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# Why Is IFN-λ Less Inflammatory? One IRF Decides

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**Type I and type III interferons (IFNs) activate similar antiviral transcriptional programs, but the type I IFN response is more inflammatory. In this issue of *Immunity*, Forero et al. find that selective induction of the transcription factor IRF1 promotes proinflammatory chemokine expression downstream of type I IFN signaling.**

Type I interferons (IFN-α, IFN-β) and type III interferons (IFN-λ) are antiviral cytokines that activate similar signaling pathways and induce overlapping transcriptional responses but signal through distinct receptors (Lazear et al., 2019; Ye et al., 2019). Although the set of IFN-stimulated genes (ISGs) induced by type I and type III IFNs is largely similar, spatiotemporal differences in these responses lead to distinct physiological functions for the two IFN subtypes. Overall, the type III IFN response has lower potency and slower kinetics compared to the type I IFN response, and the type III IFN response predominates at epithelial surfaces, such as the respiratory and gastrointestinal tracts, supporting a model in which the type III IFN response provides front-line protection at sites in frequent contact with commensal and pathogenic microbes. Importantly, type III IFN signaling activates an antiviral response without the inflammatory pathology elicited by type I IFN signaling (Broggi et al., 2017; Galani et al., 2017). However, given the similarities in the signaling cascades and transcriptional programs activated downstream of type I and type III IFN signaling, the mechanisms underlying their distinct kinetics and immunoregulatory effects have remained unclear. In this issue of *Immunity*, Forero et al. (2019) show that, despite significant overall similarity in their transcriptional responses, induction of the transcription factor IRF1 distinguishes the type I and type III IFN responses, leading to the production of proinflammatory chemokines and leukocyte recruitment specifically in response to type I IFN signaling.

To begin this work, Forero and colleagues showed that in cells treated with recombinant IFN-β or IFN-λ, the transcriptional and antiviral response induced by type III IFNs was less potent and had slower kinetics compared to the type I IFN response, as expected from prior studies. However, they noted that although the set of ISGs induced by IFN-β and IFN-λ was largely identical, a notable exception was the chemokines CXCL9, CXCL10, and CXCL11, which were induced exclusively by IFN-β, but not IFN-λ. These chemokines recruit CXCR3-expressing leukocytes, including T cells, natural killer (NK) cells, and inflammatory monocytes, so avoiding their induction could explain the minimal inflammatory response elicited by type III IFNs. Indeed, when the authors administered IFN-β or IFN-λ to mice intranasally, only IFN-β induced *Cxcl10* expression and leukocyte recruitment in the lungs. Altogether, these observations support prior studies showing that IFN-λ is less inflammatory than IFN-β, but which signaling pathways account for this distinct response?

CXCR3 ligands can be induced downstream of IFN signaling following binding of IFN-regulatory factor (IRF) transcription factors to promoters containing IFN-stimulated response elements (ISREs). Thus, Forero and colleagues next examined IRF induction following treatment with recombinant IFN-β and IFN-λ in PH5CH8 human hepatocytes. As expected, IRF7 and IRF9 levels increased following treatment with either IFN. In contrast, IRF1 was induced exclusively by IFN-β treatment, not IFN-λ. The authors showed a similar effect in A549 human airway epithelial cells, demonstrating that this effect is not specific to hepatocytes. Since IRF1 induces the expression of antiviral and inflammatory genes, including *CXCL10* and other proinflammatory chemokines, a lack of IRF1 induction provides a plausible mechanism for the diminished inflammatory response induced by IFN-λ compared to IFN-β. Another recent study showed that basal IRF1 expression contributes to an intrinsic antiviral response in hepatocytes (Yamane et al., 2019), suggesting that higher levels of IRF1 expression, such as that induced following viral infection and IFN-β signaling, are needed to stimulate leukocyte recruitment and inflammation.

Since the canonical signaling pathways activated by type I and type III IFNs are identical (Figure 1), what allows IRF1 to be induced selectively in response to type I IFNs? Using RNA interference and gene deletion approaches, the authors found that STAT1 and STAT2 are both required for IRF1 induction following IFN-β treatment. However, only STAT1 homodimers bound the *IRF1* promoter, leading the authors to conclude that STAT2 deficiency diminished STAT1 activation, thereby transiently diminishing IRF1 induction. RNA-seq analyses of IRF1-deficient and wild-type cells following IFN-β treatment revealed that IRF1 is required for induction of *CXCL10*, *CIITA*, and *TNFSF10*, as well as genes involved in coagulation and tissue repair. Furthermore, expression of the IFNAR inhibitor *USP18* required IRF1 while IRF1-deficient cells exhibited a sustained antiviral response, suggesting a role for IRF1 in negative regulation of the type I IFN response. Altogether, these findings suggest a mechanism by which type III IFNs can induce a protective antiviral response without eliciting damaging inflammation.

