Review

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Viral recognition and the antiviral interferon response

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Abstract

Interferons (IFNs) are antiviral cytokines that play a key role in the innate immune response to viral infections. In response to viral stimuli, cells produce and release interferons, which then act on neighboring cells to induce the transcription of hundreds of genes. Many of these gene products either combat the viral infection directly, e.g., by interfering with viral replication, or help shape the following immune response. Here, we review how viral recognition leads to the production of different types of IFNs and how this production differs in spatial and temporal manners. We then continue to describe how these IFNs play different roles in the ensuing immune response depending on when and where they are produced or act during an infection.

Keywords Interferon; Interferon induction; Interferon Regulatory Factor 3 (IRF3); viral recognition

Subject Category Immunology

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Introduction

An effective immune response starts with the recognition of an invading pathogen by the innate immune system, which is mediated by a set of receptors called pattern recognition receptors (PRRs) (Mogensen, 2009; Chow *et al*, 2015). Once the PRRs detect a virus, they initiate a series of signaling events, leading to the establishment of an immune response towards the intruding pathogen. The key mammalian PRRs responsible for the induction of IFN production are shown in Fig 1. This concept is conserved throughout metazoan evolution (Holleufer *et al*, 2021; Slavik *et al*, 2021) and early components of this system are found in bacteria as well (Morehouse *et al*, 2020). In this review, we focus on the mammalian response to viral infections but with a particular focus on interferon (IFN), a group of cytokines that acts as signaling molecules and triggers antiviral defenses in cells. IFNs are divided into three types, depending upon their receptor usage: Type I IFNs encompass multiple subtypes but here we will only be discussing IFN-α and IFN-β. Type II IFN only has one member, IFN-γ, which does not play a major role in the induction of an antiviral state, and it is not discussed further here. Finally, there are type III IFNs, which are also called IFN-λ. Table 1 lists the different subtypes of IFN and classifies them into types I, II, or III IFNs.

Mammals are complex animals and viral recognition can lead to widely different outcomes depending on the kind of tissue in which it happens. The implications of this on the spatial/temporal distribution of IFN production and the ensuing consequences will be discussed in detail throughout this review, as we suggest the following working model. Recognition of a viral infection in barrier tissues leads to local IFN responses driven by the local production of IFN-β and IFN-λ, whereas recognition of viruses that have penetrated the barrier tissue leads to large-scale production of IFN-α and the result is a systemic IFN response. In the first case, IFN production generally originates from infected cells whereas in the latter case, immune cells, such as plasmacytoid dendritic cells (pDCs), can contribute with large amounts of IFN. Notably, pDCs do not need to be productively infected (defined as an infection that leads to viral progeny) themselves in order to initiate IFN production (Swiecki & Colonna, 2015). Finally, microbiota found at mucosal surfaces also drives a tonic IFN response (Bradley *et al*, 2019).

Viral recognition

As the name implies, PRRs recognize specific molecular patterns called pathogen-associated molecular patterns (PAMPs) originating from the pathogen. The primary PAMP for viruses is nucleic acids, including foreign nucleic acids structures, like 5′-phosphorylated RNA and dsRNAs that are specifically associated with viral infections, and generic nucleic acids that may be perceived as foreign when located in a cellular compartment where they are not normally found. In mammals, there are three major pathways for the recognition of a viral infection (see Fig 1). (i) The endosomal toll-like receptors (TLRs), which can recognize either dsRNA (TLR3) (Alexopoulou *et al*, 2001) or a variety of single-stranded nucleic acids present within endosomes or phagosomes (TLR7, 8 or 9; Lund *et al*, 2003; Diebold *et al*, 2004; Heil *et al*, 2004; Akira *et al*, 2006). (ii) The cytosolic RIG-I-like receptors (RLR), which recognize dsRNA, 5′-phosphorylated RNAs or a combination thereof (Hornung *et al*, 2006; Kato *et al*, 2006; Pichlmair *et al*, 2006). (iii) Cytosolic cyclic GMP–AMP synthase (cGAS), which recognizes dsDNA

