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Type I Interferon: The Ever Unfolding Story

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Abstract Since the discovery of interferon 50 years ago, the understanding of the mechanism of the virus-mediated induction of type I IFN and its function has been under intensive investigation. Remarkable progress has been made in recent years both in the identification of cellular receptors detecting the viral infection and in the understanding the signaling pathways resulting in the induction of interferon and interferon-induced genes. In this review of type I interferon, we aim to summarize not only the historical site of the interferon induction and its antiviral function, but also the complexity of the signals that lead to activation of expression of interferon genes and the expanding repertoire of this multifunctional protein.

1
Introduction and Historical Perspective

The innate antiviral response developed as a rapid and regulated defense mechanism of the host against the viral intruder. Antiviral cytokines and chemokines, activated as an early response to viral infection, play a critical role in both the outcome of the infection and its pathogenicity. It has been 50 years since the antiviral protein interferon (IFN) was first described (see the chapter by Lindenmann, this volume). When it was found that the activity of IFN is species-specific, but not virus-specific, it was assumed that IFN will have broad antiviral therapeutic application. However, the IFN system was more complex than originally anticipated and it was later shown that IFN preparations had not only antiviral (Paucker and Cantell 1962), but also many anticellular effects (Gresser et al. 1969). It was also shown that there were at least two antigenic distinct subtypes of type I interferon induced by viral infection: IFNα, produced in leukocytes and IFNβ produced in fibroblasts (Havell et al. 1975). Another antiviral protein was found to be induced in mitogen-activated T lymphocytes and was named IFNγ, or type II IFN (Whellock and Sibley 1965) (see the chapter by Young and Bream, this volume).

Surprisingly, it was found that in fibroblasts, IFNβ could be induced not only by infection with different types of viruses but also by treatment with synthetic double-stranded (ds) RNA, poly rI.rC (Field et al. 1967). Interestingly enough, only ds polyribonucleotides and not single-stranded (ss) polyribonucleotides or polydeoxynucleotides were effective IFN inducers (Colby and Chamberlin 1969; Pitha and Carter 1971; Carter et al. 1972). The structural modification of polyrI.rC aimed at generating a super inducer generally failed (Pitha and Pitha 1971, 1972), leading to the assumption that the IFN-inducing entity in infected cells was the dsRNA intermediate of viral replication (Long and Burke 1971; Sekellick and Marcus 1982). It took another 30 years before the molecular mechanism of the cellular response to dsRNA was uncovered and some of the original observations about the structure of nucleic acid recognized by the cells were challenged.

In precloning times, the biological assay of IFN was the only available endpoint measurement, and therefore it was not clear whether virus- or poly rI.rC-mediated stimulation occurred at the transcriptional or post-transcriptional level (see the chapter by Vilcek, this volume). The first evidence that stimulation of IFN synthesis in infected cells occurs at the transcriptional level was suggested by studies with actinomycin D (Wagner 1964), and it was shown later that IFN mRNA is present only in poly rI.rC-induced and not in uninfected cells. Since this was done before cloning techniques were developed, IFN

mRNA was detected by transfection and translation of cellular RNA in chick cells (de Maeyer-Guignard et al. 1972; Reynolds and Pitha 1974) and later in *Xenopus* oocytes (Reynolds et al. 1975).

