The IRF Family Transcription Factors at the Interface of Innate and Adaptive Immune Responses

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The interferon-regulatory factor (IRF) family, originally identified as transcriptional regulators of the type I interferon system, consists of nine members in mammals. A large number of studies have revealed the versatile and critical functions performed by this transcription factor family in immunity and other biological processes. Most notably, the advances in the study of signal transducing innate immune receptors have placed many IRF members as central mediators in the regulation of innate immune responses. In parallel, mechanistic studies have made it clearer that many IRFs exert their function either in cooperation or competition with other factors. In this article, we discuss current advances on the multipurpose and critical functions of IRFs in the regulation of innate immunity, particularly as they instruct adaptive immunity.

Prompt and tightly regulated cellular responses are central to host immunity. Such responses are coordinated by various intricate gene-regulatory networks, which mediate the rapid alterations in gene-expression programs within the cell. Such regulatory networks are controlled, in part, through the differential expression, posttranslational modification, and interconnection of transcription factors within a stimulated cell (Tjian and Maniatis 1994). The prototypical and most extensively studied transcription factor is nuclear factor- κ B (NF- κ B). The importance of NF- κ B to gene-regulatory networks of immune responses, including the induction of the genes that encode interferons (IFNs) and proinflammatory cytokines, is well documented (Hayden and Ghosh 2008; Grivennikov et al. 2010). More recently, members of the interferon-regulatory factor (IRF) family of transcription factors have gained much attention for their regulation of the development and responses by immune cells because of their markedly diverse roles in regulating gene-expression networks within the immune system.

The mammalian IRF family consists of nine members: IRF1, IRF2, IRF3, IRF4 (also known as PIP, LSIRF, or ICSAT), IRF5, IRF6, IRF7, IRF8 (also known as ICSBP), and IRF9 (also known as ISGF3 γ) (Honda and Taniguchi 2006; Tamura et al. 2008). Each IRF protein contains a conserved amino-terminal DNA-binding domain, which recognizes DNA similar in sequence to the IFN-stimulated response element (ISRE; $^A/G$ NGAAANNGAAACT) (Darnell et al. 1994; Escalante et al. 1998; Fujii et al. 1999). Structural analyses of IRF3 revealed that the His40 residue within loop 1 (L1 loop) binds an upstream “AA” dinucleotide sequence, whereas the α -helix 3 motif binds the downstream “GAAA” sequence (Panne et al. 2007) and, expectedly, these structures are well-conserved among IRFs.

The primary amino acid sequences of the carboxy-terminal region, responsible for the regulation of IRFs’ transcriptional activities, show more diversity within the family as compared to the amino-terminal region. This region is critical for specifying interactions between IRFs and with other transcription factors or cofactors, thereby conferring specific transcriptional activities and biological functions upon each IRF protein (Panne et al. 2007; Tamura et al. 2008; Chen and Royer 2010). Of note, two types of association modules have been identified within the carboxy-terminal region of IRFs: IRF-associated domains 1 and 2 (IAD1 and IAD2). IAD1 is conserved in all IRFs except for IRF1 and IRF2 (Sharf et al. 1997) and possesses structural similarities with the Mad-homology 2 (MH2) domain of the Smad family of transcription factors, which are crucial mediators of transforming growth factor- β signaling (Ikushima and Miyazono 2010). IAD2 is shared by IRF1 and IRF2. The nature of the protein–protein interaction dictated by these domains may determine whether the protein complex functions as a transcriptional activator or repressor, and specify the DNA sequences adjacent to ISRE to which the complex can bind (Honda and Taniguchi 2006).

With the discovery of signal-transducing pattern-recognition receptors (PRRs), used by the innate immune system to recognize pathogen-associated molecular patterns (PAMPs) (Janeway and Medzhitov 2002), IRFs have gained much attention as essential regulators for the activation of immune cells. Thus far, many classes of PRRs have been identified: Toll-like receptors (TLRs), retinoic acid-inducible gene-I (RIG-I)-like receptors (RLRs), NOD-like receptors (NLRs), C-type lectin receptors (CLRs), and other nucleic acid-sensing receptors (Blasius and Beutler 2010; Takeuchi and Akira 2010; Kawai and Akira 2011; Sancho and Reis e Sousa 2012).

Depending on the nature of the pathogen and responding cell type, these recognition systems are differentially activated and elicit distinct cellular responses. In recent years, an extensive number of studies have revealed that IRFs function as important mediators of these responses. Also of note are critical roles of IRFs in the regulation of cellular responses linked to oncogenesis, for which we refer the reader to related review articles published elsewhere (Takaoka et al. 2008; Yanai et al. 2012).