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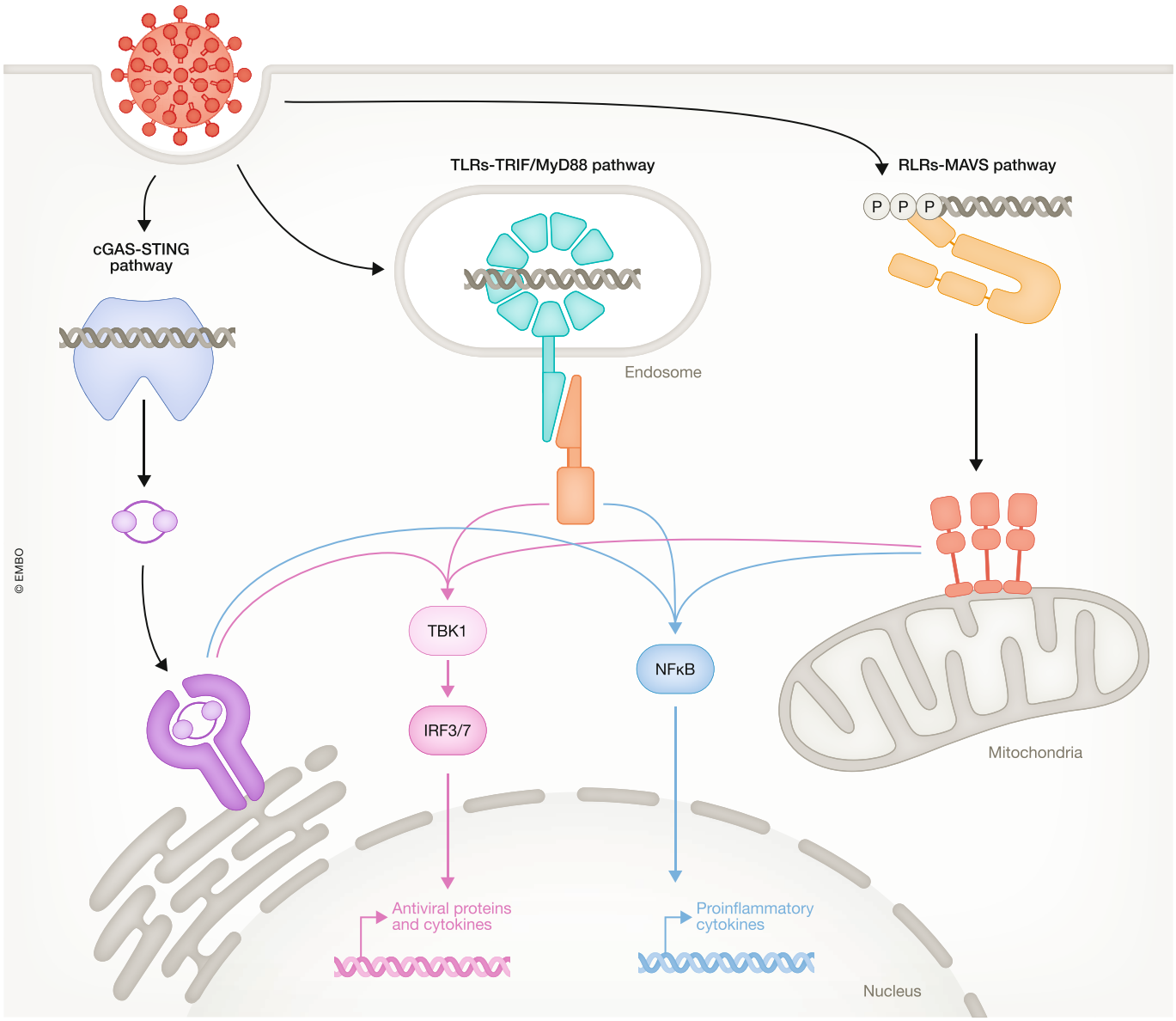

Figure 1. Recognition of viral infection by pattern recognition receptors leads to interferon production.
Viral recognition leads to interferon induction via three principal pathways that are characterized by the adaptor proteins used to connect the upstream pattern recognition receptors with the downstream signaling machinery. Signaling via those pathways is orchestrated by the adaptor proteins STING, MAVS and either TRIF or MyD88. All the three pathways activate the kinase TBK1, which in turn phosphorylates the transcription factors IRF3 and IRF7. Notably, IRF7 is constitutively expressed in pDCs but needs to be induced by IFN signaling in other cells. In addition, these pathways also activate the NF–κB family of transcription factors, yet the molecular mechanism behind this activation remains poorly characterized.

present in the cytoplasm and perceives it as a sign of viral infection (Civril *et al*, 2013; Sun *et al*, 2013; Gao *et al*, 2013a). Upon binding of the PAMP, the receptor conveys a signal to a distinct downstream adaptor protein (Fig 1). The TLRs signal via the TIR-domain-containing adapter-inducing IFN-β (TRIF) or the myeloid differentiation factor 88 (MyD88) (Liu & Ding, 2016), the RLRs signal via mammalian mitochondrial antiviral signaling protein (MAVS; Yoneyama *et al*, 2015), and cGAS produces the secondary messenger cyclic GMP–AMP (cGAMP) to signal via stimulator of interferon genes (STING; Ishikawa & Barber, 2008; Wu *et al*, 2013; Gao *et al*, 2013b). Once activated, the adaptor proteins recruit and activate several cellular kinases, like the TANK-binding kinase 1 (TBK1), which leads to the activation of the transcription factors interferon regulatory factor (IRF) 3 and 7 (IRF7). The abovementioned PRRs also activate signaling through the NF-κB pathway but our understanding of the underlying molecular mechanisms remains incomplete and will therefore not be discussed any further here. Thus, activation of PRRs ultimately leads to a transcriptional response creating an antiviral state in the infected cell. IRF3 is central in this response as it initiates two defensive mechanisms. Firstly, activation of IRF3 leads to the production of IFNs, in particular, IFN-β and IFN-λ1 (Osterlund *et al*, 2007), and other cytokines

Table 1. Provides an overview of the different subtypes of IFN found in humans, key references and main characteristic is listed.

