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Immunity
Review

Interferon-λ: Immune Functions at Barrier Surfaces and Beyond

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When type III interferon (IFN-λ; also known as interleukin-28 [IL-28] and IL-29) was discovered in 2003, its antiviral function was expected to be analogous to that of type I IFNs (IFN-α and IFN-β) via the induction of IFN-stimulated genes (ISGs). Although IFN-λ stimulates expression of antiviral ISGs preferentially in cells of epithelial origin, recent studies have defined additional antiviral mechanisms in other cell types and tissues. Viral infection models using mice lacking IFN-λ signaling and SNP associations with human disease have expanded our understanding of the contribution of IFN-λ to the antiviral response at anatomic barriers and the immune response beyond these barriers. In this review, we highlight recent insights into IFN-λ functions, including its ability to restrict virus spread into the brain and to clear chronic viral infections in the gastrointestinal tract. We also discuss how IFN-λ modulates innate and adaptive immunity, autoimmunity, and tumor progression and its possible therapeutic applications in human disease.

Introduction

Interferon-λ (IFN-λ), also termed type III IFN or interleukin-28 (IL-28) and IL-29, belongs to a cytokine family that shares functional similarities with the family of type I IFNs (IFN-α and/or IFN-β [hereafter IFN-α/β]). IFN-λ and IFN-α/β are multi-gene families composed of closely related cytokines each with specific heterodimeric receptors: IFNLR (IFNLR1 and IL10Rβ) for IFN-λ and IFNAR (IFNAR1 and IFNAR2) for IFN-α/β. Humans have genes encoding four IFN-λ proteins (IFN-λ1 [IL-29], IFN-λ2 [IL-28A], IFN-λ3 [IL-28B], and IFN-λ4) as well as 17 IFN-α/β proteins (13 IFN-α subtypes, IFN-β, IFN-ω, IFN-ε, and IFN-κ). In contrast to the IFN-α/β family, which was described almost 60 years ago (Isaacs and Lindenmann, 1957), the IFN-λ family was discovered more recently. Human IFN-λ1, IFN-λ2, and IFN-λ3 were identified in 2003 (Kotenko et al., 2003; Sheppard et al., 2003), and *IFNL4* at that time was considered a pseudogene. In 2013, it became clear that many humans have a functional *IFNL4* and that a common SNP results in a frameshift mutation that ablates IFN-λ4 production in some populations (Hamming et al., 2013; Prokunina-Olsson et al., 2013). Genes encoding type I IFNs characteristically lack introns (with the exception of *IFNK*) and are syntenic in a single locus on human chromosome 9 and mouse chromosome 4. Genes encoding IFN-λ are located on human chromosome 19 and mouse chromosome 7 and share the 5-exon gene structure characteristic of IL-10 cytokine family members (Sabat, 2010).

As discussed herein and in other recent reviews (Donnelly and Kotenko, 2010; Durbin et al., 2013; Egli et al., 2014c; Griffiths et al., 2015; Hermant and Michiels, 2014; Hoffmann et al., 2015; Koch and Finotto, 2015; Kotenko, 2011; O'Brien et al., 2014; Odendall and Kagan, 2015), several aspects of IFN-λ biology differ from IFN-α/β biology. First, the effects of IFN-λ are most evident in epithelial cells, suggesting that it contributes to the specialized immune mechanisms that protect epithelial surfaces, which undergo constant exposure to commensal and pathogenic microbes (Durbin et al., 2013; Hermant and Michiels, 2014; Mahlakõiv et al., 2015). Second, because of the more focused nature of its signaling effects, IFN-λ might share the therapeutic benefits yet avoid many of the side effects that have limited the clinical use of IFN-α/β (Donnelly et al., 2011; Hermant and Michiels, 2014; Markowitz, 2007; Pagliaccetti and Robek, 2010; Pestka, 2007). Third, genome-wide association studies (GWASs) have revealed multiple *IFNL* polymorphisms that are linked to clearance of hepatitis C virus (HCV) infection and possibly improved outcomes with other viral infections, including hepatitis B virus (HBV), human cytomegalovirus (HCMV), and herpes simplex virus 1 (HSV-1) (Egli et al., 2014c; Galmozzi et al., 2014; Griffiths et al., 2015; Lampertico et al., 2013; Manuel et al., 2015).

In this review, we discuss new insights into the antiviral functions of IFN-λ, especially those distinguishing it from IFN-α/β. We also describe how IFN-λ modulates adaptive immunity, autoimmunity, and tumor progression, as well as possible therapeutic applications in human disease. We highlight a specialized role for IFN-λ in providing antiviral activity at anatomic barriers, including epithelial surfaces and the blood-brain barrier (BBB). Because epithelial surfaces experience constant microbial exposure, IFN-λ might provide more targeted antiviral protection at key barrier sites without activating a systemic pro-inflammatory immune response.

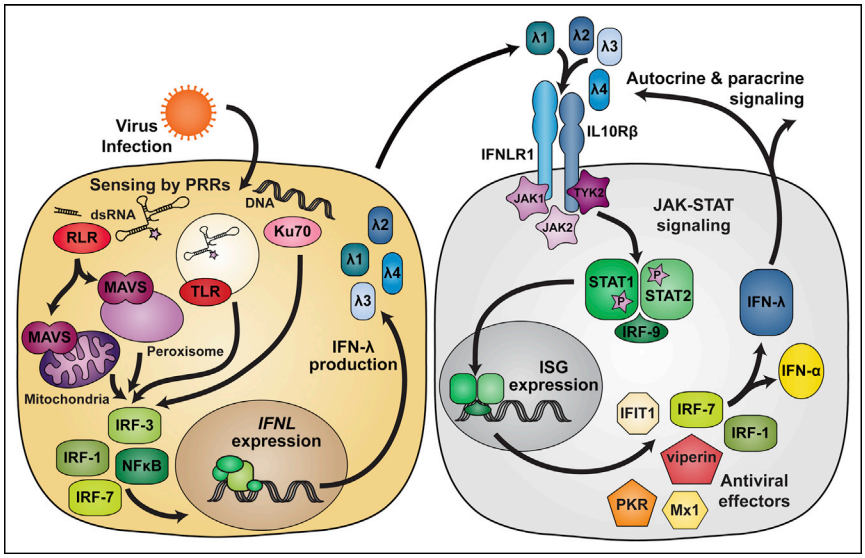

Figure 1. IFN-λ Induction and Signaling Pathways

IFN-λ production is induced when viral infection is sensed by pattern recognition receptors (PRRs), including members of the RIG-I-like receptor (RLR) and Toll-like receptor (TLR) families, as well as the DNA sensor Ku70. Whereas IFN-λ is induced via many of the same signaling pathways that induce IFN-α/β, Ku70 and peroxisome-localized MAVS preferentially induce IFN-λ. IFN-λ signals through its heterodimeric receptor, IFNLR, which is composed of IFNLR1 and IL10Rβ subunits. Canonical signaling through IFNLR activates JAK1 and TYK2 kinases, which phosphorylate STAT1 and STAT2. However, IFNLR signaling also can activate JAK2 and other downstream signaling pathways (not depicted). JAK-STAT signaling induces expression of IFN-stimulated genes (ISGs) and the production of effector molecules that inhibit viral infection. Among the ISGs induced by IFN-λ are *IRF1* and *IRF7*, encoding transcription factors IRF-1 and IRF-7, respectively, which amplify IFN production.