The oocyte translation assay was very efficient and facilitated the cloning of human IFNα cDNAs and IFNβ cDNA (Nagata et al. 1980; Derynck et al. 1980a). The cloning of the *IFN* genes opened a Pandora's box. Not only could IFN now be produced in sufficient amounts for clinical studies, but unexpectedly, instead of a single IFN*A* gene, both in humans and mice, the type I IFN family consisted of multiple IFN*A* genes and pseudogenes, and only one IFN*B* gene (Derynck et al. 1980b; Gray et al. 1982; Kelly and Pitha 1985; Zwarthoff et al. 1985) (see the chapter by Pestka, this volume). Both IFN*A* and IFN*B* lack introns (Kelly and Pitha 1985; Weissman and Weber 1986) and are clustered on the short arm of human chromosome 9, or mouse chromosome 4 (Diaz et al. 1994; Kelly et al. 1985). The human IFN*A* family is composed of 15 active genes and 11 pseudogenes, which share about 96% homology. Expansion of the IFN*A* cluster is likely to have occurred by unequal crossing over of the duplicated *IFN* genes (Henco et al. 1985). Although all IFNα are antiviral, functional diversity between individual variants is starting to emerge (Ortaldo et al. 1984; Hilkens et al. 2003). In addition, the human IFN*Ω* family consists of about five pseudogenes and one full gene expressed in leukocytes (Hauptmann and Swetly 1985) and one IFN*K* gene expressed in keratinocytes (LaFleur et al. 2001). These *IFN* genes are also part of the interferon cluster on chromosome 9. All type I IFNs share a common receptor (see the chapter by Uzé et al., this volume). The type II, IFN*G*, gene which contains three introns and maps to the long arm of chromosome 12, signals through its own distinct receptor (see the chapter by van Boxel-Dezaire and Stark, this volume). Lastly, a new group of IL10-related antiviral proteins, type III IFN, were identified in infected cells and named IFNλ (λ 1–3) (Kotenko et al. 2003; Sheppard et al. 2003). While these proteins share some antiviral and immunomodulatory functions with type I IFN, they signal via a distinct receptor (Kotenko et al. 2003).

There has been remarkable progress made in recent years in the identification of the cellular receptors detecting viral infections and in the understanding of the signaling pathways leading to the stimulation of type I *IFN* gene expression. The importance of type I IFN to the activation of effector cell populations and adaptive immunity is also emerging. In this review, we will focus on type I IFN, with an emphasis on IFNα, and we will attempt to summarize our present knowledge about the regulation of IFN*A* transcription, its cell type-specific expression and its general role in the innate immune response against viral infection. We will also discuss the possible harmful effects IFNα and the adverse interplay between the expression of IFNα and autoimmune disorders.

2
Regulation of Type I IFN Gene Expression

2.1
Cellular Recognition of Infection

Type I IFN is rapidly produced in response to viral infection in almost all nucleated cells. Two classes of cellular receptors recognize intracellular viral nucleic acids. Toll-like receptors (TLR), present in the endosomal compartments of the immune cells, detect viral RNA or DNA (Akira et al. 2006; O'Neill 2006). TLR3 detects dsRNA, a common replication intermediate of both DNA and RNA viruses, as well as viral dsRNA released from the apoptotic cells (Alexopoulou et al. 2001; Schultz and Williams 2005). Single-stranded viral genomic RNA, which is enriched in uridine or guanosine residues, is detected by TLR7 and TLR8 (Diebold et al. 2004) and DNA viruses are recognized by TLR9, which detects the unmethylated CpG regions in viral genomic DNA (Lund et al. 2003). The endosomal localization of TLR is essential for the discrimination between viral and cellular nucleic acids (Barton et al. 2004). The second classes of receptors that detect dsRNA produced in infected cells are RNA helicases containing the caspase recruitment domain (CARD), RIG-I and MDA5 (Yoneyama et al. 2004) (Fig. 1). They are expressed ubiquitously in all cell types (see the chapter by Onomoto et al., this volume). Binding of the nucleic ligand to its respective receptor activates signaling pathways leading to the activation of latent transcription factors of the NFkB and interferon regulatory factor (IRF) families that are involved in the activation of the early inflammatory genes (see the chapter by Severa and Fitzgerald, this volume)

2.2
The IRF Family

The molecular mechanism of virus-mediated type I IFN gene induction served as a model for the study of inducible transcription. The 5′ regions of both human and mouse IFN*A* and IFN*B* contain a domain called the interferon regulatory element or virus responsive element (VRE), which has multiple GAAANN repeats (Weidle and Weissman 1983; Goodbourn et al. 1986; Fujita et al. 1987). In addition, IFN*B* VRE contains an NFkB site that binds NFkB/Rel transcription factors (Thanos and Maniatis 1995). Stimulation of IFN*B* transcription is mediated by a ternary complex enhanceosome consisting of NFkB, activated interferon responsive factors (IRFs), and activated protein 1 (AP-1), which are recruited to the VRE of the *IFN* promoter (Du et al. 1993; Merika et al. 1998; Thanos and Maniatis 1995). Since not all IFN*A* subtypes are expressed at the same levels, it was not initially clear whether their levels were regulated