Type	Subtypes	Receptor	Comments	References
Type I IFNs				
IFN-α	IFN-α1, -α2, -α4, -α5, -α6, -α7, -α8, -α10, -α13, -α14, -α16, -α17, -α21	IFN-αR1/ IFN-αR2	IFN-α is primarily produced by pDCs	Cella *et al* (1999), Barchet *et al* (2002), Dai *et al* (2004), Hardy *et al* (2004), Jaks *et al* (2007)
IFN-β		IFN-αR1/ IFN-αR2	IFN-β is produced by most infected cells	Platanias (2005), Khaitov *et al* (2009), Ioannidis *et al* (2013)
IFN-ε		IFN-αR1/ IFN-αR2	IFN-ε is associated with the female reproductive tract	Fung *et al* (2013), Marks *et al* (2019)
IFN-κ		IFN-αR1/ IFN-αR2	IFN-κ is selectively expressed in keratinocytes	LaFleur *et al* (2001)
IFN-ω		IFN-αR1/ IFN-αR2	One of the least studied IFNs but the presence of neutralizing auto-antibodies against it in severe COVID-19 patients suggests its role in antiviral immunity may be underappreciated	Hauptmann and Swetly (1985), Bastard *et al* (2020)
Type II IFNs				
IFN-γ		IFN-γR1/ IFN-γR2	Not discussed here	
Type III IFNs				
IFN-λ	IFN-λ1, -λ2, -λ3, -λ4	IFN-λR1/ IL-10R2	IFN-λ is produced by infected cells at barrier tissues such as epithelial cells in the respiratory tract	Meager *et al* (2005), Sommereyns *et al* (2008), Jewell *et al* (2010), Mordstein *et al* (2010), Crotta *et al* (2013), Wack *et al* (2015), Ye *et al* (2019)

working in a paracrine manner to warn surrounding cells of the infection. Secondly, the IRF3 transcriptional response leads to the production of antiviral proteins that help combat the virus within the affected cell (Grandvaux *et al*, 2002). These responses enable cells to limit viral infection within the affected cell and simultaneously establish a strong defensive state in neighboring cells.

Molecular mechanisms of IFN production

Activation of IRF3

In this section, we focus on how STING activates IFN production as this is our research area, but we believe that the mechanism is largely conserved among the different adaptor molecules. In the model of IRF3 activation proposed by Chen and colleagues, the activation of IRF3 is initiated by it docking to a phosphorylated pLxIS motif on an adaptor molecule (Liu *et al*, 2015). This docking is mediated by electrostatic interactions between the positively charged surface on IRF3 and the phosphorylated pLxIS motif (Fig 2). The positively charged surface of IRF3 can be divided into 5 patches consisting of residues R211/R213, R255/R262/H263, R285/H288/H290, K313/K315, and K360/R361, and mutation of each of these patches abolishes activation of IRF3, thereby supporting the current model of IRF3 activation (Takahasi *et al*, 2003; Liu *et al*, 2015). Structural work using peptides representing the adaptors suggested that IRF3 docks to the adaptor proteins by forming a direct contact between R285 of IRF3 and the phosphorylated serine in the pLxIS motif (Zhao *et al*, 2016). The importance of residue R285 in IRF3 activation was further supported by the finding of a R285Q mutation in a patient suffering from viral encephalitis as well as by our detailed analysis of IRF3 docking to the adaptor (Andersen *et al*, 2015; Dalskov *et al*, 2020). The docking to an adaptor molecule positions IRF3 for phosphorylation by TBK1 on S386 (Liu *et al*, 2015). The importance of phosphorylation at either S386 or S396 has been debated in the literature. On one hand, the phosphorylation of S386 has been shown to be critical for IRF3 activity in several publications (Mori *et al*, 2004; Takahasi *et al*, 2010; Dalskov *et al*, 2020), but mutation of S396 and surrounding serine residues to the phosphomimetic aspartic acid was shown to cause a constitutively active phenotype, which suggests that S396 also plays a role in the activation of IRF3 (Lin *et al*, 1998; Servant *et al*, 2003). However, it is now clear that mutation of S396 to aspartic acid leads to a conformational change within IRF3, which facilitates the phosphorylation of S386. Thus, phosphorylation of S386 is required for the ability of IRF3 to dimerize and become transcriptionally active. Furthermore, mutation of S396 to alanine does not impact the activity of IRF3 (Dalskov *et al*, 2020) but a role for phosphorylation of S396 in facilitating S386 phosphorylation and thereby activation cannot be excluded.

Once IRF3 is phosphorylated, it forms the transcriptionally active dimer, and this dimerization is driven by the interaction between the phosphorylated residue S386 and R211. This view is supported by structural evidence (Qin *et al*, 2003; Takahasi *et al*, 2003) and was confirmed by mutational studies. Furthermore, the fact that mutation of R211 still resulted in IRF3 phosphorylation but no dimer formation also supports this model (Dalskov *et al*, 2020). (Dalskov *et al*, 2020). S396 is found in a position, where if phosphorylated, it can potentially interact with R385, as shown in Fig 2. However, S396 is dispensable for the activity of IRF3. Analysis using dimeric IRF3 with phosphomimic substitutions at position S386 and S396 and the structure of murine dimeric IRF3 with a natural phosphoserine at position S386 (murine S379) provided further support for this model (Zhao *et al*, 2016; Jing *et al*, 2020). Finally, the manner upon which phosphorylation of S386 drives dimerization of IRF3 also offers a putative mechanism for avoiding aberrant activation of IRF3 by unspecific phosphorylation. Once it is phosphorylated, IRF3 will leave STING but the phosphoserine 386 is vulnerable to dephosphorylating enzymes as long as IRF3 is in the monomeric state.

