Virus-induced Interferon α Production by a Dendritic Cell Subset in the Absence of Feedback Signaling In Vivo

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Abstract
An effective type I interferon (IFN-α/β) response is critical for the control of many viral infections. Here we show that in vesicular stomatitis virus (VSV)-infected mouse embryonic fibroblasts (MEFs), the production of IFN-α is dependent on type I IFN receptor (IFNAR) triggering, whereas in infected mice early IFN-α production is IFNAR independent. In VSV-infected mice type I IFN is produced by few cells located in the marginal zone of the spleen. Unlike other dendritic cell (DC) subsets, FACSP®-sorted CD11c+CD11b−GR-1− DCs show high IFN-α expression, irrespective of whether they were isolated from VSV-infected IFNAR-competent or -deficient mice. Thus, VSV preferentially activates a specialized DC subset presumably located in the marginal zone to produce high-level IFN-α largely independent of IFNAR feedback signaling.

Key words: IFN type I • virus infection • dendritic cell subsets • IFN regulatory factor 7 • type I IFN receptor

Introduction
Type I IFNs (IFN-α/β) constitute a family of cytokines comprising in the mouse at least 11 IFN-α isoforms and one IFN-β, which were identified by their ability to protect cells against viral infection (1). For a broad spectrum of viruses a functional type I IFN response is critical for the survival of the infected host (2, 3). Beyond the direct antipathogenic activity, type I IFNs synergize with other proinflammatory stimuli to activate innate effectors such as NK cells, macrophages, and dendritic cells (DCs) (4) and may modulate antigen presentation and the adaptive immune response (5).

The biologic activity of all IFN-α subtypes and of IFN-β is mediated by binding to the common type I IFN receptor (IFNAR), a heterodimer consisting of the α-chain (IFNAR-1) and the β-chain (IFNAR-2, references 6 and 7). IFNAR signaling activates a multitude of IFN-inducible genes whose products may drastically alter cell homeostasis; in order to impede viral replication, cell proliferation is halted and both transcription and translation are strongly reduced. High-level expression of type I IFN, that can be detrimental to the host (8, 9), is tightly regulated (10) and usually restricted to the state of acute viremia.

The molecular mechanism of type I IFN induction was intensively investigated by analyzing virus-stimulated mouse embryonic fibroblasts (MEFs) derived from gene-targeted mice deficient of factors involved in the type I IFN signaling cascade. MEFs from mice lacking IFN-β, IFNAR-1, signal transducer and activator of transcription 1, or IFN regulatory factor (IRF)-9 showed a defective IFN-α response (11–14). This suggested that after the production of early IFN-β, and possibly IFN-α4, expression of other IFN-α genes was dependent on IFNAR feedback signaling. Two members of the IRF family, IRF-3 and IRF-7, have been shown to play a key role in the sequential induction of type I IFN genes. Virus-mediated serine phosphorylation leads to IRF-3 activation and translocation to the nucleus (15). There, IRF-3 is part of an enhanceosome complex promoting the expression of IFN-β, and presumably of IFN-α4. Early type I IFN is secreted and triggers IFNAR signaling in an autocrine fashion. Among
other type I IFN–induced genes, IFNAR signaling strongly upregulates IRF-7 expression (12, 13). Virus infection leads to IRF-7 activation by phosphorylation that drives the expression of the majority of IFN-α subtypes, and hence amplifies the type I IFN response. It is not known, however, which serine kinase(s) is/are responsible for the activation of IRF-3 and IRF-7. It appears that several pathways may lead to IRF-3 and IRF-7 activation. Some viruses, such as type I herpes simplex virus (HSV), induce an IFN response independent of viral replication whereas others, e.g., vesicular stomatitis virus (VSV), lose the ability to induce type I IFN upon inactivation. Furthermore, a variety of nonviral IFN inducers has been described, including prokaryotic DNA motifs (CpG-DNA), synthetic double-stranded RNA (poly[I:C]), lipopolysaccharide, and imiquimod derivatives (16, 17).

