The unique regulation and functions of type III interferons in antiviral immunity
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Type I interferons (IFNs) were long considered to be the sole IFN species produced by virus-infected cells until the discovery of type III IFNs (IFNλs), decades later. Like type I IFNs, type III IFNs are induced by and protect against viral infections, leading to the initial conclusion that the two IFN species are identical in regulation and biological functions. However, the two systems differ in the tissue expression of their receptor, resulting in different roles in vivo. The unique nature of IFNλs has been further demonstrated by recent studies revealing differences in the regulation of type I and III IFN expression, and how these proteins elicit specific cellular responses. This review focuses on the distinctive features of type III IFNs in antiviral innate immunity.

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Introduction
Interferons are secreted proteins that are defined by their ability to confer resistance to viral infections. Historically type I IFNs were thought to be the only species produced by non-lymphoid cells in response to viral infections, and able to activate innate and adaptive immunity. However, many different examples of antiviral signaling occurring in a type I IFN-independent manner have been described [1–6]. The specific mechanisms that govern these processes are unclear but could be explained, at least in part, by the actions of type III IFNs [7,8**]. The type III IFN family is composed of three genes: IFNλ1 (IL29), IFNλ2 (IL28A) and IFNλ3 (IL28B). A fourth member, IFNλ4, was identified more recently and is a poorly understood frameshift variant of IL28B that predicts Hepatitis C virus clearance and response to IFN therapies [9]. In humans, IFNλ1 is the most prominent and best studied species, but it is a pseudogene in mice [10]. Type III IFNs signal through the IFNλ receptor (IFNλR) which is composed of two chains: IL28Ra, a unique subunit, and IL28Rβ, shared with cytokines of the IL10 family. Like the type I IFN receptor (IFNAR), binding of the IFNAR results in the activation of JAK/STAT signaling, expression of interferon-stimulated genes (ISGs) and induction of an antiviral state. However, unlike IFNAR, which is expressed on virtually all cell types, IL28Ra is only expressed on specific tissues such as epithelia. Initial reports have focused on the similar activities of type I and III IFNs, but recent work has revealed unique properties of IFNλs and have established them as the primary regulators of antiviral immunity at mucosal surfaces, especially in the intestine [11**]. This review focuses on the fundamental differences between type I and III IFN biology.

IFNλR signaling
Similar to type I and II IFNs, ligation of the IFNλR leads to the activation of kinases of the JAK family and phosphorylation of several members of the STAT family of transcription factors [7,12*,13]. Once phosphorylated, STAT1 and 2 associate with a third protein called IRF9 to form a transcription complex termed IFN-stimulated gene factor 3 (ISGF3). ISGF3 translocates to the nucleus to induce the expression of ISGs [14]. The IL28Rβ chain is shared with cytokines of the IL10 family and signals via Tyk2, leading to the speculation that Tyk2 mediates IFNλ signaling. Original studies on the IL28Ra carried out before the identification of IFNλs concluded that JAK1, but not JAK2, mediate IFNλ signaling [12*]. However, it was recently shown in a more physiological context that JAK2 is indeed phosphorylated by type III IFNs [15*,16*]. In addition, inhibitor and RNAi studies have shown that JAK2 is necessary for STAT1 phosphorylation in response to IFNλs, and that JAK2 mediates antiviral signaling in cells that only produce type III IFNs [15**]. In these studies, JAK1 was confirmed as a mediator of IFNλ signaling, but further work is needed to determine whether Tyk2 is involved in this pathway. Therefore, type I and III IFNs appear to signal via different JAK/STAT pathways, but no unique ISG among the 500 produced by IFNλ has been identified [13,17,18].