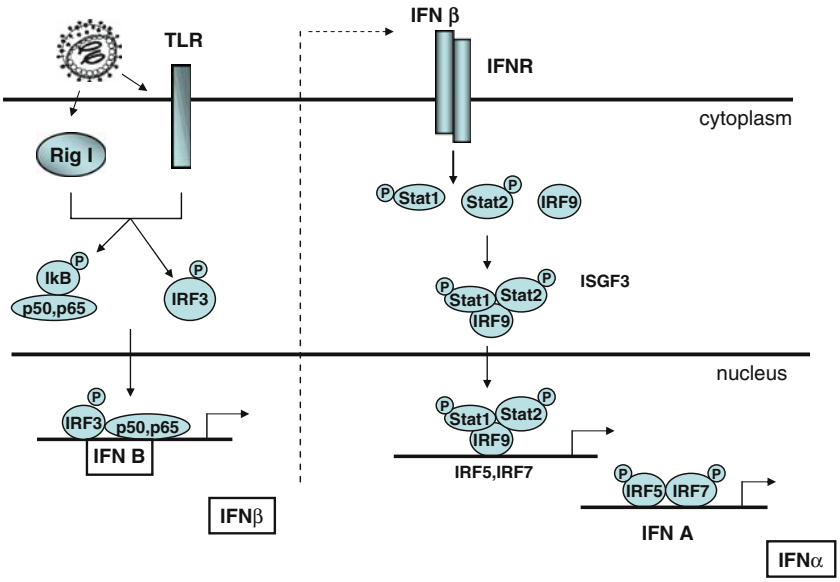

Fig. 1 The IRF family plays a critical role in the antiviral response. In most cells, the antiviral response occurs in two phases. In the first phase, the viral or bacterial infection stimulates phosphorylation of constitutively expressed IRF-3, which together with NFkB binds to VRE in the interferon promoter, resulting in the expression of the IFN*B* gene. In the second phase, the IFNβ binds to the type I IFN receptor, which results in the phosphorylation of STAT1 and STAT2 and their interaction with IRF-9, forming the ISGF3 complex that activates expression of large number of interferon stimulated genes including IRF-7 and IRF-5. Activated IRF-7 binds either alone or together with IRF-3 to the VRE of the IFNA promoters and induces expression of IFN α, which leads to the amplification of the antiviral effect

at the transcriptional or posttranscriptional levels (Kelly and Pitha 1985; Yeow et al. 2000; Zwarthoff et al. 1985). It was eventually shown that each IFN subtype contains a distinct promoter that regulates expression of individual IFN*A* in infected cells (Bisat et al. 1988). In contrast to IFN*B*, the VRE of the IFN*A* promoters does not contain an NFkB site, but shows the presence of multiple repeats of the AANNGAA sequence that can bind activated IRFs. Thus while the inducible activation of IFN*B* transcription is dependent on both NFkB and IRF, activation of IFN*A* seems to depend mainly on IRF.

The IRF family consists of nine cellular *IRF* genes (*IRF-1*to *IRF7*, *IRF-8/ICSBP* and *-IRF-9/p48/ISGF3*) (Honda and Taniguchi 2006). In addition, Kaposi's sarcoma herpes virus (KSHV) encodes viral analogs of IRF (Chang et al.

1994). Cellular IRFs share a significant homology in the N-terminal region. This region comprises a helix-turn-helix DNA-binding domain containing five tryptophane repeats, which bind the GAAA and AANNGAA domains in the VRE of type I IFN promoters (Escalante et al. 1998). The C-terminal of most IRFs contains an IRF association domain (IAD) that is important for homo- and heterodimeric interactions and a gene activation domain (Moustakas and Heldin 2003). The first member of the IRF family, IRF-1, was discovered through its ability to bind the VRE of the IFN*B* promoter (Miyamoto et al. 1988). While ectopic overexpression of IRF-1 in undifferentiated EC cells stimulated the expression of type I *IFN* genes, it failed to bind the VRE of IFN*A* (MacDonald et al. 1990; Au et al. 1992). Furthermore, homozygous deletion of IRF-1 did not impair the virus-mediated induction of IFN*A* (Ruffner et al. 1993). An IRF binding site (IRF-E) in the VRE of IFN*A* promoters plays an important role, as a single nucleotide mutation in the IRF-E abolishes the inducibility of the murine IFN *A4* promoter. Also, priming with IFN restores IFN*A* induction in IRF-1-null cells, indicating that another IFN-induced protein is the critical factor, later shown to be IRF-7 (Au et al. 1998).

