Temporal and Spatial Resolution of Type I and III Interferon Responses In Vivo§

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Although the action of interferons (IFNs) has been extensively studied in vitro, limited information is available on the spatial and temporal activation pattern of IFN-induced genes in vivo. We created BAC transgenic mice expressing firefly luciferase under transcriptional control of the Mx2 gene promoter. Expression of the reporter with regard to onset and kinetics of induction parallels that of Mx2 and is thus a hallmark for the host response. Substantial constitutive expression of the reporter gene was observed in the liver and most other tissues of transgenic mice, whereas this expression was strongly reduced in animals lacking functional type I IFN receptors. As expected, the reporter gene was induced not only in response to type I (α and β) and type III (λ) IFNs but also in response to a variety of IFN inducers such as double-stranded RNA, lipopolysaccharide (LPS), and viruses. In vivo IFN subtypes show clear differences with respect to their kinetics of action and to their spatial activation pattern: while the type I IFN response was strong in liver, spleen, and kidney, type III IFN reactivity was most prominent in organs with mucosal surfaces. Infection of reporter mice with virus strains that differ in their pathogenicity shows that the IFN response is significantly altered in the strength of IFN action at sites which are not primarily infected as well as by the onset and duration of gene induction.

Type I interferons (IFNs), a single IFN-β and more than 13 α subtypes in humans and mice, were discovered as antiviral proteins that are typically induced by viruses (38). Other members of the type I IFN family were discovered later and comprise IFN-ω, -ε, and -κ (37). These IFNs all use the same heterodimeric receptor (IFNAR1/2) (47). Another class of antiviral IFNs, the type III IFNs that comprise three λ subtypes, signal through a different receptor complex composed of the interleukin (IL)-10Rβ and the IL-28Rα subunits (29, 40). Despite the use of different receptors, both IFN types share an interferon regulatory factor (IRF)-dependent induction pathway, induce a similar set of genes (ISGs), and exhibit similar biological activities (1). This is due to their ability to activate the transcription factors STAT1 and STAT2 (10, 43). Type I IFNs have been shown to activate natural killer cells, modulate the activity of T cells as part of their innate immune activity, and also serve as activators of the adaptive immune system (4, 5). The receptor for type I IFNs is expressed on essentially all and also serve as activators of the adaptive immune system (4, 5). Primary signaling makes use of NF-κB and IRF activation, ending with transcriptional activation of the IFN genes (23). Primary induction leads predominantly to the upregulation of IFN-β and IFN-α4 in most cell types. However, in cells that have been pretreated with IFNs and which have induced ISGs, other IFNs, mainly IFNs of the α subtype, are induced as well by infection, leading to a super-stimulation of ISGs, a phenome-
non that is called “IFN type I receptor mediated feed-forward” (34). This action relies on the ISG IRF-7 (22, 34). pDCs constitutively express IRF-7 and secrete a large amount of IFNs upon primary infection. Recently, Kumagai et al. (30) analyzed the IFN-α response to viral infection using a reporter system in which a green fluorescent protein gene was knocked into the IFN-α6 gene. They found that both the infection site and the virus strain define the cell types that become dominant IFN producers. The fact that in all infections mobile cells from the hematopoietic compartment are involved predicts a distribution of the IFNs to different sites in the body, with blood and lymphoid tissue being specifically affected.

In addition to virus-induced IFN production, low constitutive expression of type I IFN has been reported (11, 21). A recent publication making use of a reporter mouse, in which the IFN-β structural gene is replaced by the firefly luciferase gene, confirms this constitutive expression and demonstrates its origin in the thymus and other sites (31).

IFNs are produced at different positions and by different cells in the body depending on the nature and site of infection. To identify not only IPC but subsequently cells and tissues reacting to IFN, highly sensitive methods are needed to dissect the spatial resolution of the gene expression triggered by IFNs in vivo. At least as important is the dynamic behavior of these IFN responses. The molecular and physiological consequences of IFNs depend on the nature (affinity), the concentration, and the time course of exposure to these cytokines. It may induce either antiviral activity (short exposure and weak binders) or antiproliferative activity (long exposure, strong binders) (26).