Type III IFN targets and biological activities
Recent studies have revealed important differences in the biological functions of type I and III IFNs. These differences primarily stem from the fact that all cells respond to type I IFNs, while only a small subset responds to type...
III. This functional tissue-specificity is due to the expression of the IL28Rα subunit, which is only expressed on epithelial cells of the gastrointestinal, intestinal and reproductive tracts and some immune cells [11**,19,20]. Consequently, type III IFNs are not able to confer protection against systemic virus infections. Rather, these IFN are most effective at controlling viral infections at mucosal surfaces [21,22**,23]. Evidence supporting this idea comes from recent work comparing the relative importance of the IFNAR and IFNαR in the control of rotavirus, an RNA virus that primarily infects intestinal epithelial cells. Whereas IFNAR-deficient mice controlled viral replication in the intestine, IL28Rα-deficient intestinal epithelial cells could not mount an effective IFN response and were unable to control rotavirus infection. Furthermore, systemic treatment of infected animals with IFNα, but not IFNβ, repressed rotavirus infection in the intestine [22**]. In vitro studies of human intestinal epithelial cells support the idea that type III IFN induction and responsiveness are key aspects of the biology of these cells. For example, the differentiation state of intestinal epithelial cells dictates the quality of the IFN response, with increasing IFNs produced as cells polarize [15**], suggesting that epithelial cell biology is intimately linked with the type III IFN system.

Regulation of type III IFN expression

Type I and III IFNs are produced following recognition of viral ligands, most prominently nucleic acids, by a wide range of pattern recognition receptors. On endosomes, viral nucleic acids are recognized by Toll-like receptor (TLR) 3, 7/8, and 9. cGAS and RIG-I like receptors (RLRs) perform the same functions in the cytosol. TLR ligands induce type III IFNs concomitantly with type I, but the pathways activated are unknown [24–27**]. Similarly type III IFNs are induced by, and protect against DNA viruses [28–30], but the precise mechanisms driving these processes have yet to be studied. Type III IFN expression in response to RNA viruses is best understood, and involves RLRs, MAVS and TBK1, like type I IFNs [15**,31–33]. However, the subcellular localization of MAVS determines which IFN species is produced. MAVS is an adapter of the RLR pathway that was first identified as being localized on mitochondria [34] and was later shown to also localize to peroxisomes [1] and mitochondrial-associated endoplasmic reticulum membranes [35]. From peroxisomes, MAVS is able to induce ISGs and control viral infections independently of type I IFNs [1]. Indeed, transgenic cell lines preferentially expressing MAVS on peroxisomes only induced type III IFNs in response to a variety of intracellular ligands [15**]. In addition, the function and abundance of peroxisomes and mitochondria determines the quality of the IFN response. Increasing peroxisomal abundance or inhibiting mitochondrial function favors the expression of type III over type I IFNs. This observation could reflect what happens physiologically in epithelia. Indeed, polarization of epithelial cells increases peroxisome abundance and type III IFN responses to viral infections, while the number of mitochondria and type I IFN expression are unaffected [15**].

Type I and III IFNs are also differentially regulated at the transcriptional level. IFNβ, the prototypical type I IFN, is induced by the combined actions of the transcription factors AP-1, interferon regulatory factor (IRF) 3 and IRF7 and NFκB. The binding sites for each set of transcription factors are localized in close proximity to each other and IFNβ expression requires the cooperative binding of all activators in a complex termed the enhanceosome [36,37]. The IFNA1 promoter contains binding sites for the same sets of transcription factors. However, only IRF3, 7 and NFκB are required for type III IFN induction [15**,32,33]. In fact, MAP kinases that activate AP-1 are not required for type III IFN production in response to RNA viruses, demonstrating that AP-1 is dispensable in the IFNA1 pathway [15**].

Both IFN families also contain binding sites for IRF1, the first IRF identified. Although first reports concluded that IRF1 bound the IFNβ promoter, IRF1 KO mice and cells are unimpaired in their ability to induce type I IFN expression [38]. However, IRF1 is known to be important in antiviral defense, and was thought to act in an interferon-independent manner [1,2]. Recent studies have confirmed that IRF1 does not induce IFNβ [15**], but does instead control type III IFN expression in response to RNA viruses [15**,39,40]. In addition, while IFNβ regulation requires all components of the enhanceosome, it appears that type III IFNs can be induced through the independent action of IRFs and NFκB [41]. This finding has important implications: first, the limited number of transcription factors it requires explains the very high inducibility of type III IFNs. Also, if NFκB and IRFs can independently induce type III IFNs, one can predict that this pathway is less susceptible to be successfully targeted by pathogens. These findings define unique pathways for the induction of each IFN family: IRF3/7 and NFκB are activated by both mitochondrial and peroxisomal MAVS to mediate the expression of both IFN classes. However, MAVS on peroxisomes activates IRF1 to only regulate type III IFNs while MAVS on mitochondria can activate MAP kinases to induce type I IFNs (Figure 1).