The search for another IRF-E binding protein has led to the identification of IRF-3, IRF-7, and IRF-5 (Au et al. 1995, 1998; Barnes et al. 2001; Marie et al. 1998). The identification of IRF-3 and IRF-7 and their role in the activation of type I IFN genes had a major impact on the understanding of the inducible expression of type I IFN (Juang et al. 1998; Sato et al. 2000; Sobel and Ahvazi 1998; Weaver et al. 1998; Yeow et al. 2000; Yoneyama et al. 1998) (Fig. 1). Ubiquitously expressed IRF-3 is activated in the TLR-3, TLR-4, or RIG-I/MDA5 signaling pathways by two noncanonic IkB kinases: TBK-1 and IKKε (Fitzgerald et al. 2003; McWhirter et al. 2004; Sharma et al. 2003). IRF-3 homodimerizes or heterodimerizes with IRF-7 and translocates to the nucleus, where it associates with CREB binding proteins CBP/p300. Activated IRF-3 stimulates transcription of IFN*B*, as well as of some interferon-stimulated genes (ISG) (Grandvaux et al. 2002; Juang et al. 1998; Lin et al. 1999). While expression of IRF-3 alone is sufficient to activate the promoter of IFN*B*, the IFNB enhanceosome contains not only IRF-3, but also IRF-7 (Wathelet et al. 1998). Mice with a homozygous deletion of IRF-3 show impairment in the NDV-mediated induction of type I IFN and an increased susceptibility to EMCV infection (Sato et al. 2000).

IRF-7, which is constructively expressed only in some lymphoid cells and pDC (Izaguirre et al. 2003), is critical for the induction of IFN*A*. Reconstitution of IRF-7 expression in infected human fibroblasts that expressed only IFNβ conferred expression of several IFN*A* genes (Yeow et al. 2000). Mice with a homozygous deletion of IRF-7 were unable to express type I IFN genes upon viral infection or activation of TLR9 by CpG-rich DNA, indicating that IRF-7 is a master regulator of type I IFN expression (Honda et al. 2005). Virus-induced

expression of distinct IFN*A* subtypes is determined by the organization of IRF-3 and IRF-7 recognizing domains in the VRE of the IFN*A* promoters. Distortion in the GAAA core sequence of these binding domains affects the cooperativity of IRF-3 and IRF-7 binding and their synergistic activation. The differential expression of the individual IFN*A* subtypes has been shown to be due to a distinct nucleotide substitution in these domains (Au et al. 1993, 2001; Civas et al. 2006; Morin et al. 2002) and by the presence of negative regulatory sequences (DNRE) located in the upstream regulatory region of some IFN*A* subtypes (Lopez et al. 2000). IRF-3 and IRF-7, together with histone transacetylases, have been shown to be part of the transcriptionally active human IFNA1 enhanceosome (Au et al. 2001), whereas the murine IFN *A11* promoter, which that is not activated by IRF-3, binds only IRF-7 homodimers (Civas et al. 2006). These data indicate that the level of IRF-3 and IRF-7 in cells determines the relative levels of expression of individual IFN*A* subtypes.

2.3 The Role of IRF-5

An unexpected finding was that type I IFN, together with number of cytokines, could be induced in PBMCs not only by dsRNA or viral infection, but also by a compound of the imidazoquinoline family, imiquimod-(R848). The profile of R848-induced cytokines was very similar to that induced by Sendai virus infection (Megyeri et al. 1995). Although it was shown that this compound activates NFkB, the mechanism of induction was unclear and it was not until the TLR7-null mice became available that R848 stimulation of the TLR7-mediated pathway was shown (Hemmi et al. 2002). In contrast to TLR3 and TLR4, TLR7 activates IRF-5 and IRF-7, but not IRF-3 (Schoenemeyer et al. 2005) (see the chapter by Severa and Fitzgerald, this volume). The question therefore arises as to what role is played by IRF-5 in the antiviral response. Many splice variants of human IRF-5 have been identified, yet not all of these were transcriptionally active (Mancl et al. 2005). Like IRF-7, ectopic expression of IRF-5 (AY 504946) rescued induction of several IFN*A* subtypes; however, the subtypes of IFNα induced by IRF-5 and IRF-7 were distinct. While IFNα1 was the major subtype induced by NDV in IRF-7-expressing cells, IRF-5-expressing cells expressed IFNα 8 as the major subtype. Like IRF-1 and IRF-3, IRF-5 induced apoptosis and expression of several pro-apoptotic genes (Barnes et al. 2002). Interestingly, IRF-5 overexpression also upregulated a number of early inflammatory proteins including RANTES, MIP-1β, I-309, MCP-1, and IL-8. This suggested an important role for IRF-5 in the regulation of the expression of the early inflammatory cytokines and chemokines (Barnes et al. 2004). IRF-5 expression could be induced not only by viral infection and type I IFN, but also by the