Further, the target cells and organs will define the biological activity. Therefore, we have created reporter mice by which several of these questions can be solved, including local versus systemic distribution of IFNs, the definition of the main target cells and organs, the dispersion and availability dynamics of IFNs, and the course of the IFN response during infection of individual living animals.

To generate reporter mice for type I as well as type III IFNs we chose the Mx2 gene. Both Mx genes, Mx1 and Mx2, are specifically induced by type I and type III IFNs (2, 19, 20, 35). It is noteworthy that the Mx genes in inbred mouse strains are specifically induced by type I and type III IFNs (2, 19, 20, 35). This action relies on the ISG IRF-7 (22, 34). The fact that in all infections mobile cells from the hematopoietic compartment are involved predicts a distribution of the IFNs to different sites in the body, with blood and lymphoid tissue being specifically affected.

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FIG. 1. Characterization of transgenic Mx2-Luc reporter mice. (A) Flow scheme of Mx2-Luc recombineering. The BAC RP24-71I6 harboring the mouse Mx2 gene (upper line) was targeted by homologous recombination with a cassette carrying the gene encoding firefly luciferase 2 (open box) followed by the SV40 virus poly(A) signal, the FRT-flanked (gray ovals) fragment of prokaryotic and eukaryotic (PGK) promoter (black arrow) controlling expression of the kanamycin/neomycin phosphotransferase gene (light gray box), and the poly(A) signal of the bovine growth hormone gene (dark gray box). The complete Mx2 ORF located on the BAC was replaced by this cassette. (B) Integration of Mx2-Luc into genomes of three transgenic mouse lines. BamHI endonuclease-restricted mouse genomic DNA, isolated from adult ear fibroblasts, was blotted on nylon membrane and probed with a [32P]dCTP-labeled luc2-ORF probe. BAC DNA used for pronuclear injection is shown as a control. A single 4.1-kb fragment shows complete insertion of luc2 and at least 2.2 kb of the upstream promoter region of Mx2 into the mice’s genomes. HindIII-digested λ DNA was used as a marker (M). (C) Bone marrow-derived macrophages from Mx2-Luc mice (line 2) were treated with IFN-β (500 U/ml) and IL-6 (0.1 μg/ml). Fold induction represents relative luminescence units of stimulated compared to untreated cells (cont.). All values were expressed as means ± standard deviation (SD) (n = 3).
RESULTS

Generation of Mx2 transgenic reporter mice and their validation. To investigate which cell populations or organs react to type I and type III IFNs, we generated BAC transgenic mouse strains that contain the optimized firefly luciferase 2 reporter gene under transcriptional control of a characteristic type I IFN-regulated gene. Therefore, we chose the mouse Mx gene locus. In the BAC clone RP24-71I6, the Mx2 gene is flanked by 5’ and 3’ sequences of 103 and 75 kb, respectively. In our BAC construct the coding region of the Mx2 gene was replaced by the sequence of the reporter gene (Fig. 1A). Three transgenic mouse lines (designated Mx2-Luc) were obtained by pronuclear injection. A molecular characterization of the core regulatory region of the integrated BACs, including the proximal promoter region and the reporter gene, revealed that an intact reporter locus is present in all three mouse lines (Fig. 1B). To show specificity of reporter gene induction, macrophages from Mx2-Luc reporter mice were isolated and stimulated with different types of IFN as well as with IL-6 and tumor necrosis factor alpha (TNF-α) (Fig. 1C). Quantitative determination of luciferase expression showed strongly increased reporter gene activity in cells stimulated with IFN-α and IFN-β for 12 h. In contrast, stimulation of macrophages with IFN-α3, TNF-α, and IL-6 did not induce significant Mx2 promoter induction while treatment of cells with IFN-γ resulted in a marginal induction of the reporter gene. Next, we studied the dose response of IFN-β on primary macrophages (Fig. 1D). IFN-β markedly stimulated the reporter, depending on the concentration. Dose-dependent induction of luciferase activity was also observed in fibroblasts derived from ear biopsy specimens of adult mice (data not shown). Thus, we conclude that the Mx2-Luc BAC construct represents a specific and highly sensitive marker to quantify the response toward IFNs.