Induction of IFN-λ: Mechanisms Shared with and Distinct from Those of IFN-α/β

The stimuli that induce expression of IFN-λ-encoding genes, including a range of viruses, are similar to those inducing expression of genes encoding IFN-α/β (Ank et al., 2008; Ank et al., 2006; Durbin et al., 2013; Kotenko et al., 2003; Sheppard et al., 2003). Nonetheless, there are differences in transcription factor requirements between IFN-α/β and IFN-λ. Given that some of these differences have been reviewed previously (Durbin et al., 2013; Iversen and Paludan, 2010), we highlight recent advances in our understanding of transcriptional regulation of IFN-λ-encoding genes.

IFN expression occurs after host detection of pathogen-associated molecular patterns by specific pattern-recognition receptors (PRRs) (Swiecki and Colonna, 2011; Figure 1). Although most PRRs responsible for inducing expression of IFN-λ overlap those triggering IFN-α/β expression, the cytosolic DNA sensor Ku70 activates the expression of IFN-λ but not IFN-α/β (Zhang et al., 2011). Transcription factors activated downstream of PRR signaling include interferon regulatory factors (IRFs) and NF-κB. The suite of PRRs and transcription factors expressed by a cell contributes to the specific capabilities and magnitude of the IFN response following infection (Hillyer et al., 2012). For example, IFN-β is induced early after activation of PRRs because its promoter is bound by IRF-3, which is constitutively expressed, whereas most IFN-α subtypes are induced preferentially by IRF-7, which is itself encoded by an IFN-stimulated gene (ISG) (Génin et al., 2009). Initial characterization of promoter regions upstream of *IFNL1* and *IFNL3* identified binding elements for IRF-1, IRF-3, IRF-7, and NF-κB, and the combined activity of IRFs and NF-κB was required for maximal gene induction (Onoguchi et al., 2007; Osterlund et al., 2007; Thomson et al., 2009). Subsequent analysis of the response to TLR9 agonists showed that IFN-λ induction exhibits a greater dependence on NF-κB than does IFN-α/β induction (Iversen et al., 2010). These findings suggest that, despite similarities, there are promoter features that discriminate *IFNL* and *IFNA/B* induction.

Additional factors distinguish the regulation of *IFNL* and *IFNA/B* induction. Med23, a component of the Mediator complex, interacts with IRF-7 and enhances transcription from the *IFNL1* promoter, but not the *IFNB* promoter; the resulting increase in IFN-λ production inhibits HSV-1 replication (Griffiths et al., 2013). A bioinformatic and biochemical analysis of the region upstream of human *IFNL1* identified binding sites for the transcription factors ZEB1 and BLIMP-1. Experiments using chromatin immunoprecipitation and gene silencing established that ZEB1 and BLIMP-1 bind the *IFNL1* promoter and repress transcription in airway and intestinal epithelial cell lines (Siegel et al., 2011; Swider et al., 2014). ZEB1 activity is specific to *IFNL1* and does not regulate *IFNB* expression. BLIMP-1 functions as a repressor by displacing binding of IRF-1 (Siegel et al., 2011), which is required for *IFNL1*, but not *IFNB*, transcription (Odendall et al., 2014). Although both IFN-λ and IFN-β are induced downstream of PRR sensing and activation of mitochondrial antiviral signaling protein (MAVS), IFN-λ production is favored when activated MAVS localizes to the peroxisome, whereas IFN-β production is dominant when MAVS localizes to the mitochondria (Odendall et al., 2014). The relative abundance of peroxisomes in epithelial cells suggests a mechanism for preferential production of IFN-λ instead of IFN-β in response to viral infection at epithelial surfaces. In hepatocytes, HBV infection stimulated production of IFN-λ but not IFN-α/β, and this IFN-λ induction depended on recognition by RIG-I and signaling through MAVS (Sato et al., 2015). Similarly, HCV infection preferentially induced IFN-λ over IFN-α/β (Marukian et al., 2011; Park et al., 2012; Thomas et al., 2012), suggesting that liver-specific factors might promote IFN-λ production in response to these unrelated hepatotropic viruses.

Although epithelial cells produce IFN-λ, myeloid-lineage cells are major sources in response to double-stranded RNA (poly I:C) or viral infections (Lauterbach et al., 2010). In the small intestine, epithelial cells and immune cells both respond to poly I:C stimulation, suggesting that multiple cell types produce IFN-λ cooperatively (Mahlakõiv et al., 2015). Although gut immune cells treated with poly I:C produce IFN-α5, IFN-β, IFN-λ2, and IFN-λ3,

gut epithelial cells exclusively generate IFN-λ2 and IFN-λ3 (Mahlakõiv et al., 2015), perhaps reflecting their peroxisome content (Odendall et al., 2014). There also are differences in the IFN induction profile within the myeloid cell compartment after stimulation with PRR agonists. Whereas plasmacytoid dendritic cells (DCs) produce nearly all IFNs (including IFN-λ), monocytes and myeloid DCs more selectively express IFN-β, IFN-λ1, and IFN-λ2 (Hillyer et al., 2012). Similarly, CD8α$^+$ DCs are the major IFN-λ-producing myeloid cell type in response to poly I:C in mice (Lauterbach et al., 2010).

IFN-λ Signaling

The proximal signaling events and downstream transcriptional responses are similar between IFN-α/β and IFN-λ, even though the cytokines and their receptors are structurally and genetically distinct. The structure of IFN-λ resembles that of members of the IL-10 family, although the primary amino acid sequence is more similar to that of IFN-α/β (Gad et al., 2009; Miknis et al., 2010). Whereas all type I IFNs signal through a shared heterodimeric receptor, IFNAR (IFNAR1 and IFNAR2), type III IFNs bind to IFNLR, a unique heterodimeric receptor. IFNLR consists of one subunit that it shares with other IL-10 family cytokines (IL10Rβ) and a second that is specific to IFN-λ (IFNLR1, also called IL28Rα). Despite the structural similarities between IFN-λ and IL-10 family cytokines (i.e., IL-10, IL-19, IL-20, IL-22, IL-24, and IL-26), the crystal structure of IFN-λ1 bound to IFNLR1 revealed a distinct receptor-binding interaction, including a 1:1 stoichiometry between IFN-λ1 and IFNLR1 (compared to 2:1 between IL-10 and IL10Rα) (Miknis et al., 2010). The impact of the shared IL10Rβ chain on interactions between IFN-λ and other IL-10 family cytokines remains unclear. IFN-λ activity was enhanced by an IL-10-blocking antibody and was inhibited in the presence of IL-10 (Jordan et al., 2007a), suggesting possible competition for IL10Rβ interaction. In contrast, IL-22 (which signals through IL10Rβ and IL22Rα) enhanced the signaling and antiviral effects of IFN-λ (Hernández et al., 2015). Although IFNAR and IL10Rβ are expressed broadly on many cell types and tissues, IFNLR1 is expressed preferentially on epithelial cells (Mahlakõiv et al., 2015; Sommereyns et al., 2008). Consistent with this pattern, the antiviral effects of IFN-λ are most evident against pathogens targeting epithelial tissues.