tumor suppressors p53, thus connecting IRF-5 and p53 induced pro-apoptotic pathways (Mori et al. 2002). Like p53, IRF5 has tumor suppressing activity as well. Not only does IRF5 stimulate the cyclin-dependent kinase inhibitor p21, while repressing cyclin B1, but it also stimulates the expression of the pro-apoptotic genes Bak1, Bax, caspase 8, and DAP kinase 2, thus indicating an ability to promote cell cycle arrest and apoptosis independently of p53. As a consequence, it was suggested that IRF5 might provide an additional line of therapeutic intervention, in particular in the case of tumors resistant to apoptosis due to a loss of p53 function (Hu and Barnes 2006).

However, unlike IRF-7-null mice that show a major defect in type I IFN induction, pDC from IRF-5-null mice did not show any defect in the MyD88-mediated induction of type I IFN, instead demonstrating downregulation of IL-6, TNFα, and IL-12p40 expression (Takaoka et al. 2005). This supports our earlier observation that in cells expressing ecotopic IRF-5, viral infection induced the expression of pro-inflammatory cytokine genes (Barnes et al. 2004). Whether IRF5 is required only for the synthesis of pro-inflammatory cytokines and not for type I IFN is not clear yet. There are, however, a few differences between the human IRF-5 (variant 4) and mouse IRF-5. Unlike the human IRF-5, which is expressed in multiple spliced variants (Mancl et al. 2005), C57BL/6 J mice express only a single , dominant transcript and very low levels of one IRF-5 splice variant in bone marrow. This variant was not express in spleen or established mouse cell lines and shows a transactivation capability similar to full-length IRF-5. Also, the mouse IRF-5, while activated by MyD88 and TBK-1, does not seem to be activated efficiently by NDV infection (P.M. Pitha, unpublished results). The differences in the IRF-5-mediated activation of human IFN*A* genes in vitro and in MyD88-stimulated induction in mice, as well as the discordant effect of IRF-5 on the activation of *IFN* genes in cells expressing ectopic IRF-5 and mouse cells lacking IRF-5 expression, are unexpected. These results indicate that in the presence of high levels of activated IRF-7, the contribution of IRF-5 to the induction of IFN genes may be negligible, and its role may be limited to the induction of inflammatory chemokines and cytokines that are not stimulated by IRF-7. The MyD88-mediated activation of both IRF-5 and IRF-7 involves the formation of a tertiary complex consisting of MyD88, TRAF6, and IRF-5 or IRF-7 (Takaoka et al. 2005), and it is likely that this complex preferentially assembles with IRF-7 than with IRF-5. It was also shown that IRF-4 completes the binding of IRF-5 to MyD88 (Negishi et al. 2005) and therefore in cells expressing IRF-4 such as pDC or B cells, IRF-5 may be not efficiently activated. Thus the role of IRF-5 in the stimulation of type I IFN genes may be limited to the cells that do not express IRF-4 or activated IRF-7 and may also depend on a distinct, concentration-dependent activation of IRF-5 and IRF-7. While NDV activates IRF-7 by the

RIG-I pathway, this pathway does not seem to lead to the activation of IRF-5 (P.M. Pitha, unpublished results). Thus additional studies of differences in the MyD88-dependent and -independent signaling pathways leading to the activation of IRF-5 are clearly warranted. Lastly, a connection between IRF-5 and IFNα induction has been suggested by the observation that one of the genetic risk factors of systemic lupus erythematosus (SLE), which is associated with a constitutive IFNα production, was identified as a mutated IRF-5 haplotype, which drives an increased expression of IRF-5 (Graham et al. 2006) (see the chapter by Crow, this volume).