In cell culture, virtually any cell type can produce type I IFN in response to appropriate stimulation, yet, depending on the expression of pattern recognition receptors distinct cell types are stimulated by different IFN inducers. For example CpG-DNA but not poly(I:C)–treated human CD11c– DCs produce type I IFN, whereas a reverse correlation was found for CD11c+ DCs (17, 18). Accordingly, CD11c– DCs express the Toll-like receptor (TLR)-9 and CD11c+ DCs express TLR-3 (17, 19), which have been shown to be involved in the recognition of CpG or poly(I:C), respectively (20, 21). So far, the major IFN–producing cell (IPC) has not been defined in the context of infection with specific pathogens. Several studies addressed the nature of the human IPC (22–24), which eventually was defined as the subset of CD11c+ plasmacytoid DCs (25, 26). There are indications that upon stimulation of mice certain macrophages (27) or DC subsets can produce type I IFN (28–30).

Considering the above information, in an acutely infected host fibroblasts presumably do not produce the majority of type I IFN. Therefore, we investigated whether the positive feedback regulation of type I IFN induction, as identified in MEFs, also plays a role in viral pathogenesis in vivo. Surprisingly, VSV- and UV-HSV–treated mice mount early IFN-α responses largely independent of IFNAR feedback signaling. We provide evidence that unlike fibroblasts, mouse IPCs can produce early IFN-α independent of IFNAR signaling. These data are discussed in the context of an extended model of IFN-α induction based on the cell type involved in the production of IFN-α.

**Materials and Methods**

**Mice and Viruses.** IFNAR–deficient mice (IFNAR^−/−) on the SV129 background (3) and IFN-β^−/− mice on the Balb/c background (14) were bred under specific pathogen free (SPF) conditions. Unmutated SV129 mice (referred to as wild-type [WT]) were obtained from the SPF-breeding colony of the European Mutant Mouse Archive (EMMA), Monterotondo, Italy. C57BL/6 mice were purchased from IFFA-Credo. Experimental mouse work was done under SPF conditions in compliance with regulations of the Ministerio della Sanità, Rome, Italy (decreto ministeriale n° 15/2001–b, 3/3.1). VSV-IND (Mudd-Summers isolate) was originally obtained from D. Kolakofsky, University of Geneva, Geneva, Switzerland. VSV was grown on BHK-21 cells (31) and plated on Vero cells. Stocks were maintained in MEM containing 2% heat-inactivated FCS. HSV strain F originally obtained by American Type Culture Collection was grown on Vero cells. HSV was purified from cell culture supernatant by PEG 40,000 (Serva) precipitation. After several pelleting steps for 1 h at 43,000 g virus was resuspended in PBS and titrated on Vero cells. After UV irradiation (1.2 Joule/cm²) HSV inactivation was verified by plaquing on Vero cells.

**Cell Culture.** WT and IFNAR^−/− MEFs were derived from day 13 embryos (32) and maintained in DMEM supplemented with 10% FCS. Cell monolayers were infected with VSV at a multiplicity of infection (MOI) of 10. After 1 h of incubation at 37°C the virus suspension was replaced by DMEM 10% FCS.

**Expression Analysis.** Preparation of total RNA was performed using the TRIZOL reagent (GIBCO BRL) according to the manufacturer’s instructions. Tissue samples were snap-frozen and homogenized in Trizol using a Polytron (Kinematica). On cultured cell monolayers and FACs®-sorted cells Trizol was applied directly. To eliminate possible DNA contamination total RNA was incubated for 15 min at 37°C with 10 U of DNase (Boehringer Mannheim). cDNA was prepared using Superscript II (GIBCO BRL) according to the manufacturer’s instructions. To estimate relative amounts of specific mRNAs, PCRs were performed with serially fivefold diluted cDNA using published primers for IFN-α (consensus primers annealing with all IFN-α subtypes) and IRF-7 (13). The mRNA content was normalized by RT-PCR analysis with GAPDH–specific primers from the same publication. The absence of contaminating DNA was verified by PCR analysis of RNA preparations not treated with reverse transcriptase.