IRFS IN TYPE I IFN-INDUCING CYTOSOLIC PRR SIGNALING

Numerous PRRs that can detect cytosolic nucleic acids have been identified and most of them can evoke type I IFN responses via activation of IRFs, particularly IRF3 and IRF7. Of note, these nucleic acids, as well as those that activate TLRs, all need to bind to HMGB family of proteins to exert their immunogenic activities (Yanai et al. 2009). The type I IFN-inducing cytosolic PRRs include the RLR family (comprising melanoma differentiation-associated gene 5 (MDA5) and RIG-I), stimulator of interferon genes (STING), DNA-dependent activator of IRFs (DAI), DEAD box polypeptide 41 (DDX41) interferon-gamma-inducible protein 16 (IFI16), RNA polymerase III (Pol III), and leucine rich repeat interacting protein 1 (LRRFIP1) (Fig. 1) (Fritz et al. 2006; Takeuchi and Akira 2010; Keating et al. 2011; Holm et al. 2013). The RLR proteins are composed of two amino-terminal caspase recruit domains (CARDs), a central DEAD box helicase/ATPase domain, and a carboxy-terminal regulatory domain (Yoneyama et al. 2005; Loo and Gale 2011). They are localized to the cytoplasm and are essential sensors for cytosolic RNAs (Andrejeva et al. 2004; Yoneyama et al. 2004; Kato et al. 2006). In general, double strand RNA (dsRNA) or 5'-triphosphate RNA derived from RNA viruses as well as 5'-triphosphate RNA from DNA viruses via Pol III are recognized by RLRs (Ablasser et al. 2009; Chiu et al. 2009). Specifically, RIG-I is critical for the recognition of shorter dsRNA (<1 Kbp) and 5'-triphosphate RNA, whereas MDA5 is critical for detection of longer dsRNA (>2 Kbp) (Kato et al. 2008; Takeuchi and Akira 2009). There is also evidence that RLRs contribute to the direct recognition of cytosolic DNA (Choi et al. 2009). The helicase domain is responsible for the detection of nucleic acids, whereas the CARD domain, exposed upon ligands binding to these receptors, triggers signaling cascades by interacting with the amino-terminal CARD-containing adaptor IFN- β -promoter stimulator 1 (IPS-1, also known as MAVS, CARDIF, or VISA) (Kawai and Akira 2006a). IPS-1 also contains a transmembrane region that is associated with the mitochondrial outer membrane, an event critical to triggering downstream signaling events. LGP2 is another, as yet poorly characterized, member of the RLR family that may play a role in sensitizing MDA5 for its activation by dsRNA (Childs et al. 2013).

The cytosolic DNA-sensing system also evokes protective and pathological immune responses (Paludan and Bowie 2013). The first discovered sensor, DAI (also

known as ZBP1 or DLM1), activates cytosolic double strand DNA (dsDNA)-mediated type I IFN responses in a cell type-specific manner (Takaoka et al. 2007; Wang et al. 2008). Although DAI is necessary for cytosolic DNA-mediated type I IFN gene induction in mouse L929 cells, it is dispensable in other cells such as mouse embryonic fibroblasts (MEFs) or bone marrow-derived dendritic cells (Ishii et al. 2008; Wang et al. 2008). It is also reported that DAI is critical for cytomegalovirus-induced type I IFN gene expression in human fibroblasts (DeFilippis et al. 2010). Further study is required to fully determine cell type-specific roles of DAI in cytosolic DNA-mediated type I IFN responses.

Thereafter, an adaptor protein termed STING (also known as MITA, ERIS, or TMEM173) was identified (Ishikawa and Barber 2008; Zhong et al. 2008). Cells and mice lacking *Sting* show impaired IFN production in response to both DNA and RNA stimulation. However, recent data have suggested that STING is not the primary sensor of cytosolic DNA. Rather, DDX41, another cytosolic DNA sensor, functions as a direct receptor for cytosolic DNA (Parvatiyar et al. 2012). IFI16, a PYHIN protein, was also identified as a cytosolic DNA sensor that mediates the induction of IFN- β (Unterholzner et al. 2010). DDX41 and IFI16 directly associate with IFN-inducing viral DNA motifs, and then are recruited to STING to induce type I IFN gene expression. More recent reports have showed that cyclic guanosine monophosphate-adenosine monophosphate (cGAMP), which is produced in response to cytoplasmic DNA by cGAMP synthase (cGAS), binds to STING and then induces type I IFNs (Sun et al. 2013; Wu et al. 2013). Consistent with this, STING was shown to be critical for the recognition of cyclic diadenosine monophosphate (c-di-AMP) and cyclic diguanosine monophosphate (c-di-GMP) derived from bacteria, data which further indicate that STING is not a direct sensor of DNA but that of cyclic nucleotides (Burdette et al. 2011; Parvatiyar et al. 2012; Yin et al. 2012).