2.4 Interferon-Stimulated Genes: Mediators of Antiviral Effects

The binding of type I IFN to its cellular receptor initiates receptor-mediated signaling pathways, resulting in the induction of IFN-stimulated genes (*ISG*) (Darnell et al. 2004). The major signaling pathway involves the activation of two JAK kinases (JAK1 and Tyk2), which are associated with the type I IFN receptor, and the consequent tyrosine phosphorylation of the preexisting signal transducers and activators of transcription (STAT). Upon phosphorylation, STAT1 and STAT2 assemble together with interferon responsive factor 9 (IRF-9) into a multimeric complex (ISGF3), which interacts with interferon-responsive elements (ISRE) present in the 5′ flanking region of *ISG* (Darnell et al. 2004; Improta et al. 1994) (see the chapter by Uzé et al., this volume). Type I IFN also stimulates the formation of STAT1 homodimers, which bind to a slightly different DNA domain, the IFN-γ-activated site (GAS), present in the promoters of *ISG* that can be induced both by type I IFN and IFNγ. In addition, the STAT2-IRF-9 heterodimer is also an activator of transcription (Kraus et al. 2003) and in LCMV infection, induction of type I IFN has been shown to depend only on STAT2 (Ousman et al. 2005). Signaling by type I IFN can also activate both the MAPK and PI3K pathways (Platanias 2005); however, the contribution of these two pathways to the antiviral response in vivo is not clear. Interestingly, while IFNλ binds to a different receptor than IFNα/β, it nonetheless signals through the JAK/STAT pathway to ISRE domains (Kotenko et al. 2003; Sheppard et al. 2003). Analysis of the transcription signature of type I IFN-induced genes shows that IFN stimulates a major upregulation of cellular genes expression, which encode proteins with diverse functions including antiviral properties, pro-apoptotic functions and modulators of ubiquitination pathways (de Veer et al. 2001).

Although the antiviral function of the majority of ISG has yet to be determined, several of the interferon-induced antiviral proteins have been identified (Samuel 2001). The earliest characterized ISG were 2′, 5′-oligoadenylate

synthetase (2′, 5′-OAS), RNA-dependent protein kinase (PKR), Mx-GTPase, and the RNA-specific adenosine deaminase (ADAR). The 2′,5′-OAS pathway leads to RNA degradation. It consists of 2′,5′-OAS, which, when activated by dsRNA, polymerizes ATP into pppA(2′p5′A)n, (2′,5′A oligoadenylates); in turn it activates a cellular endonuclease (Kerr and Brown 1978). RNase L degrades both cellular and viral RNAs at UU or AU nucleotides. Expression of 2′, 5′-OAS in cells leads to the establishment of an antiviral state, which results in the selective inhibition of the replication of picornaviruses such as encephalomyocarditis virus (EMCV) (Chebath et al. 1987). Another endonuclease induced by IFN is ISG20, which has specificity for ssRNA. When overexpressed, ISG20 inhibits replication of VSV, influenza virus, EMCV, and HIV-1 (Espert et al. 2003). The interferon-induced antiviral gene *PKR* has been given a lot of attention. *PKR* is activated by dsRNA-mediated autophosphorylation. Activated PKR catalyzes phosphorylation of several substrates, including the α subunit of the initiation factor eIF-2 (eIF-2α) (Samuel 1979), which is implicated in the inhibition of viral protein synthesis, as well as the transcription factor inhibitor IκB (Kumar et al. 1994). PKR-deficient mice exhibit an increased susceptibility to VSV infection (Stojdl et al. 2000), whereas their antiviral response to influenza virus and Vaccinia virus (VV) is not impaired, again demonstrating viral specificity among the ISG (see the chapter by Sadler and Williams, this volume).

The Mx proteins are GTPases induced by IFNα/β but not by IFNγ. Overexpression of Mx confers a high degree of antiviral activity and resistance to infection by a large group of viruses, including influenza A and C, Hantana virus and measles virus, but not picorna viruses. The inhibition of influenza virus replication by Mx proteins is due to the inhibition of primary transcription mediated by a virion-associated polymerase.