We further examined whether the transgenic Mx2-Luc allele recapitulates endogenous Mx2 mRNA expression. We correlated the response of macrophages from transgenic mice to IFN stimulation by comparing mRNA levels of the endogenous Mx2 gene and the luciferase reporter at different time points (Fig. 1E). In these cells Mx2 and luciferase mRNA levels peaked around 8 h after IFN treatment. Afterwards, the levels of both mRNAs declined. Importantly, the luciferase mRNA expression pattern resembles regulation of the endogenous Mx2 gene. Taken as a whole, these results showed that expression of luciferase faithfully mimics Mx2 induction. Furthermore, due to the relatively short lifetime of the luciferase protein, the transcriptional activation period of the Mx2 gene can be deduced in the reporter mice.

Tissue-specific response to interferons in vivo. To analyze the distribution of the IFN response in the whole organism, IFN-β as the major “initial” IFN was administered into the tail vein of reporter mice. At several time points after IFN injection, the mice were anesthetized and luciferase activity was imaged after administration of luciferin (Fig. 2A). Two observations deserve attention. First, the reporter gene activity had already reached a maximum at 3 h posttreatment and started to decline afterwards. Second, most of the signal was focused on the area of the liver. When various organs from untreated and IFN-β-treated mice were excised and imaged individually, the liver was confirmed to be the major responder to intravenously (i.v.) injected IFN-β (data not shown). Comparison of the three Mx2-Luc reporter lines in this experimental setting showed identical induction ratios for all lines, although the absolute levels of light emission differed significantly (see Fig. S1 in the supplemental material).

Despite the fact that the type I IFNs constitute a family of related cytokines that all recognize the same receptor, differences in action have been shown in vitro and in vivo (48). We compared the in vivo action of IFN-α4 with that of IFN-β (Fig. 2B and A, respectively). Although the main target organs seemed to be identical, the kinetics of reporter induction by IFN-α4 was slower, with a delay of about 3 h. Thus, we conclude that type I IFNs show different response kinetics in the same target organs. This conclusion is compatible with the fact that all IFNs bind to the same receptor but with different properties (24).

Several organs from untreated and from mice treated for 3 h with IFN-β were isolated and homogenized, and luciferase activity was quantified by standard luminometric measurement (Fig. 2C). By far, the highest level of luciferase activity (per mg tissue) was found in the liver. All other organs investigated showed significantly lower absolute levels of the luciferase reporter. Within those organs, high induction levels (>10-fold) were revealed in blood, liver, and kidney (compare with Fig. 3D). Some other organs, including lung and spleen, were found to be moderately induced. All other organs showed a comparably low induction ratio or absolute luciferase activ-
ity. Thus, it seems that the strongest response toward exogenously applied IFN can be detected in organs which are intensively supplied with blood.

The different magnitudes and rates of induction during in vivo stimulation raised the question to which extent the investigated organs and cells would respond to IFN if they were equally exposed. It was of particular interest to estimate the strong response of the liver compared to other organs: whether this is a consequence of high sensitivity toward type I IFN or due to an organ-specific accumulation of the inducer. To answer this question, primary cells from different organs were prepared and cultivated in the presence of IFN-α/β/H9252 for 8 h. The response was determined at different time points (Fig. 2D). All examined cell types are able to react to IFN-α/β/H9252 by activating the reporter gene. The fold induction was found to vary between different cell types. Reproducibly, fibroblasts and macrophages showed significantly higher induction levels than splenic T cells and hepatocytes. These results suggest that the strong in vivo reaction in the liver is not due to highly sensitive cells but rather depends on increased exposure to IFN. Taken together, the observed luciferase activities in the reporter mouse reflect a real-life IFN response.