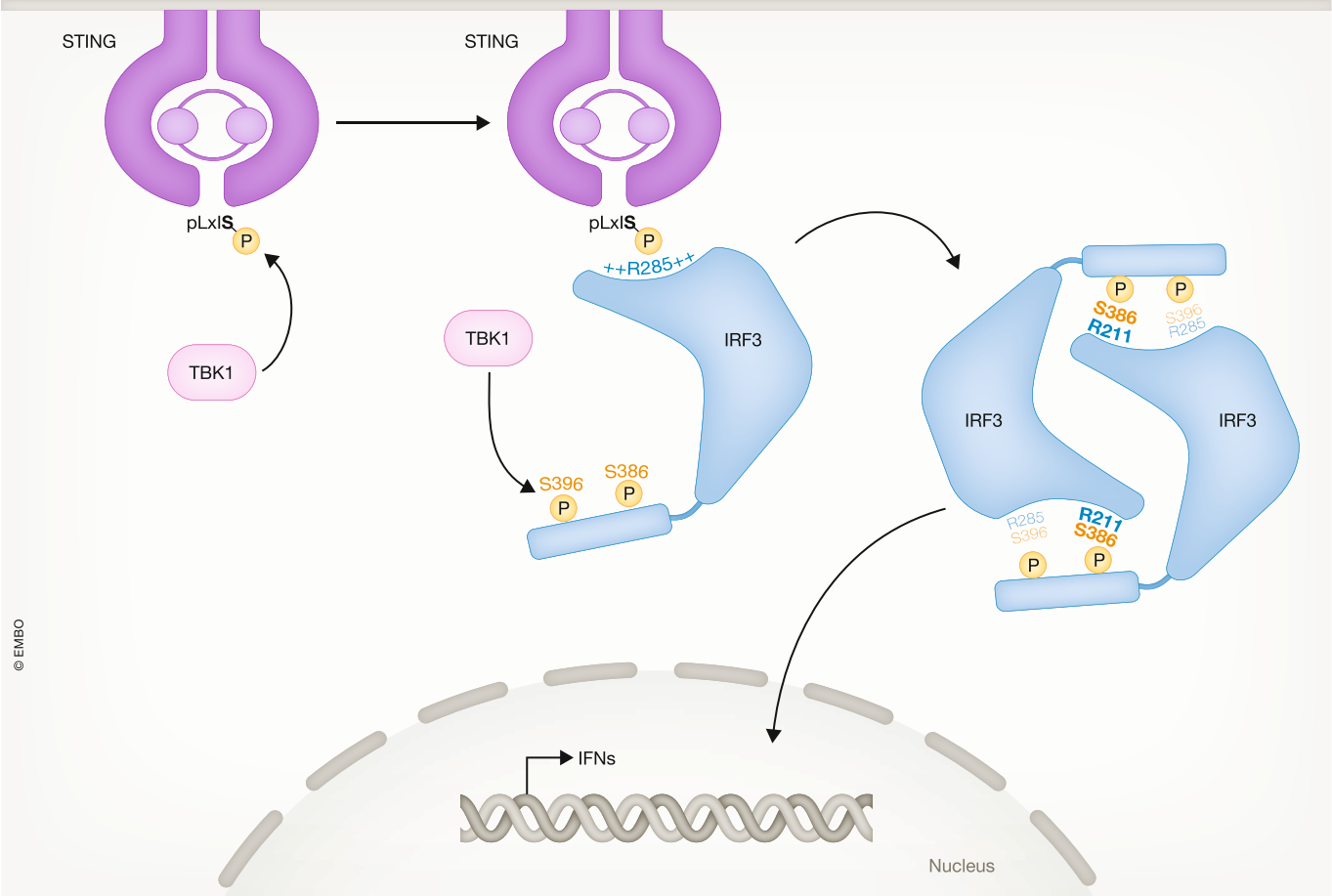

Figure 2. The activation mechanism of IRF3.
IRF3 docks to the phosphorylated form of the adaptor proteins illustrated here by STING. Once docked at the adaptor proteins, TBK1 phosphorylates IRF3 at key serine residues where residue 386 is particularly critical for IRF3 activation. Upon phosphorylation, IRF3 dimerizes and forms the transcriptionally active dimer, which then translocates to the nucleus where it drives transcription of IFNs and certain antiviral genes. The available data suggest that IRF7 is activated through a similar mechanism.

A characteristic of adaptor proteins, like STING, is the formation of higher order oligomers (Zhang *et al*, 2019). This means that the local concentration of phosphorylated IRF3 monomers is higher around those structures and thus IRF3 can rapidly find an equally phosphorylated partner to dimerize with. In the dimeric form, the phosphoserine is buried and is not accessible to phosphatases.

The activated dimeric form of IRF3 described above is transcriptionally competent and can induce the transcription of a number of genes (Grandvaux *et al*, 2002; Schoggins *et al*, 2011). A key role for IRF3 is to induce different IFN genes, but IRF3 also induces a series of antiviral genes within the infected cells, thus there is a significant overlap between the gene set induced by IFN and the gene set induced by IRF3. Notably, IFNs cannot induce their own transcription. The ability of IRF3 to induce a variety of antiviral genes (which are also under the control of IFN) hint at an ancestral role of the IRF family of transcription factors. We believe that prior to the emergence of IFN, the role of IRF was likely to directly induce antiviral genes upon detection of the virus by the PRRs. However, the strong phenotype seen in different model systems with impaired or completely inactivated IFN signaling suggests that the direct induction of antiviral genes by IRF3 plays a lesser role in vertebrates. The overlap between IFN-induced genes and IRF3-induced genes is also important to keep in mind when using modern bioinformatics tools to analyze transcriptomic data, as the programs struggle to differentiate between the signatures left by IRF3 and IFN.