Two nucleic acid-editing enzymes, adenosine deaminase acting on RNA (ADAR) and APOBEC3G, have also been shown to be ISG. ADAR-mediated transition of nucleotides from A to I disrupts base pairing and the AU base pair is replaced by the less stable IU pair, which destabilizes dsRNA. This A-I editing has been found in multiple viral RNA sites of negative-strand RNA viruses, and it has been associated with persistent infection (Murphy et al. 1991). APOBEC3G is a cytosine deaminase that converts cytidine to uridine in single-stranded proviral DNA, which results in hypermutation of the HIV-1 genome. Expression of APOBEC3G has been shown to be upregulated by IFNα (Chen et al. 2006; Yu et al. 2003). Thus both APOBEC3G and ADAR are IFN-induced antiviral proteins that can induce hypermutation of the viral genome and decrease viral fitness.

During the early days of IFN research, it was assumed that the interferon-mediated inhibition of viral replication was caused by a common mechanism affecting a large number of viruses. Instead, it has become clear that the antiviral

effect is due to the combinatory effects of many proteins and that any given antiviral protein may show specificity for a distinct group of viruses.

3 Innate Response to Pathogens

3.1 Cell Defense Against Viral Offense

The use of genetically modified mice deficient for the type I IFNAR or components of the IFN signaling pathway, such as STAT1, clearly establish the importance of type I IFN in the resistance to viral infection in vivo. Both IFN-α/β and STAT1 knockout mice are highly susceptible to viral infection and unable to establish an antiviral state. Similarly, the availability of genetically manipulated mice lacking either individual TLR receptors or cytoplasmic receptors has advanced the understanding of the cellular recognition of invading pathogens. However, it is still not completely clear what determines the specificity of the recognition and whether the receptor recognizes both the viral genome and replication intermediate. As shown with HSV-1, which is an effective IFN inducer, recognition may be complex. The unmethylated HSV-1 DNA genome is a very effective inducer of IFNα in human pDC, and this induction is dependent on TLR9 (Lund et al. 2003), while in human PBMCs, HSV-1 glycoprotein D alone can induce synthesis of IFNα. Another virus of the herpes group, mouse cytomegalovirus (MCMV), induces type I IFN through recognition of both TLR9 and TLR3 (Krug et al. 2004), but the recognition by CMV is through TLR2 (Compton et al. 2003).

dsRNA has been long considered the recognition entity for viral infection, and in the majority of the cells, RIG-I and MDA5 are important for the recognition of most RNA virus infections. However, not all viruses generate a significant amount of dsRNA intermediates and still are recognized by RIG-I (Pichlmair et al. 2006). The RIG-I pathway is induced by most of the viruses tested, whereas MDA5 is required for the response against picornaviruses (Kato et al. 2006). Recent observations indicate that this distinction is based on the specific recognition of the 5′-triphosphate viral RNA structure (Hornung et al. 2006). The uncapped 5′-triphosphate end of ssRNA and positive-strand RNA viruses or their transcripts is recognized by Rig I (Kato et al. 2006; Hornung et al. 2006). In contrast, the 5′ end of picornavirus transcripts remains associated with the protein used as a primer and therefore uncapped 5′ RNA is absent during picornavirus replication (Lee et al. 1977). RIG-I also plays a major role in the induction of the antiviral response to the hepatitis C virus (HCV) RNA

replicative intermediate in cultured hepatocytes (Sumpter et al. 2005) (see the chapter by Loo and Gale, this volume).

There are several indications that in the central nervous system (CNS), where TLR3 is expressed at high levels, the inflammatory response is initiated by TLR3. For instance, the inflammatory response initiated by TMEV and West Nile virus is dependent on TLR3 (Wang et al. 2004). The antiviral response to the ssRNA genomes of VSV and influenza virus in pDC was shown to depend on TLR7 (Lund et al. 2003) and there are some indications that the viral envelope can also be recognized by TLR4, which is a primary receptor for lipopolysaccharides (LPS). A TLR4-mediated antiviral response was induced by the fusion protein of RSV (Kurt-Jones et al. 2000) and by the envelope protein of MMTV (Burzyn et al. 2004). Altogether, these data indicate that both viral nucleic acids and glycoproteins are capable of generating the antiviral response and that the multiple patterns of recognition may enhance the antiviral response and its duration. However, in order to be able to replicate and establish infection, viruses develop various strategies for evading the innate immune response of the host, as is discussed by Haller in this volume.