Despite engaging different heterodimeric receptors, the post-receptor signaling events after IFN-α/β and IFN-λ binding exhibit remarkable overlap. These signaling pathways have been reviewed in detail elsewhere (Donnelly and Kotenko, 2010; Durbin et al., 2013; Hoffmann et al., 2015; Kotenko, 2011) and include activation of JAK-family kinases, phosphorylation of STAT1 and STAT2, and association between activated STAT complexes and IRF-9 to form ISGF3, which translocates to the nucleus and induces expression of hundreds of ISGs. Similar to IFN-α/β signaling, IFN-λ signaling induces JAK1 and TYK2 phosphorylation (Dumoutier et al., 2004; François-Newton et al., 2011; Ma et al., 2009). Additionally, JAK2 phosphorylation is induced specifically by IFN-λ (Odendall et al., 2014; Odendall and Kagan, 2015), suggesting that distinct upstream signaling events might differentiate IFN-λ activity from IFN-α/β activity. In addition to activating STAT1 and STAT2, IFNAR and IFNLR ligand engagement can activate other STAT family members (STAT3, STAT4, and STAT5) and STAT-independent signaling cascades (MAPK and ERK) (Cohen and Prince, 2013; Koch and Finotto, 2015).

The transcriptional responses induced by IFN-λ and IFN-α/β are similar (Bolen et al., 2014; Doyle et al., 2006; Kohli et al., 2012; Lazear et al., 2015; Marcello et al., 2006; Shindo et al., 2013; Zhou et al., 2007). No transcriptional signatures unique to IFN-λ have been identified, and the genes induced by IFN-λ typically represent a subset of the total induced by IFN-α/β. The IFN-λ transcriptional response generally is of lower magnitude than that of IFN-α/β and might exhibit a delayed peak and longer duration (Marcello et al., 2006), although different expression levels of IFNAR and IFNLR might confound comparisons. Differential effects of negative regulators might contribute to the sustained signaling pattern observed for IFN-λ in comparison to that of IFN-α. For example, the ISG *USP18* desensitizes cells to further IFN-α stimulation but does not inhibit IFN-λ signaling (François-Newton et al., 2011). Among the IFN-λ subtypes, IFN-λ3 (followed by IFN-λ1 and IFN-λ2) has the most potent bioactivity (Bolen et al., 2014; Dellgren et al., 2009). These differences in potency are unexpected, given that IFN-λ2 and IFN-λ3 are nearly identical (96% amino acid identity) (Sheppard et al., 2003). IFN-α/β and IFN-λ signaling both amplify IFN production, for example, via the induction of the transcription factors IRF-1 and IRF-7 (Bolen et al., 2014; Kohli et al., 2012; Lazear et al., 2015). Because of these shared positive-feedback systems, transcriptional profiling in cells lacking IFNAR or IFNLR (or in the presence of protein-synthesis inhibitors) might be needed for distinguishing bona fide IFN-α/β- and IFN-λ-induced genes. Independent of whether there truly are IFN-λ-specific transcriptional signatures, the ability to induce a narrower set of ISGs in a more targeted set of cells suggests possible therapeutic applications in which IFN-λ could promote a more focused antiviral or immunomodulatory response with fewer side effects than IFN-α/β (Hermant and Michiels, 2014; Muir et al., 2014). Given that most IFN-λ transcriptional profiling experiments have focused on human liver cells, further studies are needed in other tissues where antiviral effects of IFN-λ have been reported.

Antiviral Effects of IFN-λ

IFN-λ exhibits antiviral activity against many viruses in vitro, but its in vivo activity has been more apparent for viruses that infect epithelial cells of the respiratory, gastrointestinal, and urogenital tracts, as well as the liver (Table 1). Because the antiviral effects of IFN-λ have been reviewed recently (Egli et al., 2014c; Hermant and Michiels, 2014; O'Brien et al., 2014; Sorgeloos et al., 2013), we will briefly describe the role of IFN-λ in the respiratory tract and liver and then highlight new phenotypes in the gastrointestinal tract, as well as at an unexpected site, the BBB.

IFN-λ in the Respiratory Tract

IFNLR is expressed at relatively high levels in respiratory epithelial cells, and mice treated with IFN-λ prior to infection with human metapneumovirus (HMPV) develop lower viral titers and reduced inflammatory responses (Baños-Lara et al., 2015). Accordingly, *Ifnlr1*$^{-/-}$ mice exhibit increased susceptibility to respiratory viral infections, including influenza virus, HMPV, respiratory syncytial virus, and SARS coronavirus (Crotta et al., 2013; Hermant and Michiels, 2014; Mahlakõiv et al., 2012; Mordstein et al., 2008; Mordstein et al., 2010b). Human myeloid and bronchial epithelial cells produce IFN-λ in response to rhinovirus

Table 1. Antiviral Effects of IFN-λ In Vivo

Virus	Phenotype	Reference
Mouse Models		
West Nile virus	more permeable BBB and increased neuroinvasion in *Ifnlr1*$^{-/-}$ mice; treatment with recombinant IFN-λ prevents lethality in wild-type mice	Lazear et al., 2015
Norovirus	increased titers and shedding in *Ifnlr1*$^{-/-}$ mice; treatment with recombinant IFN-λ prevents infection and cures persistent infection	Nice et al., 2015
Reovirus	increased growth in intestinal epithelial cells and increased viral shedding in *Ifnlr1*$^{-/-}$ mice; increased fatal liver disease in suckling *Ifnlr1*$^{-/-}$ mice	Mahlakõiv et al., 2015
Rotavirus	increased titers in *Ifnlr1*$^{-/-}$ mice; treatment with recombinant IFN-λ reduces viral titers; IL-22 acts synergistically with IFN-λ to control infection	Hernández et al., 2015; Pott et al., 2011
Influenza virus	increased titers in *Ifnlr1*$^{-/-}$ or *Ifnlr1*$^{-/-}$ × *Ifnar1*$^{-/-}$ mice; treatment with recombinant IFN-λ prevents lethality in wild-type mice	Crotta et al., 2013; Mordstein et al., 2008; Mordstein et al., 2010b
SARS coronavirus	increased titers and shedding in *Ifnlr1*$^{-/-}$ mice	Mahlakõiv et al., 2012; Mordstein et al., 2010b
Human metapneumovirus	increased titers in *Ifnlr1*$^{-/-}$ × *Ifnar1*$^{-/-}$ mice; treatment with recombinant IFN-λ reduces titers in wild-type mice	Baños-Lara et al., 2015; Mordstein et al., 2010b
Respiratory syncytial virus	increased titers in *Ifnlr1*$^{-/-}$ × *Ifnar1*$^{-/-}$ mice	Mordstein et al., 2010b
Herpes simplex virus 2	treatment with recombinant IFN-λ decreases viral titers and shedding	Ank et al., 2006
Lymphocytic choriomeningitis virus	no change in viral titers in *Ifnlr1*$^{-/-}$ mice; increased T cell response to acute infection and decreased T cell response to chronic infection in *Ifnlr1*$^{-/-}$ mice	Ank et al., 2008; Misumi and Whitmire, 2014
Human Patients		
Hepatitis C virus	SNPs rs8099917, rs12979860, rs4803217, and rs368234815 correlate with spontaneous clearance and sustained response to IFN therapy	Bibert et al., 2013; Ge et al., 2009; Griffiths et al., 2015; Hamming et al., 2013; Prokunina-Olsson et al., 2013; Suppiah et al., 2009; Tanaka et al., 2009; Thomas et al., 2009
Hepatitis B virus	SNP rs12979860 correlates with response to IFN therapy	Galmozzi et al., 2014
Human cytomegalovirus	SNP rs368234815 correlates with HCMV retinitis in AIDS patients; SNPs rs368234815 and rs8099917 correlate with HCMV reactivation in transplant recipients	Bibert et al., 2014; Egli et al., 2014a; Egli et al., 2014c; Griffiths et al., 2015; Manuel et al., 2015
Herpes simplex virus 1	SNP rs12979860 correlates with the severity of reactivation disease	Griffiths et al., 2013
Influenza virus	increased viral titers in epithelial cells with *IFNLR1* inhibition; SNP rs8099917 correlates with vaccine response in immunosuppressed patients	Egli et al., 2014b; Kim et al., 2013
Rhinovirus	increased production of IFN-λ correlates with reduced viral replication in bronchial epithelial cells	Contoli et al., 2006