Monitoring the IFN type III response. Reporter mice from all three lines showed constitutive reporter activity (see Fig. S1 and S2 in the supplemental material). This activity is unevenly distributed in the mouse and has, similar to the situation after IFN administration, a focus on the liver. Quantification of this prevalence of IFN activity in different organs is depicted in Fig. 2C. Systemic administration of an antibody directed against mouse IFN-α/β/H9252 reduced the reporter activity to about 50% without changing the overall distribution (data not shown). Further, a comparison of reporter mice with animals crossed to IFNAR deficiency demonstrated that less than 20% of the constitutive response is independent of functional type I IFN signaling (Fig. 3A). This indicates that 80% of the reporter activity in unstimulated mice has to be attributed to constitutively produced type I IFN. Since the half-life time of the reporter signal is in the range of a few hours, we conclude that the constitutive activity is permanently available. According to the finding of Lienenklaus et al. (31) that the main source of constitutive IFN-β is the thymus, our data indicate that this IFN is also rapidly distributed and arrives in the same target organs as the injected IFN-β.

Though the activity was significantly reduced, the IFNAR−/−
reporter mice still showed some constitutive luciferase activity (Fig. 3A, right panel). The distribution of this activity differs from that in IFNAR\(^{+/+}\) reporter mice, as shown by analysis of the IFN response in various organs (Fig. 3C). Constitutive expression of IFN-\(\lambda\) is presumably not responsible for the remaining activity, as similar background values were observed in reporter mice lacking functional type I and type III IFN receptors (data not shown). However, the differential organ responses argue against unspecific reporter gene activity. Since type III IFNs, like type I IFNs, can induce Mx proteins, it is possible to use the Mx2-Luc reporter mouse for monitoring IFN-\(\lambda\) responses. To demonstrate this, IFN-\(\lambda\) was injected into the tail vein of Mx2-Luc reporter mice and whole-animal imaging was carried out at different time points after injection (see Fig. S3A in the supplemental material). The response was fast (3 h), and the maximum was reached by 9 h after injection. In order to avoid overlaps with type I IFN signaling, the same experiment was carried out in IFNAR\(^{−/−}\) Mx2 reporter mice (Fig. 3B). A detailed analysis of the organs showed that IFN-\(\lambda\) mainly targets large and small intestine, lung, and salivary gland. Stomach, kidney, spleen, and heart showed moderate responses. Thus, the dominant target organs of type I and type III IFNs are clearly distinct (Fig. 3D).

**Production and fate of interferon after virus infection.** Several stimuli induce IFN production which, in turn, leads to specific IFN responses. In order to assess the time course of this process, we first used the double-stranded RNA (dsRNA) analogue poly(I:C) as a ubiquitous inducer of the IFN system. Poly(I:C) was injected into the tail vein of Mx2-Luc reporter mice (Fig. 4A). Furthermore, the response dynamics to LPS, a bacterially derived inducer of IFN-\(\beta\), was examined (Fig. 4B). Treatment of mice with poly(I:C) or LPS induced an IFN

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**FIG. 2—Continued.**

![Graph C: Luciferase activity](image)

**Graph D: IFN-\(\lambda\) fold induction**

- Black: 0 h
- Gray: 4 h
- Light gray: 8 h

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response in the liver that successively increased from the very beginning to reach a maximum at 6 or 12 h, respectively. The IFN response to poly(I:C) and LPS was compared to the response with direct injection of IFN-α/H9252. The data showed that about 3 h for poly(I:C) and 6 h for LPS distinguish the maximal reporter activation from IFN-α/H9252 injection experiments. This is seen in the quantification graphs for the luminescence of the whole animal (Fig. 4C). The rapid appearance of the IFN response in peripheral organs after poly(I:C) injection suggests that in situ synthesized IFN must rapidly enter the blood circulation and confer antiviral effects to the organs. However, the delay in maximal induction of the Mx2 reporter depends on the kinetics of type I IFNs production upon stimulation with the different inducers.

