-β promoter to detect cells by FACS analyses (30). Upon THOV infection, we detected YFP-positive (YFP +) cells in the peritoneal cavity when gating on CD11b + cells in both IFN-β (iβmob/mob) and IFN-β (iβmob/mob) IFNAR −/− mice (Fig. 4A). In con-
trast, with gating on CD11c<sup>+</sup> cells, only a slight increase in YFP<sup>+</sup> cells was detectable in IFN-β<sup>mob/mob</sup> mice upon THOV infection, but this was absent in IFN-β<sup>mob/mob IFNAR</sup><sup>−/−</sup> mice (Fig. 4A). YFP<sup>+</sup> CD11b<sup>+</sup> cells were also greatly positive for the surface marker F4/80, again indicating their myeloid/macrophage phenotype (Fig. 4B). In line with this, YFP<sup>+</sup> CD11b<sup>+</sup> F4/80<sup>+</sup> cells were mostly negative for CD11c and B220. As already shown for WT and IFNAR-deficient mice (Fig. 2C), cells from WT reporter mice also showed strong expression of the activation marker CD69, which was less pronounced in cells from IFNAR-deficient reporter mice (Fig. 4B). Interestingly, within the spleen, no YFP<sup>+</sup> cells could be detected upon gating on either CD11b<sup>+</sup> or CD11c<sup>+</sup> cells (data not shown).

In order to verify these data within a second reporter mouse system, we used the luciferase reporter mouse model. Here, the firefly luciferase is expressed under the control of the endogenous IFN-β promoter (29). We enriched CD11b<sup>+</sup> cells from the peritoneal cavity of THOV-infected IFN-β<sup>β<sub>loc/lac IFNAR</sub>/β<sub>loc</sub> luc</sup> and IFN-β<sup>β<sub>loc/lac IFNAR</sub>/β<sub>loc</sub> luc</sup> animals by the MACS technique. Analyzing luciferase activity in total PEC before MACS enrichment or in the CD11b-negative and CD11b-positive cell fractions demonstrated that, indeed, CD11b<sup>+</sup> cells from both WT and IFNAR-deficient reporter mice showed enhanced IFN-β promoter activity upon virus infection (Fig. 4C). Again, CD11b<sup>+</sup> cells isolated from spleens of the same mice did not show any IFN-β promoter activity (data not shown).

Finally, we verified these data within a second reporter mouse system. We infected mice with THOV, separated CD11b<sup>+</sup> cells from CD11b<sup>+</sup> cells by the MACS technique, and analyzed cell fractions by quantitative RT-PCR. These data show that CD11b<sup>+</sup> cells from both WT and IFNAR-deficient mice are more prone to THOV infection than their CD11b<sup>+</sup> counterparts. Consequently, they showed enhanced IFN-β and IFN-α2 transcription. Of note, only CD11b<sup>+</sup> IFNAR<sup>−/−</sup> and not CD11b<sup>+</sup> IFNAR<sup>−/−</sup> cells showed type I IFN mRNA expression upon THOV infection. In line with IFNAR-dependent CD69 upregulation on CD11b<sup>+</sup> cells (Fig. 2C and 4B), the type I IFN target gene RIG-1 was upregulated upon THOV infection in both CD11b<sup>+</sup> and CD11b<sup>+</sup> cells of WT animals but not in cells from IFNAR-deficient mice (Fig. 4D).

Thus, these data suggest that upon THOV infection, mostly CD11b<sup>+</sup> myeloid cells are infected, get activated, and produce type I IFNs IFNAR independently. In contrast, CD11b<sup>+</sup> cells are less prone to THOV infection and are incapable of IFNAR-independent type I IFN production.

**DISCUSSION**

We demonstrated that in contrast to other viruses and artificial stimuli, THOV infection and treatment with replication-incompetent THOV-VLP lead to type I IFN responses even in the absence of positive feedback amplification via the IFNAR. This was rather unexpected since it is broadly believed that mice deficient for the IFNAR are unable to sense type I IFNs and, hence, to induce full-blown type I IFN production (19, 37). It has been shown before that some viruses or artificial stimuli, such as VSV and Newcastle disease virus (NDV) or artificial double-stranded RNA poly(I:C), can induce minor type I IFN responses in an IFNAR-deficient setting (38, 39). However, levels were dramatically reduced compared to those in their WT counterparts. In particular settings, pDC were shown to be the main type I IFN-producing cell type (38, 39), which is in line with the suggestion that pDC might be capable of differential usage of the transcription factor IRF7 (40, 41). In contrast, we showed that myeloid cells such as bone marrow-derived mDC and CD11b<sup>+</sup> F4/80<sup>+</sup> cells from the peritoneal cavity, but not pDC, are most responsive upon THOV infection in vitro and in vivo, respectively (Fig. 1, 2, and 4) (23). Induction of type I IFN responses upon both THOV and THOV-VLP infection is managed primarily via the MAVS-adapted RLR rather than by MyD88/TRIF-adapter TRIF (Table 3D) (23). These findings are supported by data showing that myeloid cells use RLR while pDC use the TLR pathway for pathogen detection (42). In accordance with this, we showed that both THOV and THOV-VLP show a tropism for mDC and PEC rather than pDC (Fig. 3F).