IRF7 is activated in a similar manner as IRF3

IRF7 plays a key role in the production of IFN-α and is constitutively expressed in pDCs while its expression is inducible by IFN in other cells (Au *et al*, 1998; Marié *et al*, 1998; Sato *et al*, 1998; Ning *et al*, 2011). Our data suggests that the molecular mechanism of IRF7 activation is very similar to what we just discussed for IRF3, but the regulation of IRF7 seems stricter, and it is possible that IRF7 needs to be phosphorylated at multiple sites to gain full activity (Dalskov *et al*, 2020). We have attempted to induce IFN-α expression in a series of cell lines and primary cells that express IRF7 after IFN priming, but with little success. Thus, it appears that phosphorylation of IRF7 is insufficient to activate IFN-α production in many cell types and this suggests the existence of additional layers of regulation in IFN-α production, which may possibly involve epigenetic modifications at the IFN-α loci. In other words, substantial new research of the molecular mechanisms that drive IFN-α production is needed.

IRF3 and IRF7 differ in their ability to induce specific IFN subtypes

The structure and function of IFN promoters have been characterized in detail by others, which will be summarized in the following. The IFN-β promoter contains IRF binding sites, which can bind both IRF3 and -7. Furthermore, it also contains binding sites for factors of the NF–κB family. For full activation of the IFN–β promoter, binding of both IRF3 and NF–κB is required (Thanos & Maniatis, 1995; Sato *et al*, 2000; Panne *et al*, 2004, 2007). The IFN-λ1 promoter appears to show much of the same characteristics as the IFN-β promoter (Onoguchi *et al*, 2007; Osterlund *et al*, 2007; Thomson *et al*, 2009). The IFN-α promoter can bind several different members of the IRF family, most prominently IRF7, but IRF3 can also bind to at least some IFN-α promoters (Schafer *et al*, 1998; Andrilenas *et al*, 2018; Wittling *et al*, 2020). Likewise, IRF5 has also been suggested to play a role in induction of some IFN-α genes (Andrilenas *et al*, 2018). In contrast to the IFN-β promoter, IFN-α promoters do not appear to rely upon the NF–κB pathway (Wittling *et al*, 2020).

Naturally, the differences in promoter composition among IFNs also result in different regulatory patterns. IFN-λ1 and IFN-β have a similar mode of regulation with both being primarily controlled by IRF3 in conjunction with the NF–κB pathway. While both IRF3 and IRF7 can bind the IFN-β promoter, the rapid induction of those IFNs in virus infected cells primarily relies upon IRF3, as IRF7 is not constitutively expressed in most cells. Importantly, the relative amount of IFN-λ1 and IFN-β being produced by a given cell might vary dependent upon cell type. In terms of gene regulation, human IFN-λ3 appears to share some characteristics with IFN-α (Onoguchi *et al*, 2007; Osterlund *et al*, 2007; Kotenko, 2011; Wack *et al*, 2015), but current data on the regulation of IFN-λ3 are limited and more work is needed in this area.

Role of other IRF family members in IFN production

Besides IRF3 and -7, other IRFs have also been investigated for their involvement in IFN induction. Although IRF1 was identified as a positive regulator of the IFN-β gene, it was later shown that IRF1 is not needed for IFN induction since IRF1 deficient mice produced normal levels of IFN in response to Newcastle disease virus (Fujita *et al*, 1988; Reis *et al*, 1994; Feng *et al*, 2021). IRF5 is widely expressed by immune cells such as macrophages and pDCs where it has been shown that IRF5 increases IFN-β production. Furthermore, IRF5 has been shown to bind to a subset of IFN-α promoters (Schoenemeyer *et al*, 2005; Lazear *et al*, 2013; Yasuda *et al*, 2013; Andrilenas *et al*, 2018; Khoyratty & Udalova, 2018). IRF8 has also been implicated in enhancing IFN production, especially in the later phases of IFN induction in pDCs and monocytes, by increasing recruitment of the basal transcription machinery (Tailor *et al*, 2007; Li *et al*, 2011; Jefferies, 2019). Yet, detailed knowledge about the role of other IRFs in IFN production at the molecular level is currently lacking.

Temporal and spatial regulation of IFN production

Effect of IRF3 and IRF7 deficiency on susceptibility to viral infections

The biological importance of IRF3 versus IRF7 in IFN production has been studied primarily in mice but with the recent progress in human genetics, more and more data emerge from humans as well. IRF3 knockout (KO) mice exhibited lower IFN-β expression in response to both DNA and RNA viruses (Steinberg *et al*, 2009; Hatesuer *et al*, 2017; Yanai *et al*, 2018) whereas IRF7 KO mice exhibited an almost complete loss of IFN-α expression as well as a severe drop in IFN-λ2/3 expression (Hatesuer *et al*, 2017). Here, it is important to note that mice do not have an equivalent to the human IFN-λ1 gene. Thus, the *in vivo* data agrees with the molecular model for IFN expression discussed above. Both the IRF3 and IRF7 KO mice exhibited decreased survival upon infection with influenza A virus (IAV). However, the effect of IRF7 KO was marginally larger than for IRF3 KO (Hatesuer *et al*, 2017). A lack of IRF3 should lead to an initially lower IFN production by epithelial cells, which agrees with the observed decrease in IFN-β production at day 1 in these mice. It is unclear whether IRF7 is needed due to its ability to produce IFN-λ2/3, which is particularly important in respiratory infections, or due to its role in driving a systemic IFN-α response. Thus, the role of IRF7 in the induction of IFN-λ in respiratory infections merits further investigations. Another group investigated the role of IRF3 and IRF7 in infection with murine cytomegalovirus (CMV) and found that IRF7 was particularly important whereas KO of IRF3 had little effect (Steinberg *et al*, 2009). This is what we would expect from a systemic viral infection with broad tropism, like CMV, where the systemic IFN-α response is expected to be important.