IRFs in RLR Signaling

RIG-I and MDA5 physically interact with IPS-1 via a CARD-CARD domain interaction following binding to cognate ligands. IPS-1 then relays signals to TANK-binding kinase 1 (TBK1) and inhibitor of NF- κ B kinase ϵ (IKK ϵ), which then phosphorylate IRF3 and IRF7 (Fitzgerald et al. 2003; Sharma et al. 2003). IRF3 is constitutively expressed and initially resides in the cytosol in a latent form, owing to its carboxy-terminal auto-inhibitory region. Following cytosolic RLR stimulation, the IPS-1-activated TBK1 phosphorylates IRF3 at specific serine residues in the auto-inhibitory region, which allows IRF3 dimerization and nuclear translocation. As a result, the IRF3 dimer interacts with other transcription factors and coactivators CBP or p300 to form a holocomplex for transcriptional activation of type I IFNs and other target genes (Yoneyama et al. 1998).

Unlike IRF3, IRF7 is expressed at low levels in most cells and is strongly induced by type I IFN signaling via

Figure 1. IRFs in cytosolic nucleic acid–induced type I IFN gene expression. The presence of double-strand RNA (dsRNA) or double-strand DNA (dsDNA) in the cytosol triggers innate immune responses through the cytosolic pattern-recognition system. The interaction of dsRNA with RIG-I or MDA5 induces their interaction with the adaptor protein IPS-1. This interaction results in the activation of TBK1. The interaction of dsDNA with DDX41 and IFI16 allows STING to activate TBK1. Cyclic diadenosine monophosphate (c-di-AMP), cyclic diguanosine monophosphate (c-di-GMP), and cyclic guanosine monophosphate–adenosine monophosphate (c-GAMP) act as direct ligands for STING to activate TBK1. IRF3, IRF5, and IRF7 are phosphorylated by TBK1, resulting in induction of type I IFNs. IRF3 also cooperates with β -catenin downstream from LRRFIP1.

binding of the transcription factor ISGF3 (a heterotrimeric complex consisting of Stat1, Stat2, and IRF9) to its gene promoter. IRF7 also resides in the cytosol of resting cells and, upon cytosolic RLR stimulation, undergoes serine phosphorylation of its carboxy-terminal region, allowing it to translocate into the nucleus and induce expression of type I IFN genes. Thus, the positive-feedback regulation of IRF7 comes into effect to achieve the full-blown induction of type I IFN genes during the later phases of the response (Honda and Taniguchi 2006; Chen and Royer 2010).

In addition to IRF3 and IRF7, IRF5 is also involved in the RLR signaling pathway. Indeed, *Irf5*^{−/−} mice show a reduction in the serum levels of type I IFNs when challenged with vesicular stomatitis virus (VSV) or Newcastle disease virus (NDV) (Yanai et al. 2007; Paun et al. 2008). More recent study also showed the involvement of IRF5 in West Nile virus or murine norovirus infection-

mediated *Irfn* gene induction (Lazear et al. 2013). However, the function of IRF5 may be cell type-specific since, unlike macrophages, type I IFN response evoked by VSV infection is normal in *Irf5*^{−/−} MEFs (Yanai et al. 2007). Although IRF5 can also be phosphorylated by TBK1, the detailed activation mechanism of IRF5 in the RIG-I signaling pathway is still poorly understood (Lin et al. 2005).

There is report showing that IRF8 is also required for type I IFN induction in virus-stimulated dendritic cells, wherein it binds to the promoters of type I IFN genes and magnifies the late phase of transcription in dendritic cells (Tailor et al. 2007).

IRFs in Cytosolic DNA Sensor Signaling

In response to cytosolic DNA, STING forms a complex with TBK1 and IRF3 and serves as an adaptor protein that

links TBK1 to the activation of IRF3 (Ishikawa and Barber 2008; Zhong et al. 2008; Tanaka and Chen 2012; Paludan and Bowie 2013). Recent data indicate that DDX41 and IFI16 are required for the recruitment of STING to activate the TBK1–IRF3-dependent pathway (Unterholzner et al. 2010; Parker et al. 2011; Parvatiyar et al. 2012). LRRFIP1 was also reported to recognize cytosolic dsDNA (Yang et al. 2010), and it interacts with and activates β -catenin, which induces IFN- β expression through binding to IRF3 and recruiting the acetyltransferase p300 to the IFN- β promoter (Yang et al. 2010).

IRFS AND TLR SIGNALING

To date, 13 different TLRs (10 in human and 12 in mice) have been identified and found to recognize a variety of PAMPs derived from bacteria, viruses, fungi, and/or protozoa to trigger immune responses including the induction of type I IFNs and proinflammatory cytokines (Medzhitov 2001; Kawai and Akira 2006b). All TLRs are membrane-bound type receptors which use adaptor proteins MyD88 (myeloid differentiation primary-response protein 88) and/or TRIF (TIR domain-containing adaptor including IFN- β) to activate IRFs and other transcription factors.