**Quantification of IFN Activity.** The IFN bioassay was based on the protection of L929 cells from the cytopathic effect (CPE) of VSV (CPE inhibition assay). To inactivate potential viral contamination, 1.5 prediluted serum samples from VSV-infected mice were UV-irradiated with 1.2 Joule/cm². Doubles of serially twofold diluted sera were transferred to 24 h preincubated semiconfluent monolayers of L929 cells in 96-well plates. After 24 h incubation at 37°C supernatants were removed and VSV was added at a MOI of 0.05. After 48 h incubation at 37°C, supernatants were taken away and protected cells were stained with 0.5% crystal violet in 5% formaldehyde, 50% ethanol, and 0.8% sodium chloride. After extensive washing with water and air drying, the dye was extracted from stained cells with 100 μl/well 0.1 M sodium citrate in 50% ethanol, pH 4.2, and the crystal violet content was determined on an ELISA reader at 570 nm. In the CPE inhibition assay an international mouse IFN-α/β reference standard of 100 IU/ml (GU02–901–511; NIAID Repository) and recombinant IFN-αA standards (PBL Biomedical Laboratories) of 100, 500, 2,500, and 10,000 IU/ml were included. For IFN quantification the 50% protective serum dilution was indicative. A log₅ titer corresponded to 1,000 IU IFN-αA. The contribution of IFN-β or IFN-α to the total IFN activity was determined by preincubating sera for 1 h with excess amounts of neutralizing anti–IFN-α (clone 4E-A1; Yamasa Shoyu) or anti–IFN-β (clone 7F-D3; Yamasa Shoyu) mAbs. Protection from CPE due to IFN-γ was formally excluded by an IFN-γ Elisa (Promega) indicating that the IFN-γ content of all samples tested was below the detection threshold of the assay.

**Immunohistochemistry.** Freshly removed organs were immersed in HBSS and snap-frozen in liquid nitrogen. Tissue
sections of 5-μm thickness were cut in a cryostat, placed on sili-
conized glass slides, air dried, fixed with acetone for 10 min, and
stored at ~70°C. IFN was stained using a polyclonal sheep anti-
mouse IFN-α/β antiserum (PBL Biomedical Labs) or mono-
clonal rat anti-mouse IFN-α antibody (Serotec). The primary
sheep antibody was detected with affinity purified biotinylated
donkey anti-sheep Ig antibodies (Jackson Immunoresearch Lab-
oratories) and alkaline phosphatase labeled avidin/biotin com-
plexes (ABC/AP Dako). The primary rat mAb was detected with
affinity purified peroxidase labeled goat anti-rat Ig antibody. For
signal amplification by catalyzed reporter deposition, biotinylated
gelatin. Tissue culture experiments have shown previously
that the expression of all IFN-α genes, except IFN-α4, is
dependent on IFNAR feedback signaling in Newcastle dis-
ease virus (NDV)-stimulated MEFs (12, 13). To validate
this finding under conditions of a productive virus infec-
tion, IFNAR competent (WT) and IFNAR-deficient (IFNAR−/−)
MEFs were VSV-infected and IFN-α mRNA expression was
monitored by RT-PCR analysis. WT fibroblasts showed elevated IFN-α mRNA levels beginning 4 h after VSV infection and peak expression between 9 h and 12 h (Fig. 1 a). Similar to NDV-stimulated MEFs, VSV-infected MEFs predominantly expressed IFN-α4 as
determined by subcloning of RT-PCR products and se-
quence analysis of random clones (Fig. 2 a). In contrast, IFN-α upregulation was not observed in VSV-infected
MEFs lacking the IFNAR (Fig. 1 a). Thus, in VSV-
infected MEFs the IFN-α production is strictly dependent
on IFNAR feedback signaling.

To next examine IFN responses in vivo, IFN-α gene
expression was analyzed in spleen from WT and IFNAR−/−
mice infected with VSV. Surprisingly, IFN-α mRNA was
induced rapidly and to a similar extent in spleen of WT and
IFNAR−/− mice. At later time points, IFN-α mRNA was
upregulated in IFNAR−/− mice infected with VSV. Total
RNA of 10^6 cultured MEFs or 5 × 10^4 cells of each subset were sorted from ~10^7 cells on a MoFlo

Results

Feedback Signaling Is Required for the Expression of IFN-α mRNA in VSV-infected MEFs but not in VSV-infected Mice. Tissue culture experiments have shown previously
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cytometer (Cytomation).