To investigate the kinetics of the IFN response after viral infection Mx2-Luc reporter mice were infected with viruses which are known to induce a strong IFN response. We used two different viruses that infect either locally or systemically. Thogoto virus (THOV) is known to replicate and spread to different sites in the mouse body and will eventually kill the mouse. A THOV mutant lacking the IFN-antagonistic protein ML (THOV-delML) exhibits similar virulence but induces higher levels of IFN (25). If injected intraperitoneally into a reporter mouse (IFN-α/H9252-luc) that specifically reports IFN-α/H9252 gene activation by expressing luciferase (31), THOV-delML elicited a strong IFN-α/H9252 response starting 24 h postinfection (Fig. 5A). The IFN-α/H9252 response was most pronounced in the peritoneum at early times postinfection and then became most prominent in the liver, the site at which the virus replicates to very high titers. In a parallel experiment the Mx2-Luc reporter mouse was infected and imaged. Interestingly, initially, even after 24 h postinfection a strong induction of Mx2 reporter can be detected and most of this IFN response appeared in the liver. Compared to the induction of IFN-β no significant retardation in the IFN response was obvious (Fig. 5B). After 2 days postinfection, the IFN response further increased and was also detectable at other sites. To more quantitatively determine the contribution of different organs, extracts from various tissues at 0, 24, 48, and 72 h postinfection were analyzed (Fig. 5C). These data confirmed the early appearance of the IFN...
response and its predominance in the liver. However, other organs like spleen, kidney, and lung contribute to the IFN response picture obtained by whole-body imaging and Mx2 induction continuously increases in these organs as well as in the liver.

We next analyzed the IFN response in mice infected with influenza virus strain hvPR8 that replicates exclusively in cells of the respiratory tract (15). We first used hvPR8-delNS1, a mutant virus that lacks the IFN antagonistic factor NS1. hvPR8-delNS1 is a powerful IFN inducer that can hardly replicate in the lung of IFN-competent mice (28). The virus was intranasally applied to Mx2-Luc reporter mice, and the response toward IFN was imaged at different time points. Imaging showed a strong signal in the upper chest but did not allow us to clearly distinguish lung and liver responses (see Fig. S4 in the supplemental material). The IFN response was quantitatively measured in extracts from various tissues at 0, 24, 48, and 72 h postinfection (Fig. 6A). In accordance with the fact that type I IFN is produced in the lung, a fast induction of the Mx2 reporter can be measured at the site of production. However, a low response toward IFN is also detectable in spleen and liver. Taking into account that the liver has a 6 to 10 times higher mass than the lung, the pictures obtained from in vivo imaging
point out that substantial amounts of IFN are distributed over the whole body.

It is of particular interest to compare infection with the non-pathogenic hvPR8-delNS1 strain to a pathogenic situation. Therefore, we infected mice with the hvPR8-NS1(1-126) strain and quantified luciferase activity in tissue extracts. This strain leads to death of most infected animals within 4 to 5 days (28). In general, Mx2 reporter mice infected with hvPR8-NS1(1-126) re-