IRF3 and IRF7 in the TRIF-Dependent Pathway

TLR4 and TLR3 both use the TRIF adaptor protein to activate IRF3 and induce type I IFNs, albeit weakly as compared to RLR-mediated induction (Fig. 2A) (Negishi et al. 2012). TLR4 is a cell surface receptor that recognizes LPS from gram-negative bacteria and a variety of other PAMPs or DAMPs (damage-associated molecular patterns). TLR4 signaling results in the induction of the genes encoding IFN- β , but not the genes encoding IFN- α except IFN- α 4 (Kawai et al. 2001; Sakaguchi et al. 2003). After activation of TLR4, TRIF recruits TRAF3, NAP1, and TBK1 (Hemmi et al. 2004; Perry et al. 2004; Oganessian et al. 2006). The induction of IFN- β in response to LPS is abolished in *Ir3*^{-/-} dendritic cells, whereas this induction is almost unaffected in *Ir7*^{-/-} cells (Sakaguchi et al. 2003; Honda et al. 2005). Consistent with these results, *Ir3*^{-/-} mice show resistance to LPS-induced endotoxin shock, for which IFN- β is critical (Sakaguchi et al. 2003). Thus, IFN- β induction by TLR4 is mainly mediated by IRF3, rather than IRF7, via its phosphorylation by TBK1.

TLR3 is located within the membrane of endosomes and phagosomes or at the cell surface, in the case of endothelial and natural killer cells (Tamura et al. 2008). TLR3 recognizes the synthetic dsRNA analog poly(rI:rC) and viral dsRNA derived from either dsRNA or short strand RNA (ssRNA) viruses (Alexopoulou et al. 2001; Wang et al. 2004; Rudd et al. 2006). TLR3 is also involved in the defense against infection by some DNA viruses or parasites (Tabeta et al. 2004; Aksoy et al. 2005; Flandin et al. 2006; Zhang et al. 2007). The activation of TLR3, like TLR4, can induce type I IFN expres-

sion via a TRIF-dependent pathway. Although IRF3 plays an essential role in this induction, a weak induction of type I IFN mRNAs by poly(rI:rC) is still observed in *Ir3*^{-/-} dendritic cells. This residual induction was completely abolished in IRF3 and IRF7 doubly deficient dendritic cells (H Negishi and T Taniguchi, unpubl.). Therefore, in contrast to TLR4, IRF3, and IRF7 are both required for TLR3–TRIF-mediated induction of type I IFN genes.

IRF3 and IRF7 in the MyD88-Dependent Pathway

Plasmacytoid dendritic cells are defined by their massive expression of type I IFNs in response to ligands for TLR7 and TLR9, and unlike conventional dendritic cells, express high amounts of TLR7 and TLR9 in endosomes (Kawai and Akira 2006b). TLR7 recognizes genomic ssRNA of ssRNA viruses, whereas TLR9 recognizes hypomethylated CpG DNA motifs present in bacteria and DNA viruses. In contrast to TLR3- or TLR4-mediated, TRIF-dependent IFN gene induction, TLR7 and TLR9 exclusively use MyD88 as its signaling adaptor (Fig. 2B).

IRF7, but not IRF3, directly interacts with the death domain of MyD88 (Honda et al. 2004; Kawai et al. 2004) and is essential for the robust MyD88-dependent induction of IFN gene in plasmacytoid dendritic cells. Splenic plasmacytoid dendritic cells derived from *Ir7*^{-/-} mice show a profound defect in type I IFN gene induction upon infection by DNA and RNA viruses or treatment with synthetic TLR7 or TLR9 ligands (ssRNA or CpG-A, respectively), while the induction is normal in *Ir3*^{-/-} plasmacytoid dendritic cells (Honda et al. 2005). However, a recent study suggests that IRF3 also participates in the TLR9–MyD88-mediated induction of type I IFN gene in plasmacytoid dendritic cells after *Listeria monocytogenes* infection by a still unknown mechanism (Stockinger et al. 2009).

IRF7 also interacts with TRAF6 whose overexpression induces type I IFN genes through the activation of IRF7 (Honda et al. 2004). IRAK1 and IRAK4 are signal transducers between MyD88 and TRAF6 and are required for TLR9-mediated IFN- α induction in plasmacytoid dendritic cells. In addition, IKK α is essential for the phosphorylation of IRF7 (Hoshino et al. 2006). Therefore, the IRAK4–IRAK1–IKK α kinase cascade, which is known to operate within the NF- κ B activation pathway, also leads to IRF7 activation.

Recently, two DExD/H-box helicases, DHX9 and DHX36, were identified as MyD88-dependent sensors of CpG-containing DNA in plasmacytoid dendritic cells (Kim et al. 2010). DHX36 triggers a pathway specific for IRF7 activation to induce IFN- α expression, whereas DHX9 promotes nuclear translocation of the p50 NF- κ B subunit and subsequent induction of NF- κ B-dependent genes such as TNF- α and IL-6 (Kim et al. 2010; Keating et al. 2011).