Figure 1. VSV infection stim-
ulates IFNAR-independent pro-
duction of IFN-α in mice, but
not in MEFs. (a) MEFs derived from WT or IFNAR-deficient mice (IFNAR−/−) were in-
fected with VSV at a MOI of 10
and (b) WT and IFNAR−/− mice were intravenously injected with 2 × 10^6 PFU VSV. Total
RNA of 10^6 cultured MEFs or
of spleen tissue was extracted, and
the mRNA content of different
samples was normalized by a
GAPDH-specific RT-PCR.
The expression of IFN-α
mRNA was monitored using
these primers amplifying all
IFN-α subtypes. The analysis
was performed with serially five-
fold diluted cDNA samples start-
ing with undiluted material;
spleen derived samples were
fivefold pre-diluted.
mice showed abundant expression of IFN-α2 and 5, whereas IFN-α4 was found only rarely (Fig. 2 b and c). Thus, different IFN-α patterns found after VSV infection of MEFs and mice suggested that in vivo the majority of IFN-α is produced by a cell type different from fibroblasts. Moreover, similar IFN-α patterns found in WT and IFNAR−/− mice indicated a largely feedback-independent production of early IFN-α in VSV-infected mice.

Early IFNAR-independent and Late IFNAR-dependent IFN-α Expression in VSV- and UV-HSV–treated Mice. To correlate IFN-α mRNA levels and IFN serum activities, blood serum of VSV-infected mice was taken and analyzed in a CPE inhibition assay. As early as 4 h after VSV infection IFN activity was found in the serum of both WT and IFNAR−/− animals. Specific inhibition of IFN-α and/or IFN-β by neutralizing mAbs in the CPE inhibition assay revealed a major contribution of IFN-α to the serum IFN activity, irrespective of whether IFNAR signaling was functional or not. Serum IFN titers of WT animals increased more rapidly than IFNAR−/− mice eventually leading to ~30-fold higher IFN titers in WT animals than in IFNAR−/− mice (Fig. 3 a).

Since VSV replication is not controlled in IFNAR−/− mice, a highly elevated virus load and increased cell death could have interfered with cytokine production in IFNAR−/− mice. Thus, we next analyzed type I IFN responses after stimulation with nonreplicating virus. Since UV-inactivated VSV does not induce type I IFN responses (data not shown), mice were injected with UV-inactivated HSV. 2 h after the treatment, WT and IFNAR−/− mice showed similar peak IFN activity with ~50 and 35% IFN-α contribution, respectively. While the IFN response was short-lived in IFNAR−/− mice, IFN titers in WT mice were sustained for several hours, and a switch to a more pronounced IFN-α expression was found (Fig. 3 c). To verify the induction of early IFN-α in the absence of IFNAR-feedback signaling, mice deficient of the IFNAR, or IFN-β were intercrossed and IFNAR−/− IFN-β−/− double-knockout mice and IFNAR+/− IFN-β−/− littermates were stimulated with UV-HSV. Even in the absence of IFN-β initial IFN-α titers showed the same magnitude in receptor deficient and competent mice. At later time points IFN-α production was sustained and further enhanced in IFN receptor competent mice as compared with IFNAR−/− mice (Fig. 4). Thus, upon treatment with live VSV– or UV-inactivated HSV early IFNAR-independent IFN production is observed. Yet, at later time points IFNAR-feedback signaling is critical to sustain the production of IFN-α.

Early IFN-α Production in the Absence of IRF-7 Induction. The transcription factor IRF-7 has been shown to be upregulated upon IFNAR triggering (12, 13) and was proposed to be critically required for the expression of IFN-α subtype genes (12, 35). Therefore, we asked whether early IFNAR-independent IFN-α was produced in the absence of IRF-7 induction. The RT-PCR analysis of IRF-7 mRNA levels in VSV-infected WT and IFNAR−/− mice revealed a prompt IRF-7 upregulation only in WT mice but not in IFNAR-deficient mice (Fig. 5). These experiments indicated that in VSV-infected mice early IFN-α was induced in the absence of detectable IRF-7 upregulation. At later time points IFNAR-independent IRF-7 upregulation was observed (data not shown) which, however, did not suffice to promote a sustained production of IFN-α in IFNAR−/− mice.

Poly(I:C) Treatment of Mice Induces Late IFN-α Production. To further study the role of feedback signaling with a nonreplicating type I IFN inducer, mice were treated with poly(I:C). Sera of poly(I:C)–treated WT and IFNAR−/− mice displayed early peak IFN activity, followed by a rapid decline in the case of IFNAR−/− mice. In WT mice IFN activity was sustained at a high level for >20 h. Neutralization of IFN-α and/or IFN-β revealed an abundant contribution of IFN-β to early type I IFN activity in both WT and IFNAR−/− mice. At later time points, strong IFN-α production was observed in WT but not in IFNAR-deficient mice (Fig. 3 b).