Simple tools to study type III IFNs in mouse systems

Most studies on type III IFNs have been carried out in human systems as the tools to study mouse IFNs are lacking. As type I and III IFNs differ in their ability to activate JAK2 [15**,16*], immunoblotting against phosphorylated JAK2 can report type III IFN signaling in a given experimental system. In parallel, JAK2 RNAi, knockout and/or inhibition with pharmacological inhibitors such as AG490 or 1,2,3,4,5,6-hexabromocyclohexane
[42] will specifically block type III IFN signaling without affecting type I [15**].

As a complementary method, we have developed a very simple bioassay that enables the quick and distinct detection of mouse type I and III IFNs. Our system utilizes human 293T and mouse L929 cells that express luciferase under the control of an ISRE promoter, the sequence activated by type I and III IFNs. L929 readily respond to mouse IFNβ in a dose-dependent manner but they do not respond to IFNλ2 (Figure 2a). Therefore, these cells can be used to detect IFNβ without detecting IFNλ. To selectively detect IFNλ we took advantage of the fact that IFNβ cannot signal across species while IFNλs do (Figure 2b). Therefore human 293T-ISRE cells can be used to specifically detect mouse type III IFNs, without detecting type I. These bioassays are simple and inexpensive, and are very efficient at detecting specific IFN species.

Conclusions
Recent studies have revealed that the transcriptional regulation of type I and III IFNs are fundamentally different, and that the cellular signaling pathways that drive expression of each subtype can also differ. But perhaps the most fundamental difference between the two systems is their physiological functions in vivo, with type III IFNs being the main drivers of antiviral immunity at mucosal surfaces. This tissue-restricted function of type III IFNs has implications in IFN-mediated therapies. PEGylated IFNλ has long been used to treat chronic viral infections such as Hepatitis B/C, which infects...
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![Image of figure 2](www.sciencedirect.com)

Bioassays to specifically detect IFNβ and IFNλ in mouse cells. L929 cells do not respond to mouse IFNλ2 but enable the detection of increasing concentrations of mouse IFNβ (a). As IFNλs are able to signal across species but IFNβ is not, human 293T cells are able to detect mouse IFNλ2 but not IFNβ. Units are in ng/ml (IFNλ2) or in units/ml (IFNβ).


In this paper, the type III IFN receptor is defined as being composed of IFN28Rα and IL10Rβ, and its ligands are shown to be IFNLs1-3, also known as IL29, IL28A and IL28B. Refs. [1**, 2**, 6] were published back to back and are the first reports of type III IFNs.


In this paper IL29, IL28A and IL28B are shown to be induced in response to viral infections, and to exhibit similar activities as type I IFNs. The IFNλ receptor is shown to be a dimer of IL10Rβ and IL28Rx.


**References and recommended reading**

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest


Type III interferon regulation Odendall and Kagan


The IFN-λR is primarily expressed in epithelia, and the IFNλ system is shown to be primarily important for antiviral defenses at mucosal surfaces.

12. Dumoutier L, Lejeune D, Hor S, Fickenscher H, Renaud J-C:

This paper, published shortly after Ref.11,9,16*9.8 studies the IFN-λR without identifying IFNλs as being its ligand. Chimeras of the IL28Rx (called LICIR2 in this study) cytoplasmic domain and the IL10R ecto domain were used to study signaling by the IL28Rx.


This study describes the differential regulation of type I and III IFNs by the Rig-I/MAVS pathway. Jak2 is shown to be activated by type III but not type I IFN signaling, while IRF1 regulates type III but not type I IFN expression. MAVS localized on peroxisomes is shown to only induce type III IFNs.


IL28A is shown to induce JAK2 phosphorylation.


Mice deficient for type I (IFNAR) or the IFNλR are compared for their ability to resist rotavirus infection. IFNAR but not IFNλN mice are shown to be deficient in ISG expression, control of virus replication in epithelia and limitation of intestinal epithelial cell damage.


This paper shows that type III IFNs are produced by most cells in response to TLIR ligands and viruses, but that only plasmacytoid dendritic cells and epithelial cells are able to respond to IFNλ. This study also describes IL28Rx KO mice.