infection, and IFN-λ inhibits rhinovirus replication in bronchial epithelial cells (Contoli et al., 2006). Correspondingly, production of IFN-λ correlates inversely with viral load and disease severity in humans experimentally inoculated with rhinovirus (Contoli et al., 2006). Respiratory epithelial cells respond to both IFN-λ and IFN-α/β (Mahlakõiv et al., 2015), and cultured cells respond to IFN-λ from both the apical and basolateral surfaces (Fox et al., 2015; Hamming et al., 2013). IFN-λ is the dominant IFN produced by respiratory epithelial cells after infection with influenza virus or other respiratory viruses (Crotta et al., 2013; Fox et al., 2015;

Jewell et al., 2010; Okabayashi et al., 2011). This implies a key role for IFN-λ in mediating antiviral immunity in the respiratory tract (Figure 2A).

IFN-λ in the Liver

There has been great interest in defining the antiviral role of IFN-λ in the liver, particularly against HCV and HBV infections. IFNLR is expressed by human hepatocytes, the primary cellular targets of these viruses (Figure 2B), and both viruses preferentially stimulate IFN-λ production rather than IFN-α/β production (Marukian et al., 2011; Park et al., 2012; Sato et al., 2015; Thomas et al., 2012). GWASs have revealed *IFNL* polymorphisms that are associated with improved outcome from HCV and HBV infection, both in terms of spontaneous clearance and in response to antiviral therapy (Galmozzi et al., 2014; Ge et al., 2009; Griffiths et al., 2015; Hermant and Michiels, 2014; Lampertico et al., 2013; O'Brien et al., 2014; Russell et al., 2014; Suppiah et al., 2009; Tanaka et al., 2009; Thomas et al., 2009). The different protective alleles identified in these studies imply distinct antiviral mechanisms against HCV. One protective allele in the promoter region of *IFNL3* is associated with increased IFN-λ production, which might control infection by enhancing expression of ISGs (for SNP rs8099917, the protective allele is T/T rather than T/G or G/G) (Suppiah et al., 2009; Tanaka et al., 2009). However, an unfavorable allele also is associated with elevated ISG expression and is thought to render cells refractory to further stimulation of antiviral activity (for SNP rs12979860, the protective allele is C/C rather than T/C or T/T) (Ge et al., 2009; Sheahan et al., 2014; Thomas et al., 2009; Urban et al., 2010). In addition to its association with viral hepatitis, rs12979860 predicts the severity of liver inflammation and fibrosis of other etiologies, but with the opposite association (C/C, unfavorable allele) (Eslam et al., 2015). This disparity suggests that ISG expression might differentially affect viral and non-viral liver disease. Another protective allele alters the sequence of the 3′ UTR of *IFNL3* such that its transcript is no longer targeted for degradation by microRNAs induced after HCV infection (for SNP rs4803217, the protective allele is G/G rather than T/T) (McFarland et al., 2014). The SNP that is most highly associated with improved HCV outcome is one in which the protective allele has a frameshift insertion that ablates IFN-λ4 production (for SNP rs368234815, the protective allele is TT rather than -G) (Bibert et al., 2013; Hamming et al., 2013; Key et al., 2014; O'Brien et al., 2014; Prokunina-Olsson et al., 2013). It remains unclear why the loss of IFN-λ4 confers improved clinical outcome, but several explanations have been proposed: (1) a counter-regulatory role for IFN-λ4, (2) an IFN refractory state induced by high basal expression of IFN-λ4, (3) stimulation of regulatory T cell responses by IFN-λ4, and/or (4) reduced production of the more antiviral IFN-λ3. Because many of the polymorphisms within the *IFNL* locus exist in high linkage disequilibrium, they often segregate as haplotypes. This has made it difficult to parse out the protective functions of individual SNPs. For example, many of the protective phenotypes initially attributed to rs12979860 (C/C allele) might in fact be caused by the loss of IFN-λ4 production as a result of the highly linked and more recently identified rs368234815 (TT allele).

There was significant interest in the therapeutic potential of IFN-λ for viral hepatitis (Donnelly et al., 2011; Hayes et al., 2012), at least before the introduction of direct-acting antiviral agents to treat HCV infection (Alexopoulou and Karayiannis, 2015). Conventional pegylated IFN-α therapy is accompanied by side effects that hinder compliance. Given the more restricted expression pattern of IFNLR, and the relatively high amount of IFNLR expression in hepatocytes, IFN-λ could confer many of the antiviral benefits of IFN-α in the liver with reduced side effects (Muir et al., 2014). Although direct-acting antiviral agents are supplanting IFN therapy for HCV infection, the therapeutic advantages of IFN-λ might still have utility against HBV, other viral infections, or other hepatic conditions.

Although human hepatocytes respond to IFN-λ, mouse hepatocytes are less responsive. Instead, cholangiocytes that line the bile duct reportedly are the principal cells that respond to IFN-λ in the mouse liver (Hermant et al., 2014; Mahlakõiv et al., 2015). This observation could explain the apparent lack of an antiviral activity for IFN-λ in mouse models of hepatotropic virus infection, despite its clear role in human hepatitis (Mordstein et al., 2008). Because of their IFN-λ responsiveness, cholangiocytes could restrict excretion of hepatotropic viruses into the bile and thus control viral spread in feces.

IFN-λ in the Gastrointestinal Tract

The gastrointestinal tract is a primary site of microbial exposure, and intestinal epithelial cells form a barrier to pathogenic viruses and bacteria (Figure 2C). Initial characterization of tissue *Ifnlr1* expression in mice demonstrated relatively high expression in the stomach and intestine (Sommereyns et al., 2008), and subsequent studies revealed that epithelial cells are the predominant IFN-λ-responsive cell type in the gastrointestinal tract (Mordstein et al., 2010b; Pott et al., 2011). IFN-λ and IFN-α/β responsiveness are compartmentalized within the mouse intestine. IFN-α/β has a minimal effect on intestinal epithelial cells, whereas IFN-λ has a minimal effect on cells of the lamina propria (Mahlakõiv et al., 2015; Pott et al., 2011). This differential response might be explained by the relatively high expression of IFNLR on intestinal epithelial cells (Mahlakõiv et al., 2015), as well as the low expression and apical trafficking of IFNAR on these cells (Pott et al., 2011). Although *IFNLR1* transcripts are expressed in the human gastrointestinal tract (Sheppard et al., 2003), it remains to be determined whether IFN-λ and IFN-α/β responsiveness are similarly compartmentalized.