Preferential production of IFN-β after poly(I:C) treatment, while mainly IFN-α is produced after virus stimulation, indicates that different cell types may be involved in the

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**Figure 2.** Differential IFN-α expression profiles of VSV infected MEFs and mice. PCR products of the IFN-α expression analysis 6 h after VSV infection of (a) WT MEFs, (b) spleen of WT mice, or (c) spleen of IFNAR−/− mice were subcloned and DNA sequence of 13, 35, and 25 single clones was analyzed, respectively. IFN-α sequences were classified according to EMBL database entries. The sequence termed IFN-α10 corresponds to the IFN-α/β cDNA sequence available on the EMBL database (GenBank/EMBL/DDBJ accession no. L38698).
production of poly(I:C)− versus virus-induced type I IFN. Moreover, these results further support the hypothesis that the sustained production of IFN-α is IFNAR dependent.

**VSV Infection but not Poly(I:C) Treatment Stimulates High-Level Type I IFN Production in Cells Located in the Marginal Zone of the Spleen.** To localize IPCs, mice were infected with VSV and 9 h later, spleens were analyzed immunohistochemically with type I IFN-specific antibodies. Consecutive sections were stained with polyclonal antibody directed against type I IFN or with an IFN-α-specific mAb. While staining with the polyclonal serum appeared more sensitive and decorated a structure surrounding follicles, i.e., the marginal zone, stainings with mAb revealed distinct type I IPCs scattered in groups within the marginal zone (Fig. 6 a and b). After treatment with VSV or UV-HSV, spleens of WT and IFNAR−/− mice showed similar marginal zone stainings with polyclonal antibody, albeit staining of spleens from IFNAR-deficient mice was somewhat weaker (Fig. 6 c). Despite comparable IFN levels in the serum, no specific type I IFN staining was detected in the marginal zone of poly(I:C)−treated mice (Fig. 6 c). Thus, after injection of virus particles, irrespective of whether they are replicative or not, type I IFN production is localized to the marginal zone. The soluble inducer poly(I:C) did not induce high enough type I IFN produc-
tion in the spleen to be detected by immunohistology. Probably under these conditions type I IFN is produced at a low level by numerous cell types and in various tissues.

In situ hybridization of spleen sections of untreated mice, with an IRF-7 probe, did not reveal evidence for constitutive IRF-7 expression, neither in few marginal zone cells nor anywhere else in the spleen (data not shown). After VSV infection of WT mice a strong IRF-7 induction was observed all over the spleen. In contrast, no IRF-7 induction was detected in spleens of VSV-infected IFNAR−/− mice (Fig. 6 d). Together with the above RT-PCR data these observations indicate that virus-induced early IFN-α can be produced independent of IFNAR signaling and IRF-7 upregulation.

**Identification of the Major IPC.** The phenotype of the major IPC in VSV pathophysiology is not known. Since the marginal zone is a complex tissue containing specialized macrophages, DCs, endothelial cells, and nonrecirculating B cells, colocalization studies with markers for marginal zone cells could not further resolve the nature of mouse IPCs (data not shown). In analogy to the DC origin of human and mouse IPCs (25, 26, 29, 30) we aimed for the analysis of mouse DC subsets. Mice were VSV-infected and, 9 h later, several CD11c+ DC subsets were FACS®-sorted and analyzed for the IFN-α mRNA content (Fig. 7 a). Surprisingly, only CD11cintCD11b+ GR-I+ DCs (fraction D) were strongly positive for IFN-α mRNA, whereas neither lymphoid DCs (CD11c−CD11b−, fraction A), myeloid DCs (CD11c+CD11b−, fraction B), CD11cint CD11b+ DCs (fraction E), nor granulocytes (CD11c−GR−1−, fraction F) showed high IFN-α expression (Fig. 7 b). CD11cint
CD11b− DCs (fraction C) showed slightly enhanced IFN-α mRNA levels (which were at least 125-fold lower than fraction D), that might be due to contaminating cells of fraction D. We next asked whether CD11c+CD11b+GR-1+ cells are producers of IFN-α independent of IFNAR feedback signaling. For this purpose DC subsets were isolated from VSV-infected IFNAR−/− mice. Again only CD11c+CD11b+GR-1+ cells showed high-level IFN-α mRNA (Fig. 7 c). These results indicate that although other DC subsets are able to produce IFN-α upon in vitro stimulation (28), they do not play a major role in IFN-α production after VSV infection in vivo.