Other IRFs in TLR Signaling

IRF5 is a critical transcription factor for proinflammatory gene induction downstream from the MyD88-depen-

Figure 2. IRFs in TLR-mediated gene induction. (A) TLR4 signals through at least four adaptors: MyD88, TRIF, TIRAP (Toll/interleukin-1 receptor (TIR)-domain-containing adaptor protein), and TRAM (TRIF-related adaptor molecule). TRAM and TRIF mediate the activation of IRF3. TRIF associates with TBK1 through NAP1 (NAK-associated protein 1) and TRAF3, and then TBK1 phosphorylates IRF3. IRF1 and IRF5 interact with and are activated by MyD88-dependent signaling. IRF4 binds to MyD88 in a region that overlaps the IRF5-binding region, thus inhibiting the binding of IRF5 to MyD88 and attenuating the MyD88-mediated activation of IRF5. Activated IRFs translocate to the nucleus, where they induce transcription of their specific target genes. (B) In plasmacytoid dendritic cells, after activation of TLR7 or TLR9, IRF7 binds to MyD88 and is activated by a protein-kinase cascade that involves IRAK4, IRAK1, and IKKα. TRAF6 and TRAF3 also interact with and activate IRF7.

dent signaling pathway (Takaoka et al. 2005). Similar to IRF7, IRF5 also interacts with MyD88 and TRAF6 (Takaoka et al. 2005). However, unlike IRF7, IRF5 interacts with the central region (the intermediary domain and part of the TIR domain) of MyD88, causing the competition with IRF4 described in more detail below (Negishi et al. 2005). Following TLR activation, IRF5 translocates

from the cytoplasm to the nucleus, where it binds to ISREs within promoters of target genes such as *Il12b*. Interestingly, IRF5 also contributes to MyD88-dependent type I IFN gene induction in plasmacytoid dendritic cells stimulated with CpG-B oligonucleotides (Yasuda et al. 2013). Consistent with this report, polymorphisms of the human *Ir5* gene are suggested to be associated with type

I IFN-related autoimmune diseases, especially systemic lupus erythematosus (SLE) (Graham et al. 2006, 2007).

Although the detailed mechanism for the activation of IRF5 is still not fully elucidated, it is suggested that TRAF6-mediated K63-linked ubiquitination is important for IRF5 nuclear translocation in TLR7/9–MyD88-dependent signaling (Balkhi et al. 2008). In addition, phosphorylation of serine/threonine residues in a carboxy-terminal auto-inhibitory region was shown to be a crucial step for dimer formation of IRF5 and interaction with CBP/p300 in the nucleus (Chen et al. 2008).

Like IRF5, IRF1 also interacts with the central region of MyD88 (Negishi et al. 2006). Although IRF1 is strongly induced by type II IFN (IFN- γ), IFN- γ signaling itself is insufficient to fully activate IRF1. Rather, TLR9 engagement causes MyD88-associated IRF1 to undergo posttranslational modifications and migrate into the nucleus. Thus, IRF1 is critical for the IFN- γ enhancement of the TLR-dependent gene induction program.

IRF4 functions in the negative feedback regulation of TLR signaling. Upon TLR activation, *Irf4* mRNA is induced and IRF4 protein principally localizes in the nucleus. However, a significant fraction also exists in the cytoplasm where it colocalizes with MyD88. Because IRF4 binds to the same region of MyD88 that IRF5 binds, TLR-induced IRF4 competes with and inhibits the sustained activity of IRF5. Consistent with its role as a negative regulator of TLR signaling, induction of proinflammatory cytokines by TLR stimulation is enhanced in *Irf4*^{-/-} cells, and *Irf4*^{-/-} mice are highly sensitive to endotoxic shock induced by CpG-B (Negishi et al. 2005). IRF4 is also suggested as a negative regulator of TLR-mediated NF- κ B activation (Honma et al. 2005).

Although IRF8 binds to TRAF6 (Zhao et al. 2006), it has not been shown to bind to MyD88 (Negishi et al. 2005). Its interaction with TRAF6 suggests that IRF8 functions in the cytosol. Indeed, IRF8 participates in the TLR9–MyD88-dependent activation of NF- κ B to induce proinflammatory cytokines such as TNF- α and IL-6 (Tsujimura et al. 2004). On the other hand, IRF8 also acts as a transcription factor which is required for the induction of *Il12b* gene upon various PAMP stimuli in macrophages and dendritic cells, and for the induction of type I IFN genes by viruses and TLR ligands in dendritic cells (Savitsky et al. 2010).