The ability of IFN-β to induce specific ISGs via STAT1 homodimers that are not produced following IFN-λ signaling highlights the contribution of non-canonical signaling pathways to the overall IFN response (Figure 1). While the canonical pathway of JAK1- and TYK2-mediated

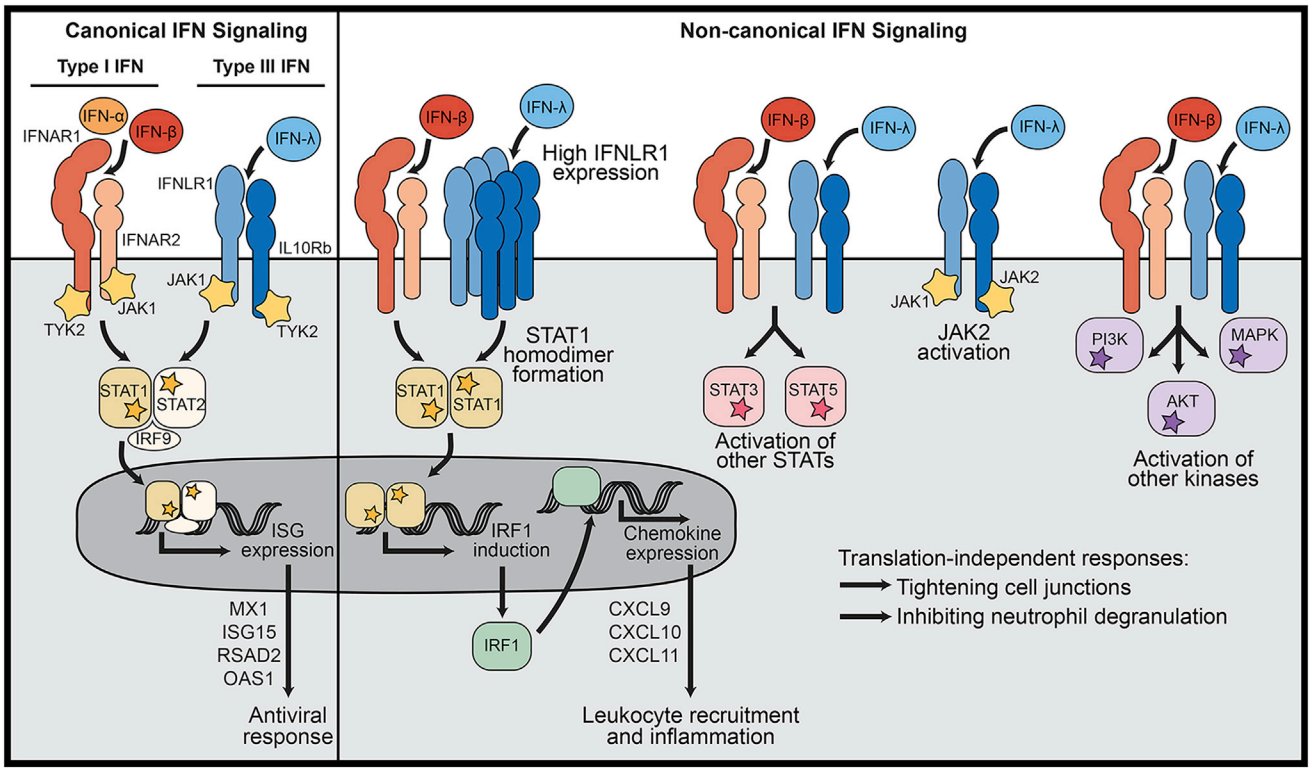


**Figure 1. Type I and Type III IFN Signaling Pathways**

Type I interferons (IFN-α, IFN-β) bind to a heterodimeric receptor comprised of IFNAR1 and IFNAR2 whereas type III interferons (IFN-λ) bind to a distinct receptor comprised of IFNLR1 and IL10Rb. In canonical IFN signaling, IFN binding activates receptor-associated kinases JAK1 and TYK2, which phosphorylate STAT1 and STAT2. Phosphorylated STAT1 and STAT2 associate with IRF9 to form a transcription factor that activates the expression of hundreds of IFN-stimulated genes (ISGs). ISGs act by a variety of mechanisms to inhibit viral replication and exert immunomodulatory functions. In addition to the canonical pathway, type I and type III IFNs can activate additional signaling pathways. Type I IFN can induce STAT1 homodimer formation, which leads to the induction of the transcription factor IRF1, expression of chemokines, and inflammation. Forero and colleagues showed that type III IFN does not induce IRF1, which may explain the diminished inflammatory response elicited by type III IFN. However, elevated IFNLR1 expression enabled IFN-λ-mediated IRF1 induction. Type I and type III IFNs also can activate additional STATs, including STAT3 and STAT5. Type III IFN can activate the kinase JAK2 instead of TYK2. Both type I and type III IFNs also activate other kinase signaling pathways, including PI3K, AKT, and MAPK. While the canonical signaling pathway requires the production of ISG proteins to mediate the antiviral response, other protective effects of IFN signaling are translation independent, including tightening cell junctions and inhibiting neutrophil degranulation. Since the canonical signaling pathway is shared between type I and type III IFNs, the relative contributions of non-canonical signaling pathways (particularly in a cell-type-dependent manner) may underlie biological differences in type I versus type III IFN responses.