**Discussion**

Here we show that after viral infection of mice not only IFN-β but also IFN-α is produced immediately and largely independent of IFNAR feedback signaling. This is in contrast to the current model of IFNAR feedback-dependent expression of IFN-α, that was established based on in vitro data. To reconcile the previous in vitro data with our in vivo observations, we hypothesized that after viral infection of mice a cell type different from fibroblasts, i.e., the murine IPC, produced the majority of type I IFN, and that in these cells IFN-α expression was regulated differently than in fibroblasts. Indeed, we found that after in vivo infection the subset of CD11c+GR-1+ DCs produced IFN-α at high level, and that early IFN-α production by this cell type was largely independent of IFNAR feedback.

The positive feedback regulation of IFN-α genes was first observed in NDV-infected MEFs that lack IFN-α expression in the absence of a functional IFN signaling cascade (11, 12). One study showed IFNAR-independent IFN-α expression in NDV-infected MEFs (13). Interestingly, IFN-α−/− was previously found to dominate IFN-α responses of NDV-stimulated L929 fibroblasts (36). However, MEFs infected with VSV (Fig. 1), or vaccinia virus (37) showed a strictly IFNAR-dependent expression of all IFN-αs. Equally, Sendai virus-infected MEFs from IFN-β−/− deficient mice were found unable to produce any IFN-α, unless stimulated by the addition of exogenous IFN-β (14).

In contrast to these in vitro data, we found that VSV-infected mice mount an early IFNAR-independent IFN-α response. Furthermore, IFN-β−/− deficient and IFN-β−/IFNAR double-deficient mice treated with UV-HSV still expressed substantial IFN-α levels (Fig. 4). While IFN-α production in IFN-β−/− mice could have been promoted by priming with natural type I IFN, produced pathogen independently (38), IFN-α production in IFN-β−IFNAR double-deficient mice indicated that IFNAR triggering was not a prerequisite for early IFN-α production. IFN-α levels reached in IFN-β−/− were sufficient to control intravenous VSV infection (data not shown). In contrast, IFN-β−/− deficient mice infected peripherally with vaccinia virus showed a markedly increased susceptibility to lethal disease (37): after intranasal infection, IFN-β−/− mice showed up to 102-fold increased virus titers in the lung as compared

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**Figure 7.** In VSV-infected mice CD11c+CD11b+GR-1+ DCs express high-level IFN-α. (a) Mice were intravenously infected with 2 × 108 PFU VSV and 9 h later spleens were removed to prepare single cell suspensions. MACS®-enriched CD11c+ cells were stained with anti-CD11c-Biot./Str.-APC, anti-CD11b-FITC, and anti-GR-1-PE, and DC subsets were FACS®-sorted using the indicated gates A–F. Cells from gate C/D were further subdivided by separating GR-1− cells (fraction C) and GR-1+ cells (fraction D). Sorted fractions were derived from spleen of (b) VSV infected or untreated C57BL/6 mice, and of (c) VSV-infected Sv129 (WT) and IFNAR−/− mice. Total RNA of ~4–10 × 104 sorted cells was prepared and analyzed by RT-PCR as described in Fig. 1.
with WT mice, and eventually succumbed to the infection. Interestingly, in these experiments virus titers in spleen of WT and IFN-β−/− mice were comparably low, indicating that upon local infection vaccinia virus replication was better controlled in the spleen than in the peripherally infected organ. Thus, it appears that depending on the route of infection virus may activate IPCs or other susceptible cell types that differ in their requirement of IFNAR feedback for the IFN-α induction. Apart from the route of infection also the nature of the stimulus may determine the cell type(s) involved in type I IFN production. Our histology data show that poly(I:C) does not induce marginal zone IPCs to produce high-level type I IFN, suggesting that the observed IFN titers were contributed mainly by nonIPCs. It is possible that in analogy to the human plasmacytoid DCs, mouse IPCs express TLR-9 but not TLR-3 that is involved in poly(I:C) recognition (19, 21), and thus mouse IPCs are not triggered by poly(I:C). IFN titers in poly(I:C)-treated mice showed an early peak of IFN-β expression that only in animals with a functional receptor feedback was followed by sustained IFN-α expression. This indicated that similar to in vitro VSV-infected MEFs, IFN-α expression by nonIPCs in vivo required IFNAR feedback signaling.