The compartmentalized response to IFN-λ in the mouse gastrointestinal tract most likely explains its activity against different viral pathogens depending on their cellular tropism. Rotavirus infects epithelial cells (Lopez and Arias, 2006), reovirus replicates in both epithelial and non-epithelial cells (Forrest and Dermody, 2003), and norovirus grows preferentially in myeloid and B cells but not in epithelial cells (Jones et al., 2014; Karst et al., 2014; Wobus et al., 2004). Rotavirus infection is controlled exclusively by IFN-λ, and there is no defined role for IFN-α/β (Angel et al., 1999; Pott et al., 2011). Reovirus is controlled cooperatively by IFN-α/β and IFN-λ, such that IFN-λ limits replication in epithelial cells, and IFN-α/β restricts infection in non-epithelial cells (Mahlakõiv et al., 2015). Norovirus infection and dissemination are controlled by IFN-α/β and IFN-λ, such that IFN-α/β prevents extra-intestinal spread, and IFN-λ inhibits the persistent shedding of virus in feces (Nice et al., 2015; Nice et al., 2013). The role for IFN-λ in norovirus infection is apparent only for strains that are persistently shed across the intestinal epithelial barrier into the gut lumen and not for strains causing only acute

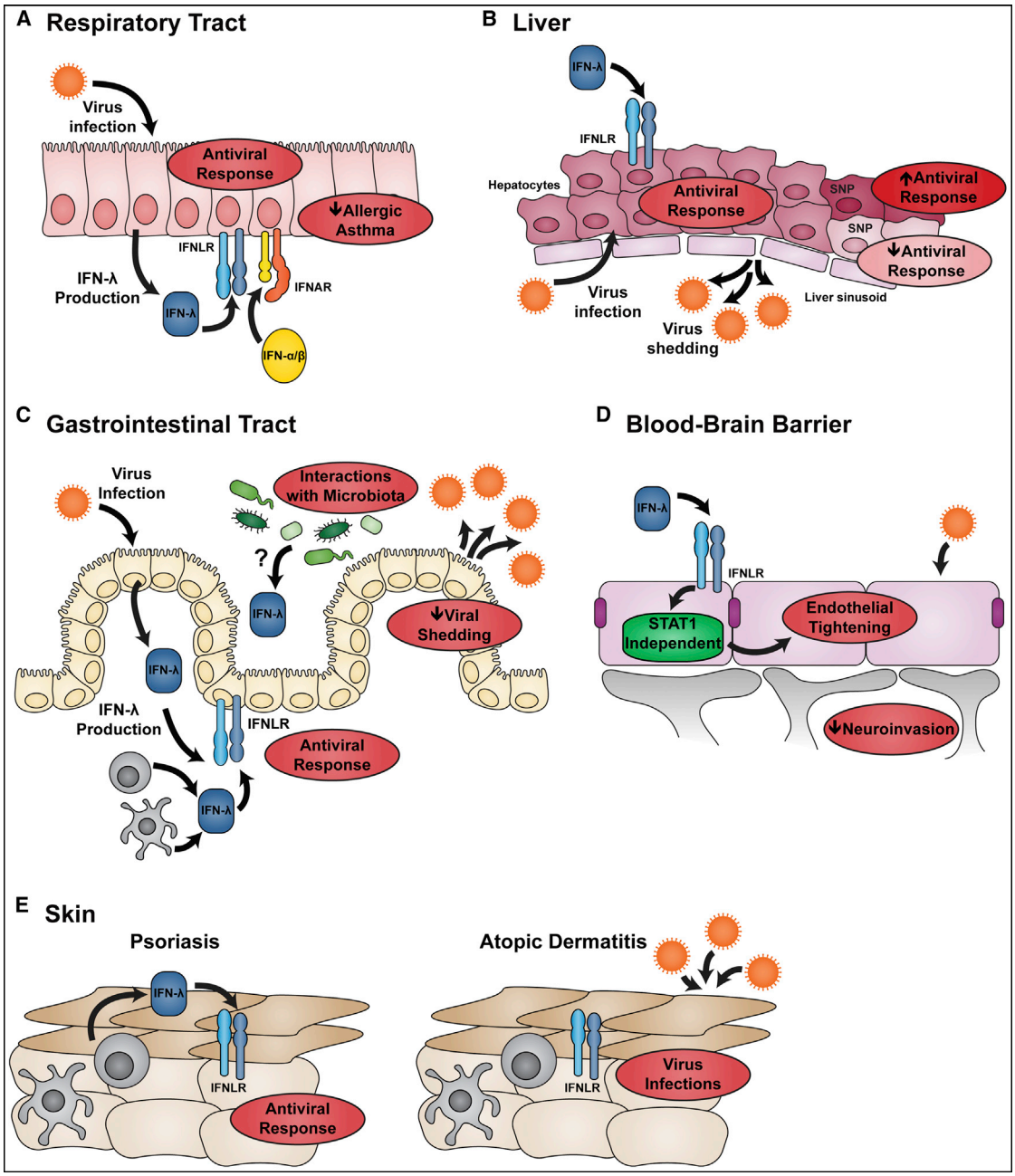

Figure 2. Antiviral Effects of IFN-λ at Barrier Surfaces

(A) IFN-λ is a dominant IFN produced after viral infection in the respiratory tract. Respiratory epithelial cells can respond to both IFN-λ and IFN-α/β to activate an antiviral response. Th1 skewing induced by IFN-λ reduces the severity of allergic asthma.

(B) The fenestrated endothelium of the liver creates a tissue architecture in which hepatocytes directly contact blood in liver sinusoids. Hepatocytes are the primary cellular targets for HBV and HCV and are highly responsive to IFN-λ. A role for IFN-λ in controlling HCV infection is suggested by the association between HCV clinical outcome and numerous SNPs within the *IFNL* locus.

(C) IFN-λ has a key role in gastrointestinal tract immunity because unlike respiratory epithelial cells, gut epithelial cells do not respond to IFN-α/β and therefore rely upon IFN-λ to activate an antiviral response. The antiviral effects of IFN-λ could be especially important for restricting the shedding and transmission of enteric viruses. Immunity in the gastrointestinal tract is shaped by the bacterial microbiome; the ability of gut microbes to promote viral persistence requires IFN-λ signaling, although the mechanism of this interaction remains unclear.

(D) IFN-λ signaling tightens the endothelial junctions of the BBB, which reduces viral neuroinvasion from the circulation. The tightening activity of IFN-λ is STAT1 independent, implicating a non-canonical signaling pathway.

(E) Psoriasis and atopic dermatitis are chronic inflammatory skin conditions characterized by breakdown of the epithelial barrier. Compared to lesions from atopic dermatitis patients, psoriasis lesions exhibit elevated IFN-λ production and enhanced expression of ISGs. This might explain why disseminated viral skin infections are common in patients with atopic dermatitis but not psoriasis.

infection. These studies with genetically and phenotypically unrelated gastrointestinal viruses reveal unique antiviral roles for IFN-α/β and IFN-λ. In particular, IFN-λ selectively prevents viral shedding into the lumen of the gastrointestinal tract and transmission of fecal-oral viral pathogens.