IRFS AND NLR SIGNALING

The NLR family is comprised of three distinct subfamilies: the NODs (NOD1-2, NOD3/NLRC3, NOD4/NLRC5, NOD5/NLRX1, and CIITA), the NLRPs (NLRP1-14, also called NALPs), and the IPAF subfamily (Schroder and Tschoop 2010). They perform cytoplasmic surveillance for PAMPs. Among NLR family proteins, NOD2 is reported to mediate ssRNA-induced type I IFN production (Sabbah et al. 2009). Although IRF3 and IRF5 were suggested to be involved in the NOD2-mediated IFN induction (Sabbah et al. 2009; Chang Foreman et al. 2012), it has not yet been fully determined whether other IRFs are involved in the gene regulation by NLRs. The inflamma-

some is a protein complex that activates caspase-1 composed of NLRs, ASC (apoptosis-associated speck-like protein containing a CARD), and caspase-1. Inflammasomes are molecular platforms activated upon cellular infection or stress which trigger the maturation of IL-1 β to activate components of innate immune system (Schroder and Tschoop 2010). The role of IRFs in the regulation of inflammasomes has not yet been clarified and further investigation is needed to uncover if and what roles IRFs play in NLR signaling and inflammasome systems.

IRFS AND CLR SIGNALING

CLRs comprise a large family of receptors that bind to carbohydrates in a calcium-dependent manner (Sancho and Reis e Sousa 2012). CLRs are expressed by most cell types including macrophages and dendritic cells. Dectin-1, a CLR specific for β -glucans, plays an important role in antifungal innate immunity. Upon binding to its ligand, Dectin-1 triggers phagocytosis and activation of Src and Syk kinases through its ITAM-like motif (Sancho and Reis e Sousa 2012). A recent study showed that IRF5, but not IRF3 or IRF7, acts as a signal mediator under Dectin-1–Syk pathway and is required for Dectin-1-induced IFN- β production (Del Fresno et al. 2013). Indeed, the production of type I IFNs in renal infiltrating dendritic cells, mediated by Dectin-1–Syk–IRF5 signaling, plays a crucial role in defense against *Candida albicans* infection (Del Fresno et al. 2013). Further studies are required to elucidate relationships between CLRs and other IRFs.

INTERACTION OF IRFS WITH OTHER TRANSCRIPTION FACTORS

The cooperation of IRFs with other IRFs and other transcription factors is thought to be an important mechanism by which they control the specificity and magnitude of a transcriptional event. The classic example is IRF9, which forms a heterotrimeric complex with Stat1 and Stat2. This complex, termed ISGF3, is essential for evoking antiviral innate responses by type I and type III IFNs (Levy et al. 2011).

IRFs can also associate with NF- κ B family members to cooperatively regulate the transcription of several cytokine genes. The best known of these is the IFN- β gene that requires the coordinated binding of NF- κ B, AP1 (a complex of ATF2 and JUN), and homodimers or heterodimers of IRF3 and IRF7, followed by binding of coactivators CBP or p300 forming a complex termed “the enhanceosome.” The interaction between IRFs and NF- κ B in this instance occurs through their mutually independent binding to the promoter (Honda and Taniguchi 2006; Panne et al. 2007). IRF3 can also form a complex directly with the NF- κ B subunit p65 (or Rel-A) by binding to the Rel-homology domain (RHD) of p65. In this case, IRF3 does not bind to an ISRE, but functions as a promoter-specific and signal-specific cofactor to activate transcription of some NF- κ B-dependent genes. It has also

been shown that IRF3–p65 complexes can support the full induction of ISRE-driven genes in response to TLR4 signaling. Thus, depending on the nature of signaling, IRF3 can either function as a coactivator of p65 for the transcription of NF- κ B-dependent genes, or p65 serves as a cofactor of IRF3 for the transcription of IRF-dependent genes (Wietek et al. 2003; Ogawa et al. 2005). Interestingly, another facet of the cooperation between NF- κ B and IRF3 was reported, in which glucocorticoid receptor complex represses TLR-dependent gene expression through IRF3 or NF- κ B. Ligand-bound glucocorticoid receptor prevents interactions between p65 and IRF3 by binding to and competing with IRF3 for the RHD of p65. As a result, the glucocorticoid receptor represses a large set of inflammatory-response genes (Ogawa et al. 2005).

Recent studies have revealed a mechanism of cell specificity for IRF4 (Glasmacher et al. 2012; Li et al. 2012; Tussiwand et al. 2012). IRF4 is a key regulator of the differentiation of mature B cells into antibody-secreting plasma cells (Shaffer et al. 2009) and contributes to the development of multiple T_H cell subsets (Pernis 2002; Lohoff and Mak 2005). The mechanism of these cell type-specific functions is partially explained by interaction of IRF4 with other transcription factors (Li et al. 2012; Tussiwand et al. 2012). In B cells, the largely B-cell-restricted Ets family transcription factor PU.1 interacts with IRF4 and allows IRF4 to regulate genes whose promoter or enhancer regions contain composite Ets-IRF consensus motif elements (EICEs, GGAAAnnGAAA), including immunoglobulin κ and λ light chain genes (Brass et al. 1996; Escalante et al. 2002). In T_H17 cells, where PU.1 expression is low, IRF4 instead functionally cooperates with an AP1 family protein BATF to act on AP1-IRF consensus motif elements (AICEs, TGAnTCA/GAAA), resulting in the activation of genes such as *Il17a* (Glasmacher et al. 2012; Li et al. 2012; Tussiwand et al. 2012). Each IRF-cofactor combination targets a particular set of genes, determined by the cognate binding sequence elements in the regulatory regions of target genes. Through this combinatorial interaction with different transcription factors, IRFs can activate or repress several target genes in a cell type-dependent manner.