In the current model of positive feedback regulation, IFNAR-dependent upregulation of the transcription factor IRF-7 plays a central role. Virus-treated mutant MEFs that failed to induce IRF-7 also lacked expression of IFN-α. The IFN-α response could be restored by ectopic expression of IRF-7 (12). In IFNAR-deficient mice, we observed early IFN-α responses in the absence of detectable IRF-7 upregulation. One possibility is that under these conditions low constitutive IRF-7 levels suffice to drive early IFN-α expression. In this context it is worth noting that in all RT-PCR experiments, unlike other DC subsets, CD11c−/CD11b+GR−1+ DCs (fraction D) showed some background IRF-7 expression already in uninfected WT mice (Fig. 7 b). Alternatively, IPCs might be able to utilize an IRF-7-independent pathway for IFN-α expression, as suggested by the recent finding, that ectopic expression of IRF-5 can substitute for IRF-7 to allow expression of certain IFN-α genes (39). Ultimately, only the generation and analysis of IRF-7-deficient mice can define the role of IRF-7 in the expression of virus-induced IFN-α in vivo.

Our immune histological analysis of VSV-infected mice revealed that IPCs are predominantly located in the marginal zone. Similarly, we and others have found the same IPC distribution in UV-HSV-treated mice (27, 40). The importance of the marginal zone for pathogen surveillance was demonstrated by the scavenger function of marginal zone cells, which can retain microscopically small particles, including pathogens, from the blood (41). Mice that were experimentally depleted of macrophages in the marginal zone (42), and osteopetrotic (op) mutant mice that are M-CSF deficient and lack the same macrophage populations, showed an increased sensitivity to pathogen infection (43). Thus, in order to be stimulated early in the course of an infection it appears “reasonable” that IPCs are located in the marginal zone. From histological colocalization studies it had been concluded that MOMA-1+ metallophilic and ERTR-9+ marginal zone macrophages might be involved in type I IFN production (27). Since we could not observe a strict correlation of MOMA-1 and ERTR-9 versus IFN-α/β stainings, and after recent evidence that the human and mouse IPC are plasmacytoid DCs (25, 26, 29, 30), we FACS-sorted several DC subsets from spleen of VSV-infected mice and analyzed them for IFN-α mRNA expression. These experiments indicated that the subset of CD11c+CD11b−GR−1+ DCs showed high IFN-α mRNA levels. Interestingly, we did not find increased levels of IFN-α message in myeloid or lymphoid DCs (fractions A and B, respectively) isolated from VSV-infected mice, as could have been expected form recent in vitro data (28).

In conclusion, we suggest to extend the current model of IFNAR feedback dependent expression of IFN-α as follows. (i) In vivo, local infections may stimulate the production of mainly IFN-β by nonIPCs to protect surrounding tissues in a paracrine fashion. These cells produce IFN-α IFNAR dependently, i.e., only if sufficient autocrine type I IFN stimulation is provided. (ii) Systemic infection and acute viremia that require a generalized antiviral state may stimulate IPCs to produce IFN-α early and at high-level independent of IFNAR feedback signaling; however, IFNAR feedback defines the magnitude and duration of sustained IFN-α production.

To our knowledge, we present the first set of ex vivo data defining IPCs in viral pathogenesis. Our results support the concept that highly specialized DCs, located in the marginal zone, produce type I IFN upon pathogen contact. The observation that early IFN-α production of mouse IPCs is largely independent of IFNAR signaling indicates a less stringently regulated IFN-α expression in IPCs as compared with other cell types. Further experiments will reveal, whether, depending on the tissue tropism and the stimulation mechanism, different pathogens may activate discrete cell populations to produce type I IFN, and whether mouse IPCs do play a role in antigen processing and presentation. A better understanding of the role of IPCs in viral pathophysiology can have important diagnostic and therapeutic implications, as exemplified by publications showing reduced IPC counts in HIV-infected AIDS patients (44, 45).

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