Turning toward humans, a patient, which was heterozygote for the IRF3 missense variant (p.Arg285Gln/WT), displayed high susceptibility toward DNA viruses, such as herpes simplex virus 1 (HSV-1), but not to RNA viruses (Andersen *et al*, 2015). *In vitro* characterization of this IRF3 variant demonstrated a selective failure in STING-mediated activation, explaining the high susceptibility toward herpes infection. Several IRF7 variants have been identified in patients with severe IAV infection. Common for all of these is a reduced IFN production and defective IFN priming (Ciancanelli *et al*, 2015; Thomsen *et al*, 2019; Zhang *et al*, 2020). More specifically, it was observed that the IRF7 variants affected IFN-α and IFN-λ production, but to a lesser extent IFN-β production (Ciancanelli *et al*, 2015; Campbell *et al*, 2022). These observations correlate with the findings in the IRF3/7 KO mice, where IRF7 plays a role not only in IFN-α production but also in IFN-λ production.

The role of pDCs in IFN production

The abovedescribed division of labor between IRF3 and IRF7 results in temporal differences in IFN production. During early stages of a viral infection, the infected cells will activate IRF3 and drive an IFN-β and IFN-λ based response, which will be largely local in nature. However, as the infection progresses, a subset of dendritic cells, namely the pDCs, becomes major producers of IFN-α (Cella *et al*, 1999; Barchet *et al*, 2002). In agreement with this, depletion of pDCs in a mouse model and/or in humans reduces IFN-α production (Rowland *et al*, 2014; Karnell *et al*, 2021). Furthermore, *in vitro* pDCs will produce large amounts of IFN-α after stimulation with a variety of viruses (Dai *et al*, 2004; Yin *et al*, 2012). There is no strict definition of a systemic response, but in our opinion, this occurs when pDCs produce sufficient IFN-α to raise the serum concentration above the critical concentration needed for a systemic response. Thus, it is the response to IFN that is systemic, not the production of it. It is possible that IFN-β also contributes to the systemic response whereas IFN-λ acts in a more targeted fashion due to the limited distribution of its receptor. The IFN-producing pDCs are

most likely localized in the lymphatic tissue around the site of infection. It is possible that induction of IRF7 by IFN in non-pDCs can lead to production of IFN-α, which may then contribute to the systemic response. However, at present the role of non-pDCs as IFN-α producers is not clear. Importantly, we need a better understanding of the molecular mechanisms driving IFN-α production and enabling pDCs to produce large amount of IFN-α in order to understand how IFN-α production is regulated.

Physiological effects of IFN and their therapeutic use

IFN signaling at a cellular level

The primary role of IFN is to warn uninfected cells of an approaching virus and thereby allow them to establish a defensive state before being infected. Figure 3 summarizes the discussion above and seeks to illustrate the primary functions of an IFN response. However, IFN also has secondary roles in promoting inflammatory reactions occurring upon detection of viral infection and increasing antigen presentation in an MHC I context. For type I IFNs, signaling occurs after IFN binds a heterodimeric receptor complex consisting of the IFN-α receptor (IFN-αR) 1 and IFN-αR2 (Schreiber, 2017). The type III IFNs signal through a separate receptor complex formed by the IFN-λ receptor 1 (IFN-λR1) and the interleukin-10 receptor 2 (IL-10R2), the latter also used by the cytokines IL-10 and IL-22 (Kotenko *et al*, 2003; Sheppard *et al*, 2003). Upon binding of the ligand to the IFN receptor complexes, the receptor associated kinases, tyrosine kinase 2 (TYK2), and Janus kinase 1 (JAK1), are auto-phosphorylated and then phosphorylate specific tyrosines in the cytoplasmic part of the receptor. This then leads to the recruitment and phosphorylation of signal transducer and activator of transcription 1 (STAT1) and STAT2 proteins. Despite using two different receptor complexes, this process is rather similar for both type I and III receptor complexes (Qureshi *et al*, 1995; Zhou *et al*, 2007). Following their activation, STAT1 and STAT2 join with IRF9 to form the IFN-stimulated gene (ISG) factor 3 (ISGF3), which drives the expression of ISGs. Activation of the type I IFN receptor, but not the type III receptor, is thought to also lead to production of a phosphorylated STAT1 homodimer, which is transcriptionally competent. This might lead to physiologically significant gene expression in immune cells (Zhou *et al*, 2007; Forero *et al*, 2019) but more work is needed in this area.

The genes induced by IFN signaling are referred to as interferon-stimulated genes (ISGs) and can be divided into three functional groups: antiviral effectors, positive regulators, and negative regulators (Schneider *et al*, 2014). The antiviral effectors control and combat an infection by directly targeting the replicating virus. The positive regulators aid in the induction of the immune response by enhancing recognition or innate immune signaling and many PRRs and signaling proteins are thus ISGs. At last, the negative regulators, such as USP18, help with terminating signaling to keep the immune system tightly controlled (Veer *et al*, 2001; Schneider *et al*, 2014).