The trillions of bacteria juxtaposed to the intestinal epithelial barrier are a source of numerous microbial products that are sensed by the immune system. Thus, the IFN-mediated antiviral response in the gastrointestinal tract is modulated by the bacterial microbiome. Indeed, a recent study showed that the bacterial microbiome promotes persistent norovirus infection in an IFNLR-dependent manner (Baldridge et al., 2015). Fecal shedding of norovirus, but not viral entry into Peyer's patches or extra-intestinal spread, is reduced in mice treated with antibiotics that deplete the bacterial microbiome. This effect of antibiotics on norovirus shedding is diminished in *Ifnlr1*$^{-/-}$ mice, but not in *Ifnar1*$^{-/-}$, *Ifngr1*$^{-/-}$, *Tlr4*$^{-/-}$, *Myd88*$^{-/-}$, or *Trif*$^{-/-}$ mice, which lack other innate immune signaling components. The specific requirement for IFNLR in bacterial promotion of norovirus infection contrasts with that in poliovirus and mouse mammary tumor virus (MMTV) infections, where bacterial promotion of viral infection depends on bacterial lipopolysaccharide and TLR4, respectively (Kane et al., 2011; Kuss et al., 2011). However, an additional role of IFN-λ in poliovirus and MMTV infections remains possible because TLR4 signaling and bacterial infection induce IFN-λ (Bierne et al., 2012; Coccia et al., 2004; Contoli et al., 2006; Odendall et al., 2014). Further experiments are required to characterize the intestinal production of and response to IFN-λ in the presence and absence of enteric bacteria. Given the importance of IFN-λ to intestinal immunity in mice, studies to test for linkage between human IFN-λ SNPs and the control of enteric viral infections appear warranted. The studies in mice also suggest that IFN-λ might have therapeutic applications for humans with persistent norovirus infection and gastroenteritis (Bok and Green, 2012).

The ability of IFN-λ to confer antiviral protection at epithelial barriers is an interesting parallel to another IL-10 family cytokine, IL-22, which promotes antibacterial immunity at epithelial surfaces (Zheng et al., 2008). Similar to IFNLR1, IL22Rα associates with IL10Rβ and is expressed preferentially by epithelial cells (Wolk et al., 2004). A recent study demonstrated that IL-22 acts synergistically with IFN-λ to control rotavirus infection and prevent intestinal tissue damage in mice (Hernández et al., 2015). Further work is needed for determining whether IL-22 contributes to the antiviral activity of IFN-λ at other epithelial surfaces and against other viruses targeting the gastrointestinal tract.

IFN-λ and Chronic Viral Infection

Surprisingly, IFN-λ stimulates clearance of persistent norovirus infection independently of the adaptive immune system (Nice et al., 2015), suggesting that innate immune responses might have a greater role in determining viral persistence than previously appreciated. Because IFNLR is expressed on epithelial cells, IFN-λ could be particularly important for restricting shedding of chronic viral infections. Consistent with this idea, IFN-λ treatment decreases HSV-2 shedding from the vaginal mucosa in mice (Ank et al., 2006). In humans, SNPs in *IFNL3* and *IFNL4* have been associated with the ability to clear or control multiple chronic infections, including HCV, HBV, HCMV, and HSV-1 (Bibert et al., 2014; Egli et al., 2014a; Egli et al., 2014c; Griffiths et al., 2015; Griffiths et al., 2013; Lampertico et al., 2013; Manuel et al., 2015). Although the mechanisms by which these polymorphisms affect IFN-λ production and activity remain unclear, these associations suggest that IFN-λ contributes to the control of chronic viral infections in humans. Restricted IFNLR expression within the mucosal epithelium and other key cell types might allow targeted local antiviral responses that control chronic infection and shedding without generating a potentially deleterious systemic pro-inflammatory response. A greater mechanistic understanding of how human SNPs influence IFN-λ expression or function and the study of IFN-λ in additional models of chronic viral shedding are needed.

IFN-λ at the BBB

A recent study with West Nile virus (WNV) demonstrated an antiviral effect of IFN-λ at an unexpected barrier site: the BBB (Lazear et al., 2015). The BBB protects the CNS from harmful substances in the periphery, including viruses (Koyuncu et al., 2013). *Ifnlr1*$^{-/-}$ mice exhibited increased BBB permeability after WNV infection and sustained higher viral titers in CNS tissues. Although endothelial cells, including those composing the BBB, do not express high levels of IFNLR, administration of exogenous IFN-λ tightened the BBB, restricted viral neuroinvasion, reduced viral titers in the CNS, and protected mice from lethal viral infection (Figure 2D). These observations are consistent with those of a study demonstrating that IFN-α/β also can exert a tightening effect on the BBB (Daniels et al., 2014). IFN-λ tightening of endothelial monolayers in an in vitro model of the BBB did not require STAT1 or de novo protein synthesis, implying that the phenotype occurred via a non-canonical signaling pathway. Although further experiments are needed for determining the specific pathway by which it exerts its effects on BBB integrity, this study suggests that IFN-λ might have therapeutic activity for pathological conditions or viral infections that involve BBB breakdown or permeability.

IFN-λ Roles in Autoimmunity, Adaptive Immunity, and Anti-tumor Responses

The most direct consequence of IFN-λ signaling is the induction of ISGs, which can act in a cell-intrinsic manner to inhibit viral infection (Figure 1). However, IFN-λ has immunologic roles beyond the innate antiviral response. There is increasing evidence that IFN-λ shapes the adaptive immune response to viral infection, alters anti-tumor responses, and affects autoimmunity.

IFN-λ and Autoimmunity

Altered expression or activity of components of pathogen sensing and IFN-induction pathways is associated with autoimmunity. For example, alterations in the PRR MDA5, signaling molecule STING, or transcription factor IRF-5 can cause severe clinical syndromes known as "type I interferonopathies" (Crow, 2015; Rigby and Rehwinkel, 2015). As overlapping stimuli, sensors, and signaling mechanisms induce expression of type III IFNs, these same alterations might enhance IFN-λ production and contribute to these clinical phenotypes.

Given that it exerted a tightening effect on the BBB, IFN-λ could have a prominent barrier effect in epithelial tissues where IFNLR expression is higher. In the skin, IFNLR is expressed predominantly on keratinocytes (Witte et al., 2009). The barrier function of keratinocytes is important, as evidenced by the pathology

of psoriasis and atopic dermatitis, two chronic conditions characterized by epithelial cell hyperproliferation, impaired barrier function, and inflammation (Figure 2E) (Clark, 2013; Leung, 2013; Perera et al., 2012). Patients with atopic dermatitis commonly develop disseminated bacterial (e.g., *Staphylococcus aureus*) and viral (e.g., HSV, human papillomavirus, and molluscum contagiosum poxvirus) infections in the skin (Christophers and Henseler, 1987; Wollenberg et al., 2003). However, these disseminated infections are not characteristic of psoriasis. Skin lesions from psoriasis patients exhibit higher amounts of IFN-λ1 and enhanced expression of ISGs than do atopic dermatitis lesions, and this occurs in the absence of differential expression of IFN-α/β (Wolk et al., 2013). Although altered epithelial barrier function is a feature of both psoriasis and atopic dermatitis, elevated IFN-λ production and ISG expression provide antiviral protection only in the context of psoriasis.

The factors that cause elevated IFN-λ production in psoriasis are not understood but might result from the Th17-driven response in psoriatic lesions rather than a Th2-driven response in atopic dermatitis. Th17 cells are the main source of IFN-λ in psoriasis lesions, and IFN-λ inhibits the production of Th2 cytokines, particularly IL-13 (Srinivas et al., 2008; Wolk et al., 2013). A protective role for IFN-λ in psoriasis is also suggested by the linkage of a SNP in the 3′ UTR of *IFNLR1* to disease outcome (for SNP rs4649203, the protective allele is G rather than A), although a mechanistic basis for this association remains uncertain (Li et al., 2013; Strange et al., 2010).