FIG. 4. Whole-body imaging kinetics of the response to IFN and IFN inducers. (A and B) Mx2-Luc reporter mice (line 2) were injected i.v. with 200 μg of poly(I:C) (A) and 50 μg of LPS (B). Mice were imaged before treatment (0 h) and then followed over time as indicated, starting 3 h postinjection. Images from representative Mx2-Luc mice are shown. (C) Signal progression of luciferase activity from mice treated with IFN-β (from Fig. 2A) (open bars), poly(I:C) (from Fig. 4A) (gray bars), and LPS (from Fig. 4B) (black bars) are presented as fold induction of the reporter signal.
revealed a much stronger luciferase activity than that of hvPR8-delNS1-infected animals. Interestingly, there was a 24-h delay in the maximal response toward IFN (Fig. 6B). While Mx2-Luc induction in the different organs was comparable for the first 24 h postinfection, at later time points luciferase expression increased dramatically not only in the lung but even more in liver and the other organs. Furthermore, after 48 h, luciferase activity stayed maximal until the animals had to be euthanized. The data also indicate that at later time points during infection, induction of the reporter gene in the liver is stronger than in the lung and even activity in the kidney is at comparable levels. Thus, it seems that the IFN response from both virus strains differs significantly in the strength of IFN action at sites which are not primarily infected and by the duration of ISG induction.

DISCUSSION

In this study, BAC transgenic mice carrying recombinant Mx2 locus containing firefly luciferase under the control of natural promoter elements were generated. Although the absolute expression strength of IFN-induced luciferase in three independent founder lines differs, the relative levels and induction kinetics are highly comparable. Our validation experiments in primary cells of the Mx2 reporter mice perfectly recapitulated the kinetics of endogenous Mx2 mRNA expression. The Mx2 gene as well as the Mx1 gene is regarded as a bona fide ISG that is representative for a typical IFN-induced antiviral response. We note that depending on the cell type not all ISGs are strictly coregulated and thus, the Mx2 induction...
cannot report truly the full spectrum of an antiviral activity in any cell type (8, 14). However, in all primary cell types tested so far the reporter system was activated by type I IFN and therefore allows a quantitative determination of which organs and cells an IFN response is elicited. Furthermore, this work showed that the Mx2 reporter system is valid not only for the response to type I IFNs but also for the response to type III IFNs. In particular, the use of the IFNAR-deficient reporter mouse is a valuable tool to distinguish the type III from type I IFN responses.

Regarding the kinetics of luciferase expression, as with all reporter genes, the measured responses primarily reflect the onset of gene induction, assuming that transcription and translation of the replaced gene are similar to those of the reporter gene. Figure 1E suggests that the mRNA level of luciferase is always higher than that of the endogenous Mx2 but the decline of both mRNAs during the continuous presence of IFN-α is paralleled by the presence of IFN in the organism. This is confirmed by analysis of the IFN concentration in the serum after a single i.v. injection, showing that most of the introduced dosage disappears from the bloodstream within a few minutes (see Fig. S5 in the supplemental material). Thus, if the reporter response in the animal is sustained it indicates ongoing IFN production, e.g., as seen in the virus infection experiments.

From previous publications it is known that the expression density of the IFN-α receptor corresponds to the main targets of an IFN-α response. For the liver-specific expression, conflicting results were published. While Doyle et al. (9) find significant functional IFN-α receptors expressed in human hepatocytic cells in vitro, Mordstein et al. (35) and Sommereyns et al. (41) find essentially no IFN-α receptor expression and very little response in the mouse liver. According to the results from this work, mouse liver tissue indeed responds to IFN-α; however, induction of Mx2 reporter in IFNAR<sup>−/−</sup> mice is only 2-fold (see Fig. S3B in the supplemental material). Clinical data suggest an important role of the IFN-α in the clearance of hepatitis C virus (HCV). A genetic polymorphism near the IFNα3 gene associates with spontaneous clearance and treatment response (13, 45). With respect to the response of the liver to IFN-α in mice containing functional type I IFN receptor, indirect or priming effects may be considered. Additional work is needed to clarify that indeed substantial type I IFN priming is required for an IFN-α response of the liver.

So far, published results suggest that the respective receptor expression is the main determinant for the site of the biological IFN response. Consequently, concentration and affinity of the ligand to the receptor as well as receptor density would be additional parameters. In this line, IFN-α, which has recently been shown to exhibit lower affinity to the IFNAR than IFN-β, should have a retarded response (24). Indeed, this could be verified by following the response by whole-animal imaging (compare Fig. 2A and B).