Performing a set of concerted in vitro, ex vivo, and in vivo experiments, we showed that while no cell type investigated produced IFN-α independently of IFNAR upon in vitro infection (Fig. 1B), minor levels of IFN-α could be detected when cells were isolated from THOV-infected IFNAR-deficient mice and cultured subsequently in vitro (Fig. 2A). In contrast, robust IFN-α production was observed in spleen and in the peritoneal cavity of IFNAR<sup>−/−</sup> mice in vivo (Fig. 2B). These data point toward the need for in vivo conditions for robust IFNAR-independent type I IFN induction upon THOV infection. It was shown that, on an individual level, the infected cell is not necessarily the type I IFN-producing cell (43). However, our own preliminary experiments with mixed cultures of PEC and spleen cells and experiments using mixtures of infected and uninfected cells did not result in robust IFN-α secretion in vivo (data not shown). Whether in vivo conditions involve the interaction of several cell types and/or the cooperation between infected and uninfected cells will be a matter of future investigations.

Using replication-incompetent but transcriptionally active THOV-VLP, we demonstrated that IFNAR-independent type I IFN induction depends on viral polymerase activity but is largely independent of viral replication (Fig. 3A). Accordingly, UV irradiation abolished the IFN-α-inducing capacity of THOV-VLP, indicating the necessity for THOV-VLP transcriptional activity (Fig. 2B). Nevertheless, compared to IFN-α levels upon THOV in vivo infection, total IFN-α levels upon THOV-VLP in vivo infection were reduced (compare Fig. 1A and 3A). This suggests that the viral load is of great importance for the magnitude of IFN-α responses. For most viruses, including THOV, it has been shown that the viral load is much higher in an IFNAR-deficient experimental setting than in WT mice or cells (23). However, this higher viral load does not lead to enhanced type I IFN responses compared to those seen with the WT in any of these settings, e.g., using VSV (Fig. 1A) (34) or NDV (39), THOV and the Rift Valley fever bunyavirus (44) being the exceptions.

The cellular mechanism used by myeloid cells for robust IFNAR-independent IFN-α production might include enhanced basal expression of pathogen sensors, thereby enhancing the first, IFNAR-independent wave of type I IFN production. Such a mechanism was suggested by Hui et al. for infection with an H5N1 influenza virus, demonstrating that virus-induced mediators upregulated RIG-I in uninfected cells by paracrine effects contributing to amplified cytokine cascades (45). However, our own quantitative RT-PCR data do not suggest enhanced RIG-I expression in IFNAR-deficient cells (Fig. 4D). On the other hand, it was shown that type III IFNs can induce a type I IFN-like response in a restricted subset of cells (46) and might therefore prime IFNAR
independent IFN-α production. However, our own preliminary results indicate that type III IFN signaling is not involved in IFNAR-independent IFN-α production upon THOV infection (data not shown). This is in line with data showing that in the mouse, type III IFNs are expressed in a tissue-dependent fashion and primarily act on epithelial cells in vivo (47). Finally, the absence of the induction of negative regulators via the IFNAR (12) may contribute to the strong IFNAR-independent IFN-α production in myeloid cells upon THOV infection as well. Why these mechanisms, which might account for the IFNAR-independent IFN-α secretion upon THOV infection, do not play a role in infection with other viruses that are unable to mount IFN-α responses in the absence of the IFNAR will be a matter for future investigations.

Using two different IFN-β reporter mouse systems, we demonstrated that CD11b+ cells from the peritoneal cavity, further characterized as being F4/80+ CD11c+ B220−, are the main cell type producing type I IFNs independent of the IFNAR. CD11b+ cells and CD11c+ cells, respectively, from both WT and IFNAR-deficient animals were rather unresponsive upon infection (Fig. 4). These results are in line with data showing a clear tropism of THOV for myeloid CD11b+ cells and much less for pDC (Fig. 1C and 4D), which were shown to be CD11c+. However, in WT or IFNAR-deficient animals as well as in both reporter mouse systems used, we never detected any type I IFN or reporter gene signal, respectively, within the spleens (data not shown). Along this line, Bauer et al. recently showed that upon treatment of IFN-β mob/mob mice with CpG, a stimulus for pDC but not mDC or PEC to produce type I IFNs (Fig. 1B), only a minor and distinct population of pDC within the spleen was stimulated to express the YFP reporter gene (48).

Collectively, our data indicate that the infected host can effectively use alternative pathways to induce type I IFN responses if the classical feedback amplification is not available. Understanding how type I IFNs can be produced in large amounts in specialized cell types independently of the IFNAR-dependent enhancement will broaden our view of host strategies to fight viral pathogens.

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