A protective role for IFN-λ in allergic asthma also has been proposed (Figure 2A) (Koch and Finotto, 2015). Cells from asthma patients have impaired IFN-λ production after rhinovirus infection (Contoli et al., 2006). IFN-λ downregulates inflammatory Th2 cytokines, including IL-4, IL-5, and IL-13 (Jordan et al., 2007b; Koltsida et al., 2011; Srinivas et al., 2008), which contribute to asthma pathogenesis. In a mouse model of asthma, administration of IFN-λ reduced the production of IL-5 and IL-13, diminished eosinophil infiltration into the lung, and decreased Th17 cell responses, all of which minimized disease (Koltsida et al., 2011). The protective activity of IFN-λ in this model depended on IL-12 and IFN-γ, implicating the Th1-skewing effects of IFN-λ.

In a recent study using a mouse model of autoimmune arthritis, IFN-λ treatment reduced neutrophil recruitment into the joints and resulted in improved disease outcome (Blazek et al., 2015). This work identifies neutrophils as IFN-λ-responsive cells and suggests therapeutic applications for IFN-λ in treating autoimmune arthritis and other inflammatory conditions.

Despite their linkage to other autoimmune conditions, human SNPs near the genes encoding IFN-λ (rs8099917 and rs12979860) or IFNLR1 (rs4649203) did not show an association with multiple sclerosis (Lopez de Lapuente et al., 2012; Malhotra et al., 2011). Furthermore, the *IFNLR1* allele that is protective for psoriasis was associated with exacerbated systemic lupus erythematosus (for SNP rs4649203, the protective allele is A rather than G) (Li et al., 2013), suggesting distinct contributions of IFN-λ signaling to different autoimmune conditions.

IFN-λ in Cancer

IFN-α is currently approved as a therapeutic agent for some cancers. The primary anti-tumor mechanisms of IFN-α/β are cell-intrinsic induction of apoptosis and cell-extrinsic stimulation and priming of immune cells (Booy et al., 2015; Di Trolio et al., 2015). Given that IFN-λ signaling triggers similar downstream gene expression, but in a restricted subset of IFNLR-bearing cells, it could serve as a more targeted therapeutic option. In support of this concept, IFNLR signaling induces apoptosis in colorectal cancer cells more potently than IFN-α/β or IFN-γ (Li et al., 2008) and inhibits growth and colony formation of several different tumor cell lines (Sato et al., 2006).

Induction of IFN-λ in response to viral infections could also promote anti-tumor immune responses. IFN-λ has been detected in human papillomavirus lesions, and lower levels of IFN-λ expression correlated with progression to cervical cancer (Cannella et al., 2014). Infection with a vesicular stomatitis virus strain intended for use as an oncolytic treatment also triggered IFN-λ expression in hematopoietic cells in vitro and enhanced anti-tumor natural killer (NK) cell responses in vivo (Wongthida et al., 2010). Loss of IFNLR expression on tumor cells prevented IFN-λ-stimulated expression of stimulatory ligands for the NK cell receptor NKG2D and associated anti-tumor NK cell responses (Wongthida et al., 2010).

IFN-λ is also present in the tumor microenvironment in the absence of a viral infection. IFNLR expression on mammary epithelial cells inversely correlated with tumor growth in a mouse model of breast cancer (Burkart et al., 2013). IFN-λ signaling on mammary epithelial cells promoted production of the chemokine CXCL10, which recruits $CD4^+$ T cells into the tumor microenvironment (Burkart et al., 2013). Tumor models with melanoma, colon carcinoma, and fibrosarcoma cells also demonstrated a role of IFN-λ in triggering anti-tumor NK and T cells (Lasfar et al., 2006; Numasaki et al., 2007; Sato et al., 2006). An increased anti-tumor immune response was apparent even when the tumor cells themselves lacked the ability to respond to IFN-λ (Lasfar et al., 2006; Numasaki et al., 2007). Recent work has demonstrated that *Ifnlr1*$^{-/-}$ mice are more susceptible to sarcoma formation induced by the carcinogen methylcholanthrene and death in transplanted tumor models (either RMA or B16F10 cells) (Souza-Fonseca-Guimaraes et al., 2015). Furthermore, IFN-λ treatment delayed lethality in the B16F10 adoptive cell transfer model and reduced sarcoma development in methylcholanthrene-treated mice (Souza-Fonseca-Guimaraes et al., 2015). Despite low levels of IFNLR expression, NK cells might respond directly to IFN-λ in vivo, resulting in enhanced IFN-γ production and anti-tumor activity (Souza-Fonseca-Guimaraes et al., 2015). Because IFN-λ can alter tumorigenesis directly and indirectly, it might have utility as an adjunctive anti-cancer therapy.

IFN-λ Modulates Adaptive Immunity

Although its innate antiviral effects have been characterized more extensively, IFN-λ also modulates adaptive immunity. During acute LCMV infection with the Armstrong strain, *Ifnlr1*$^{-/-}$ mice had increased expansion of $CD4^+$ and $CD8^+$ T cells and enhanced T cell responses to LCMV rechallenge (Misumi and Whitmire, 2014). These data suggest that IFN-λ, in contrast to IFN-α/β, inhibits T cell responses during acute viral infection. This effect was cell extrinsic given that IFNLR signaling was not required on the T cells (Misumi and Whitmire, 2014). However, IFN-λ had a different effect during chronic LCMV infection: *Ifnlr1*$^{-/-}$ mice infected with LCMV clone 13 had reduced $CD8^+$ T cell expansion and increased weight loss (Misumi and

Whitmire, 2014). In comparison, *Ifnlr1*$^{-/-}$ mice did not show defects in the magnitude or quality of antigen-specific CD8^{+} T cell responses in the spleen or brain after WNV infection (Lazear et al., 2015), indicating that virus-specific host interactions might affect how IFN-λ modulates the adaptive immune response. Additional studies are required to define the role of IFN-λ in adaptive immunity to mucosal infections, where IFNLR is more abundantly expressed.

Although there are conflicting reports on whether human lymphocytes respond directly to IFN-λ (Dickensheets et al., 2013; Gallagher et al., 2010), there is consensus that IFN-λ modulates T cell responses. The addition of IFN-λ during stimulation of peripheral-blood mononuclear cells (PBMCs) with concanavalin A or during a mixed lymphocyte reaction reduced the production of Th2 cytokines (IL-4, IL-5, and IL-13) and increased the production of IFN-γ, suggesting that IFN-λ promotes a Th1 response (Dai et al., 2009; Jordan et al., 2007b; Srinivas et al., 2008). Consistent with these data, a SNP (rs8099917, with the T/T allele) in the *IFNL3* locus was linked to increased IFN-λ3 production and skewing toward a Th1 response after PBMCs were stimulated with influenza virus (Egli et al., 2014b). Furthermore, administration of IFN-λ as a vaccine adjuvant resulted in reduced expression of the Th2 cytokine IL-4 by HIV gag-specific T cells and reduced numbers and activity of regulatory T cells (Morrow et al., 2009). Collectively, these observations suggest that IFN-λ skews T cell responses toward a Th1 cell bias and away from a Th2 cell bias.