The key difference, however, between type I and type III IFNs lies in the distribution of their receptor complexes. Whereas the type I receptor is found on all nucleated cells, the IFN-λR1 chain is expressed in a highly tissue specific manner, which restricts the response toward type III IFNs (Sommereyns *et al*, 2008). IFN-λR1 is expressed in all epithelial cells in both mice and humans, but whereas human hepatocytes do express IFN-λR1, mice hepatocytes do not (Hermant *et al*, 2014). The responsiveness of immune cells to type III IFNs is actively being debated, but it is clear that only a subset of immune cells responds to type III IFNs. However, the identity of those cells and the effect that type III IFNs has upon them is still being worked out. We refer the reader to the review by Wack *et al* (2015) focusing upon type III IFNs for an in-depth discussion of this topic (Wack *et al*, 2015).

Systemic IFN responses versus local IFN responses

As used here, the phrasing "IFN responses" covers the entirety of effects caused by the IFN system with the range of these responses being determined by a combination of how much IFN is produced and how specifically/broadly the receptor for the given subtype of IFN is expressed. Since the receptor for type I IFN is found on all nucleated cells, this means that if enough type I IFN is produced to raise the plasma level above a certain concentration, a systemic response will follow. In contrast to this, the type III IFN receptor is only expressed in specific cell types and thus even in the event of massive type III IFN production, the response will be restricted by its tissue tropism. As described above, the primary cause of systemic IFN responses is thought to be large-scale production of IFN-α by pDCs.

Observing patients receiving systemic IFN treatment (this will typically be pegylated IFN-α) provides a good idea of the global effect of IFN on the human body. Generally, patients report "flu like" symptoms, including fatigue, headache and nausea, after treatment with type I IFN. One phase II clinical trial compared pegylated IFN-α to pegylated IFN-λ for the treatment of hepatitis C virus (HCV) infection. The two IFNs had similar antiviral properties but the abovedescribed side effects were substantially lower for IFN-λ (Muir *et al*, 2014). This illustrates our current view of why two different IFN systems that regulate the same set of genes have proved to be an advantage throughout evolution. In essence, type III IFNs provide efficient control of viral replication in high-risk tissues but avoid some of the negative effect of an IFN response due to the targeted nature of the system as compared to type I IFNs.

IFN responses in the respiratory tract

As alluded to above, type III IFNs act in a much more targeted manner than type I IFNs, and here, we will discuss the IFN response at barrier tissues in more details. Our focus will be on IFN responses in the respiratory tract since this is our particular area of expertise. Since barrier tissues suffer from a particular high risk of viral infection, they have evolved specific defense mechanisms. This includes the type III IFNs, which are largely specialized in defending the mucosal surfaces in our body (Wack *et al*, 2015), but also the IFN-ε, a member of the type I IFN family, which is produced only in the female reproductive tract and act there to protect from viral infection (Fung *et al*, 2013; Marks *et al*, 2019). Primary human bronchiolar epithelial cells as well as human airway type II epithelial cells were shown to primarily produce IFN-λs (IFN-λ1 and IFN-λ2/3) and IFN-β and to a lesser extent IFN-αs following infection with respiratory syncytial virus (RSV) or IAV (Khaitov *et al*, 2009; Wang *et al*, 2009; Ioannidis *et al*, 2013). Similar observations have been made in murine alveolar epithelial cells where IFN-λ was mostly produced followed by IFN-β and at last IFN-α, which was only produced in small amounts (Ioannidis *et al*, 2013). Combining the data, we arrive at a model where production of IFN-λ provides efficient