In this work we confirmed that constitutively produced type I IFN targets organs like the liver. The question of which function this effect may have for new infections remains to be elucidated. The IFNAR<sup>−/−</sup> reporter mice show a reduced but still significant constitutive activity of reporter gene action. At this stage we cannot fully exclude unspecific background elicited by the BAC transgenes. However, since the “background values” response is not evenly distributed in the mouse body, it is tempting to speculate that IFN receptor-independent inflammatory mechanisms are responsible for this activity.

The injection of poly(I:C), a ubiquitous IFN inducer, leads to a maximal IFN response 6 h after administration. Based on the observation that IFN-β elicits an earlier maximum response and assuming that this subtype is the main type I IFN produced upon poly(I:C) administration, the maximal IFN production appears to occur at 3 h after injection. Interestingly, the response to LPS is retarded and the response upon infection with the studied viruses is found even much later. The detailed understanding of the kinetics will be of importance for the understanding of infections.

The finding that the liver is the main response organ for type I IFNs is surprising. We defined parenchymal cells as the main responding cells in this organ. Nonparenchymal cells showed a significantly lower response (data not shown). Independent of the inducing agent the type I IFN response was rapidly detectable in the liver. This is explained by two mechanisms. First,
secreted IFN entering the blood circulation is rapidly absorbed by the liver, as shown by the i.v. injection of IFN-β and IFN-α. This rapid elimination from the bloodstream explains why little IFN, even after massive infections, is found in the serum. Second, IFN-producing cells (IPCs) that become activated at the site of infection might circulate and become trapped in the interstitial space of the liver to release their load close to the parenchymal cells. These consist of virus-specific target cells, such as the lung epithelial cells for influenza virus, but as well of certain immune cells that are typically found in infected tissue. Kumagai et al. (30) concluded that in epithelial lung infection three different cell species are sequentially activated. These are the main IPCs: alveolar macrophages (AMs), conventional DCs (cDCs), and pDCs. Also, responses to the constitutively expressed type I IFN were predominantly found in the liver. According to these findings, liver tissue should be permanently in a mild antiviral state. Since IFNs induce activities other than only antiviral effects (5, 44) the constitutively expressed IFN is expected to have a profound influence on the overall immune status of the liver as well as on immune cells that are affected by the liver.

The focus of the constitutive and induced type I IFN response to the liver motivated us to speculate that a specific protection of this organ from virus propagation might have advantages for the host. It is known that many circulating pathogens are preferentially trapped in the liver (12), and the IFN-induced status might help to control massive infection. Another, although less obvious, explanation is that the liver parenchymal IPCs as major IFN targets might help to control massive infection. Such a view is strengthened by the fact that the liver parenchymal cells did not confirm this assumption (see Fig. S6 in the supplemental material). Another explanation concerns the short time for which the organs are exposed to a single infection. This would simulate a pulsed response with an earlier maximum activity.

Our observations and the new mouse lines will have implications for basic research, for clinical translation, and for the use of IFNs in therapy. The reporter mice will help to elucidate the complex kinetics and cell-specific function of type I and III interferons in normal and diseased states. Among the potential applications are mouse models for certain autoimmune diseases in which excessive amounts of IFNs are produced. Another application concerns the screening for new compounds that serve as agonists or antagonists for the IFN system. Cells derived from the reporter mouse and the mouse itself could be used to test and validate such compounds for their activity. Currently, the major use of type I IFNs concerns the treatment of multiple sclerosis and chronic hepatitis induced by hepatitis viruses B and C. The systems might help to find better injection and dosage schemes and evaluate the modification of IFN preparations. Also, new IFN subtypes could be directly compared and their kinetics and accessibilities could be defined. The differences shown in IFN-α4 and -β might explain the differences in treatment with these IFN species. Since human IFNs are species specific, the expression of human receptor proteins for such experiments in the mouse might be essential.

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