IRFS IN CROSS-INTERACTION BETWEEN PRR SIGNALING

Suppression of TLR-Activated IRF5 by RLR-Activated IRF3

The innate PRR signaling pathways are known to interact with each other in a variety of ways, including cooperation, complementation, and compensation (Nish and Medzhitov 2011). Recent studies have revealed the involvement of IRFs in the signal cross talk that regulates PRR-mediated gene induction. Cooperation of TLR-induced signaling pathways in response to pathogen is the most well documented type of interaction in situations where more than one TLR ligand is present (Nish and Medzhitov 2011). In particular, MyD88- and TRIF-dependent pathways synergistically activate proinflammatory

gene induction. This synergistic gene induction is dependent on IRF5, indicating that both pathways cooperate to activate this transcription factor (Ouyang et al. 2007).

Recently, IRF3 was shown to uniquely participate in a process termed “signaling interference,” which defines a new facet of signal cross talk. This occurs between TLRs and cytosolic nucleic acid-sensing receptor pathways, wherein virus-induced activation of the latter pathway results in the selective suppression of bacteria- or TLR-induced *Il12b* gene that encodes the common subunit of IL-12 and IL-23, which are critical for driving T_H1- and T_H17-type T-cell responses, respectively (Negishi et al. 2012; Koshiba et al. 2013). This suppression is mediated by IRF3, which is strongly activated by cytosolically derived nucleic acids. IRF3 binds dominantly to ISREs within both promoter and enhancer, and interferes with the TLR-induced assembly of a productive transcription factor complex by IRF5 (Fig. 3A,B). Consequently, RLR activation by viruses in mice attenuates TLR-induced T_H1- and T_H17-type T-cell responses and viral infection causes lethality at sublethal doses of bacterial infection.

Although the carboxy-terminal region of IRF3 determines its cooperation with other transcription factors, its amino-terminal DNA-binding region was shown to be critical for IRF3-mediated *Il12b* gene suppression (Koshiba et al. 2013). Interestingly, in the *Il12b* promoter/enhancer, the 5'AA dinucleotide sequence of ISRE, which interacts with the His40 residue within loop 1 (L1 loop) of IRF3, deviates from consensus ISRE in all enhancer and promoter ISREs known to be activated by IRF3. Of note, these deviated ISRE sequences are found among other mammalian species, which may indicate an evolutionary significance of this deviation (Koshiba et al. 2013). The alteration of this binding sequence to a genuine ISRE sequence allows the *Il12b* promoter/enhancer to be transcriptionally activated by IRF3, which further supports the importance of this binding sequence. Thus, the interaction of IRF3 with the “deviated” ISREs sequence may cause IRF3 to undergo a conformational change distinctly different from IRF3 bound to the “consensus” AA sequence and render IRF3 to induce a transcriptionally inactive state (Fig. 3C) (Koshiba et al. 2013). Under the same conditions, TLR-activated IRF3 mediates the cooperative induction of other genes such as *Il33* and *Tslp*, both of which contain “genuine” ISREs within their promoters. Since these cytokines are known to promote T_H2-type T-cell responses, the dual function of virus-activated IRF3, namely, suppression of *Il12b* gene and activation of *Il33* and *Tslp* genes may account for the enhanced T_H2 response in virally infected mice (Negishi et al. 2012).

Immunological Consequences of IRF3-Mediated Signaling Cross-Interference

The above observations may have implications for several immunopathological conditions. For instance, viral infections that activate RIG-I are implicated in triggering asthma with manifestations of a T_H2 signature, and patients infected by human immunodeficiency virus (HIV), known to activate IRF3, are more susceptible to infec-

Figure 3. Schematic illustrations of IRF3-mediated cross-interference between TLR and RLR. (A) The *Il12b* gene is induced through a TLR-IRF5-mediated pathway upon bacterial infection, causing promotion of T_H1 - and T_H17 -type antibacterial T-cell responses. (B) In the case of simultaneous infection with viruses and bacteria, RLR stimulation by viruses results in the dominant binding of IRF3 over bacteria- and TLR-activated IRF5 to the promoter/enhancer of the *Il12b* gene; this is accompanied by a loss of C/EBP β and Oct-2 recruitment, thereby inhibiting TLR-induced gene expression (Negishi et al. 2012; Koshiba et al. 2013). (C) The IRF3-mediated suppression of *Il12b* promoter/enhancer; a speculative view. Activated IRF3 binds to its target genes, such as *Irfn*, *Il33* and *Tslp*, and recruits other factors/coactivators to activate gene transcription (left panel). On the other hand, IRF3 interacts with ISREs containing the "deviated" 5'AA sequence of the *Il12b* gene and undergoes a conformational change that is different from IRF3 bound to the "consensus" AA sequence (right panel). This conformational change may render IRF3 to induce a transcriptionally inactive state, whereas IRF5 interactions to these deviated ISREs can mediate "productive" complex formation. Supporting this notion are data showing that the enhancer and promoter could be converted from "IRF3-repressible" to "IRF3-activatable" when these deviated sequences were converted to the "consensus" AA sequence. However, this view needs further elaboration.