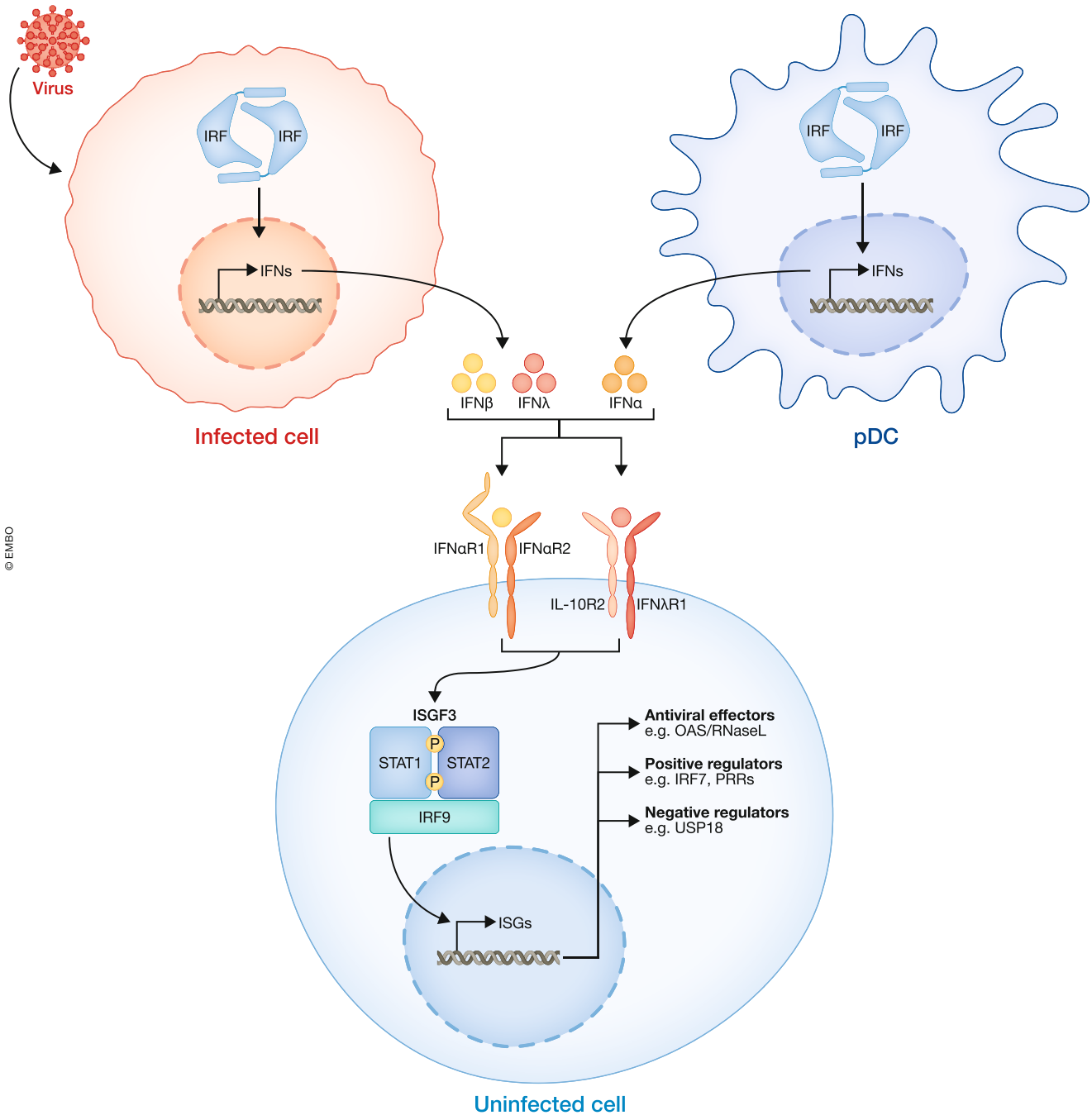

Figure 3. Interferon signaling leads to the establishment of an antiviral state in uninfected cells.
Recognition of a viral infection in infected cells leads to production of IFN-β and IFN-λ, whereas recognition of viruses by pDCs leads to production of large amount of IFN-α. The IFNs signal through their respective receptor complexes to induce the expression of ISGs in uninfected cells and thereby establish an antiviral state.

protection of the lung epithelium supplemented by limited production of IFN-β, which provides local protection of underlying tissues. Clearly, type I IFNs are critically important, as they prevent the virus from penetrating deeper into our body and offer the strength of a systemic IFN response should the virus penetrate the epithelium. Yet, their effect comes with a substantial cost caused by inflammation and therefore it is beneficial for the host only to deploy systemic type I IFN responses when absolutely needed.

In some of the important respiratory infections, such as IAV and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the inflammatory response poses more of a threat than direct cytotoxicity caused by the virus. The absence of the IFN-λR1 receptor chain on most professional immune cells means that type III IFNs have much weaker pro-inflammatory activity than type I IFNs. In contrast, the IFN-λR1 chain is expressed by all epithelial cells and therefore type III IFNs can exert the same antiviral effect as type I

IFNs during respiratory infections while causing less inflammation. Furthermore, treatment with type I IFN led to successful inhibition of IAV replication in mice, although with increased production of pro-inflammatory cytokines and increased mortality of the infected mice. In contrast, treatment with type III IFN also led to successful inhibition of IAV replication but was accompanied by a reduced inflammatory response as well as increased survival of infected mice (Davidson *et al*, 2016; Galani *et al*, 2017). Type I IFN has been used extensively as an antiviral therapy against HCV but is being replaced with modern direct acting antiviral drugs, which have substantially fewer side effects and higher success rates. Based upon the abovedescribed mice data, type III IFN was suggested as a potential therapeutic against both IAV and SARS-CoV-2 (Davidson *et al*, 2016; O'Brien *et al*, 2020; Prokunina-Olsson *et al*, 2020) with less inflammatory damage than type I IFN. Recent clinical trials using pegylated IFN-λ in SARS-CoV-2 infected patients, show a benefit if patients are treated with IFN-λ during the early phase of the infection (Reis *et al*, 2023). In turn, this then poses a clinical challenge to identify and treat patients early enough.

At higher doses, IFNs also exert an anti-proliferative effect and prolonged IFN responses can harm highly proliferative tissues, like the bone marrow or epithelial tissues (Parker *et al*, 2016). In lung epithelial cells, both type I and type III IFNs induce an anti-proliferative effect via a mechanism involving induction of p53 (Broggi *et al*, 2020; Major *et al*, 2020). Thus, at early stages of a respiratory infection, the IFN response is critical to limit viral replication but at later stages, a prolonged IFN response can prevent proper repair of the lung epithelial due to its anti-proliferative effect and thereby expose patients to secondary bacterial infections (Planet *et al*, 2016; Rich *et al*, 2019).

We would like to finish this section by alluding to some of the important unanswered questions in this area. Which cells are the primary source of IFN during viral infections? IFN production can originate both from infected cells that we presume recognize the virus through one of the cytosolic sensing pathways (STING or MAVS) or from non-infected immune cells that acquire virally derived PAMPs by phagocytosis or related mechanisms and recognize those PAMPS via TLR receptors (Bruni *et al*, 2015). Many studies address the ability of individual cell types to produce various subtypes of IFN *in vitro*, but at present we have little information on the *in vivo* importance of different cell types during an ongoing viral infection. Specifically, we need to address the role of infected versus non-infected cells as IFN producers, the balance between production of IFN-λ versus IFN-β during early stages of infection and potential differences between the mouse model and humans while keeping in mind that mice lack IFN-λ1 and -4.

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The authors declare that they have no conflict of interest.

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