activation of STAT1/STAT2 heterodimers likely drives the majority of ISG expression, it has long been recognized that IFN signaling also activates additional signaling pathways beyond those typically depicted in signaling pathway diagrams; differences in these non-canonical pathways may contribute to the distinct biological effects of type I and type III IFN (Lazear et al., 2019; Platanias, 2005; Ye et al., 2019). For example, IFN-λ can signal via JAK2, rather than TYK2, but IFN-β does not (Broggi et al., 2017; Ye et al., 2019). Other non-canonical pathways include activation of other STATs (STAT1 homodimers, STAT3, STAT5) as well as MAPK, AKT, and PI3K signaling pathways. Studies in intestinal organoid cultures found that the antiviral response induced by IFN-λ, but not IFN-β, was blocked by MAPK inhibitors, suggesting differential effects of STAT-independent signaling pathways (Lazear et al., 2019; Ye et al., 2019). Indeed, Forero and colleagues observed an IFN-λ-specific activation of the MAPK MKNK1, as well as the tyrosine kinase MERTK. Other STAT-independent IFN activities include inhibition of neutrophil degranulation and tightening of cell-cell junctions in blood-brain barrier endothelial cells, both of which occur in a translation-independent manner (Broggi et al., 2017; Lazear et al., 2015). Although the canonical type I and type III IFN signaling pathways are identical, their relative activation of non-canonical signaling pathways may represent a mechanism for exerting distinct physiologic effects. This could be especially pronounced in particular cell types or disease states where the relative contributions of non-canonical signaling pathways may vary.

A key distinction between the type I and type III IFN responses is the distribution of receptor expression: IFNLR1 is expressed preferentially on epithelial cells and some leukocytes (such as neutrophils), whereas IFNAR1 and IFNAR2 are ubiquitously expressed (Ye et al., 2019). Thus, IFNLR1 availability is a key determinant of the type III IFN response. Since the type III IFN response is less potent than that of type I IFNs, Forero and colleagues asked if IFNLR1 overexpression could enable IRF1 induction by IFN-λ. Indeed, overexpressing IFNLR1 enabled IFN-λ-mediated IRF1 induction and STAT1

activation equivalent to the levels achieved following IFN-β treatment. Accordingly, *CXCL10* was induced in IFNLR-overexpressing cells, and expression of ISGs such as *ISG15* and *MX1* also increased. These findings indicate that the reduced potency and lack of proinflammatory chemokine induction associated with the type III IFN response are threshold dependent and can be overcome by elevated receptor expression. It will be interesting to determine whether there are specific cells or tissues in which endogenous IFNLR1 expression reaches the threshold required for IFN-λ-mediated induction of IRF1 and induction of inflammatory chemokines. Notably, the authors found that in intestinal organoids, which are especially responsive to type III IFN, IFN-λ treatment did induce IRF1 expression, albeit at lower levels than that induced by IFN-β. In addition to cell-type-specific effects, there may be genetic, autoimmune, or infectious disease states that feature elevated IFNLR1 expression, which could potentiate IRF1 induction and an inflammatory response. However, the blunted inflammatory response characteristic of type III IFNs is best described in the lungs and gut, which are tissues with high IFNLR1 expression, suggesting that this model is relevant at physiological levels of IFNLR1. Forero and colleagues found no increase in *IFNLR1* expression following treatment with poly(I:C) or TNFα or after infection with Sendai virus or influenza A virus, implying that expression of IFNLR1 is not generally increased in response to infection. However, other studies have shown increased expression of IFNLR1 on neutrophils following fungal infection (Espinosa et al., 2017) or on bone-marrow-derived dendritic cells following influenza A virus infection (Hemann et al., 2019), suggesting that under some circumstances increased IFNLR1 expression could potentiate IRF1 induction and inflammatory responses.

Altogether, the study by Forero and colleagues provides mechanistic insight into the observation that the antiviral response induced by type III IFNs is less inflammatory than the type I IFN response, which may inform strategies to target these pathways therapeutically. Since limited IFNLR1 expression underpinned the lack of IRF1-mediated chemokine induction by IFN-λ, it will be important to determine under what circumstances IFNLR1 expression may exceed the threshold required for IRF1 induction. Furthermore, the observation that IFN-β, but not IFN-λ, led to the production of STAT1 homodimers, leading to IRF1 induction, highlights the importance of non-canonical IFN signaling pathways and suggests that such off-the-diagram pathways may play an important role in the distinct immune responses elicited by type I versus type III IFNs.

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