tious agents that typically generate T_H1 -type responses (Holt and Sly 2002; Manel et al. 2010). Thus, enhanced T_H2 -type and attenuated T_H1/T_H17 responses, which result from viral infections and for which the RLR-IRF3 axis is critical, may account, at least in part, for the development of these pathogenic events.

What is the evolutionary significances of the intricate regulatory mechanism that switches IRF3 to become a

transcriptional suppressor? It might have arisen and been selected to hedge the risk(s) of immune pathogenesis by which the system balances intrinsic "pros and cons" to maintain the host's delicate homeostasis. Thus, upon viral infection, a robust activation of the innate immune system, a hallmark of which is the induction of type I IFN genes by IRF3, is of immediate necessity as the first line of defense of the host. On the other hand, the antiviral

responses, if left uncontrolled, may increase the risk of excessive and sometimes life-threatening inflammation, or perhaps, under chronic conditions, to predispose the host toward autoimmunity, characterized by excessive T_H1 and T_H17 -type responses. Indeed, there is ample evidence for the involvement of type I IFN signaling per se to the development or progression of autoimmunity (Banchereau and Pascual 2006; Trinchieri 2010). Therefore, antiviral responses may be viewed as a “double-edged sword” in immunity. In this context, the induction of type I IFNs for antiviral responses fulfills the “pros,” whereas the suppression of inflammatory immune responses, by suppressing *Il12b* expression, is a means to minimize the “cons” of the responses. In short, IRF3 may serve as a guardian of homeostatic immune responses. It will be interesting to examine whether other types of signaling cross-interference also operate for the fine-tuning of the innate receptor-mediated immune responses.

CONCLUDING REMARKS

The IRF family of transcription factors was first identified as regulators of type I IFN gene induction. However, the detailed mechanisms of how IRFs exert their functions in response to viruses or their mimetics remained elusive until the discovery of signal-transducing PRRs. Indeed, the discovery of the various classes of PRRs brought about a breakthrough in the understanding of how viruses and other pathogens trigger signaling events that lead to the activation of IRFs and other transcription factors. As described in this and other articles, much is now known about how each of these IRFs participates in the gene regulation programs downstream from PRRs. However, the history of this family is relatively new as compared to other well-studied transcription factors, such as NF- κ B. Therefore, one may anticipate there are much more broadly-acting functions of IRFs in the regulation of immune responses. It is also clear that the functional diversity of IRFs is driven by their interactions with IRF and other binding partners.

In addition to inducing type I IFN gene expressions, IRFs also serve to sustain or enhance type I IFN gene induction upon viral infections. IRF3 and IRF7 are structurally related and likely evolved from a common ancestor. The IRF7 gene is under the control of IFN signaling for further amplification of the IFN response against viral infections. In fact, multiple IFN- α genes themselves may also have evolved to enhance the IFN-mediated antiviral immune response, and IFN- α and - β genes have diverged from a common ancestor (Taniguchi et al. 1980; Honda and Taniguchi 2006; Tamura et al. 2008). Of note, the induction of most IFN- α genes is dependent on IFN- β , the signaling of which induces IRF7 gene expression. The evolution of these positive-feedback mechanisms must have been beneficial or even essential for the antiviral immunity of the host (Honda and Taniguchi 2006).

Although the beneficial aspects of the type I IFN system on the host defense against viral infection are evident, data also suggest the aberrant activation of type I IFNs

contributes to the development of autoimmune diseases, such as SLE (Banchereau and Pascual 2006). SLE patients are characterized by the production of autoantibodies to cellular macromolecules, typically self-nucleic acids, and these autoantibodies to nucleic acids form immune complexes that can be taken up into endosomes of pDCs or other cells to stimulate TLR7 or TLR9 to produce type I IFNs. Thus, self-RNA and -DNA are potentially immunogenic, and the aberrant activation of these TLRs for type I IFN induction might be responsible for the pathogenesis of autoimmune diseases. IRF5 is another member whose contribution to SLE has gained much attention (Deng and Tsao 2010). Thus, better understanding of how these signaling pathways are turned on and off by the IRF system will be important for the development of new therapeutic interventions for infectious, inflammatory, and autoimmune diseases.

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