The humoral immune response might be regulated by IFN-λ, although the net effect is not clear. Increased seroconversion in response to influenza or measles vaccines correlated with SNPs in the *IFNL3* promoter (the T/T allele for rs8099917 and the G/G allele for rs10853727) (Egli et al., 2014b; Haralambieva et al., 2011). The T/T allele of SNP rs8099917 was linked to increased production of IFN-λ and decreased seroconversion after influenza vaccination (Egli et al., 2014b). In vitro experiments showed reduced antibody production by PBMCs stimulated with influenza virus in the presence of IFN-λ and increased antibody titers in the presence of an IFNLR-blocking peptide (Egli et al., 2014b). In contrast, a stimulatory effect on humoral response was observed in an HIV vaccine study comparing IFN-λ and IL-12 as adjuvants: the IFN-λ-adjuvanted vaccine resulted in a greater increase in IgG2a responses than did the IL-12-adjuvanted vaccine (Morrow et al., 2009). However, in the context of WNV or LCMV infection, antiviral antibody production was the same between *Ifnlr1*$^{-/-}$ mice and wild-type animals (Lazear et al., 2015; Misumi and Whitmire, 2014). Thus, the impact of IFN-λ on humoral immunity appears to be context dependent and requires further evaluation.

More Than Just Viral Infections

Although known for their antiviral effects, IFN-α/β and IFN-λ might function in other microbial infections as well. Whereas IFN-α/β signaling protects against viral infections, it can be detrimental in the context of infection by some bacteria (e.g., *Mycobacterium tuberculosis* and *Listeria monocytogenes*) and parasites (e.g., *Leishmania spp.*) (Stifter and Feng, 2015). IFN-λ also is induced by bacterial infection (Bierne et al., 2012; Cohen and Prince, 2013; Lebreton et al., 2011; Love et al., 2014; Odendall et al., 2014; Pietilä et al., 2010; Travar et al., 2014) and might have consequences during *Staphylococcus* and *Pseudomonas* infections: *Ifnlr1*$^{-/-}$ mice sustained lower bacterial loads and exhibited less pathology without differences in inflammatory cell infiltrates (Cohen and Prince, 2013). Further investigation into the role of IFN-λ in non-viral infections is warranted. The effects of IFN-λ in the context of helminth infections could be especially interesting given its ability to downregulate Th2 responses (Jordan et al., 2007b; Srinivas et al., 2008).

Antagonism of IFN-λ

Given the antiviral effects of IFN-λ, it is anticipated that some viruses will have evolved specific evasion strategies. IFN-α/β antagonist mechanisms have been described for many virus families (Hoffmann et al., 2015); because some of these target induction or signaling pathways shared between IFN-λ and IFN-α/β, these same evasion strategies most likely limit the effects of IFN-λ as well. Viruses targeting epithelial tissues might be especially likely to encode specific IFN-λ-evasion mechanisms. Yaba-like disease virus (YLDV) is a primate poxvirus that exclusively infects the skin. Similar to other orthopoxviruses, YLDV encodes a secreted glycoprotein (Y136) that binds to IFN-α/β and blocks IFNAR signaling. Unlike orthologs in vaccinia or variola viruses, Y136 also binds to and inhibits the antiviral effects of IFN-λ (Bandi et al., 2010; Huang et al., 2007). Because the structure of IFN-λ is distinct from that of IFN-α/β (Gad et al., 2009), it remains uncertain how a single viral evasion protein targets both IFN-λ and IFN-α/β. Because they infect epithelial cells and their large genomes enable them to encode a suite of immune evasion molecules (Epperson et al., 2012), other poxviruses and herpesviruses might have evolved analogous or unique mechanisms to antagonize IFN-λ responses.

IFN-λ across Species

The use of *Ifnlr1*$^{-/-}$ mice has provided insight into the role of IFN-λ in antiviral immunity (Hermant and Michiels, 2014; Hernández et al., 2015; Lazear et al., 2015; Mahlakõiv et al., 2015; Mordstein et al., 2010a; Nice et al., 2015). Nonetheless, differences in IFN-λ between mice and humans should be considered when one interprets its array of activities. Whereas humans have three or four functional *IFNL* genes, mice have only two (*Ifnl2* and *Ifnl3*), because the *IFNL4* genomic region is absent in mice and mouse *Ifnl1* is a pseudogene. Compared to human studies, mouse studies might underestimate some of the contributions of IFN-λ because they lack IFN-λ1. Given that most IFN-λ transcriptional profiling to date has focused on human cells, studies are needed to characterize IFN-λ responses in mouse cells to inform the interpretation of mouse models of disease.

The IFN-λ family is conserved throughout tetrapod evolution, as evidenced by synteny and structural similarity among mammalian, avian, and amphibian IFN-λ-encoding genes. In comparison, fish have genes encoding IFN-α/β but not IFN-λ (Qi et al., 2010). Antiviral effects of avian IFN-λ have been documented (Reuter et al., 2014), and in tadpoles, IFN-λ rather than IFN-α/β dominantly inhibits infection by frog virus 3 (Grayfer et al., 2015).

In contrast to the preferential epithelial expression pattern of IFNLR in humans and mice, in the bat *Pteropus alecto*, IFNLR

is expressed in a broader range of cells and tissues (Zhou et al., 2011a). Infection of *P. alecto* splenocytes with the paramyxovirus Tioman virus induced the expression of IFN-λ1 but not IFN-α/β (Zhou et al., 2011b). It will be interesting to determine whether broader tissue responsiveness to IFN-λ in bats contributes to their ability to serve as reservoir hosts for diverse viruses (Baker et al., 2013). For example, enhanced IFN-λ responsiveness might enable bats to control viral infections without developing disease. Alternatively, sustained IFN-λ signaling might impede the activation of more potent antiviral responses and prevent bats from clearing viral infections.

Although the mouse and rat genomes lack *IFNL4*, this gene is conserved in most mammals and displays evidence of positive selection, implying functional significance (Key et al., 2014). In some human populations, however, *IFNL4* is undergoing pseudogenization with the acquisition of a frameshift insertion in the *IFNL4* open reading frame that ablates IFN-λ4 production (Hamming et al., 2013; Prokunina-Olsson et al., 2013). The frequency of this polymorphism varies, such that the ancestral (IFN-λ4-producing) allele dominates in African populations, and the pseudogene allele has reached near fixation in East Asian populations (Key et al., 2014). The selective pressure driving *IFNL4* pseudogenization in humans remains unknown, but GWASs suggest a strong association between the pseudogene allele of *IFNL4* and improved outcome after HCV infection (Griffiths et al., 2015; Hamming et al., 2013; Prokunina-Olsson et al., 2013). Because HCV infection is unlikely to provide sufficient selective pressure to drive this evolutionary process, the loss of IFN-λ4 production might be advantageous in the context of other infections or inflammatory conditions.

Conclusions

Since its discovery in 2003, many groups have identified contributions of IFN-λ to antiviral and other immune responses. These studies have prompted a growing appreciation of the unique features that distinguish IFN-λ from IFN-α/β, and these features provide possibilities for more targeted therapeutic intervention. The ability of IFN-λ to provide antiviral protection at specific tissue sites (e.g., epithelial surfaces) could explain the maintenance of a system that appears somewhat redundant with the IFN-α/β response. Epithelial surfaces could require greater innate immune protection because of their constant exposure to commensal and pathogenic microbes. Constant microbial stimulation at epithelial barriers could require local control without broadly activating a systemic immune response. Thus, IFN-λ signaling could control frequent or persistent low-level infections at epithelial barriers, and the more potent and inflammatory systemic IFN-α/β response could be reserved for severe infections. This compartmentalized tissue responsiveness to IFN-λ could be utilized therapeutically as a means of achieving antiviral benefit while minimizing systemic side effects associated with inflammation.

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