Type I IFNs are not only essential for antiviral defense, but they also exert a number of immunoregulatory effects. They modulate the expression of major histocompatibility complex (MHC) antigens, and it is via this mechanism that IFN-α/β increases susceptibility of vaccinia virus (VV), or lymphocytic choriomeningitis virus (LCMV) -infected fibroblasts to lysis by cytotoxic T lymphocytes (CTL) (Bukowski and Welsh 1985). IFNα/β can also downregulate expression of IL-12 in human dendritic cells (DCs) and monocytes (Karp et al. 2000), stimulate expression of IFN-γ in response to influenza virus infection (Sareneva et al. 1998), and induce expression of IL-15 (Durbin et al. 2000). IFNα has also multiple effects on the function of immune cells (Garcia-Sastre and Biron 2006) including enhancement of NK cell activity (Biron et al. 1999), activation of $CD8^+$ T cells during the early steps of infection (Zhang et al. 1998), and protection of $CD8^+$ T cells from antigen-induced cell death (Marrack et al. 1999). Human IFNα promotes the differentiation of dendritic cells (Santini et al. 2000), the upregulation of IFN-γ expression, and stimulation of B cell differentiation in both a DC-dependent and -independent manner (Biron 2001; Santini et al. 2000). Recent data demonstrate that type I IFN can directly stimulate the B cell response during the early stages of influenza virus infection (Coro et al. 2006). IFNα has been also shown to induce the differentiation of human monocyte-derived DCs, which are able to induce Th1 polarization both in vitro and in vivo (Cella et al. 2000; Santini et al. 2000) and stimulate B cell proliferation and Ig class switching (Le Bon and Tough 2002). Thus, while in the past the IFN system was considered as only a part of the host innate immunity,

recent data indicate that type I IFN has an important role in bridging innate and acquired immunity (Biron 2001).

3.2 Positive and Negative Role of Type I IFN in Bacterial Infection

Although synthesis of type I IFN was originally associated with viral infection, the production of type I IFN is also induced as an immediate innate response to bacterial infection, where interferon has been shown to modulate an innate antibacterial response (Bogdan et al. 2004). Like viruses, bacteria can be recognized by membrane-bound receptors and cytoplasmic receptors. The binding of a specific ligand to a given TLR recruits specific adaptors and initiates cellular signaling pathways, leading to the activation of the IRFs and the NFκB family of transcription factors. LPS present on Gram-negative bacteria is recognized by TLR4 and initiates the association of TLR4, either with TRIF and TRAM adaptor proteins, leading to the activation of IRF-3 and IRF-7 or with MyD88 adaptor, which leads to NFκB activation. Several Gram-negative bacteria, such as *Salmonella typhimurium*, *Shigella flexneri* and *Escherichia* spp., stimulate type I IFN synthesis after the invasion of the cell (Bogdan et al. 2004). The unmethylated bacterial DNA is recognized by endosomally expressed TLR9. Binding of dsDNA to TLR9 occurs in the endosomal compartment and results in the activation of IRF-5 and IRF-7. However, there is also cytoplasmic recognition of B-form DNA, which occurs in the cytosol and results in the activation of IRF-3. Thus an intracellular Gram-positive bacterium that has a cytoplasmic life cycle phase, such as *Listeria monocytogenes*, probably activates the IFN response via the cytosolic DNA recognition pathway (O'Connell et al. 2005; Stetson and Medzhitov 2006). Bacterial flagellin is a TLR5 ligand, which mediates signaling through MyD88, resulting in the activation of NFκB factors and the induction of inflammatory cytokines (Hayashi et al. 2001). Whether TLR5 also activates IRFs and type I IFN has not been yet determined.

Type I IFN can also modulate the outcome of bacterial infection. It is important to realize, however, that interferon can be both protective and detrimental to the host. Type I IFN inhibits intracellular replication of *Legionella pneumophila* (Schiavoni et al. 2004) and contributes to the clearance of pathogens in *Leishmania* infection (Diefenbach et al. 1998). Type I IFN also increases resistance against Gram-positive bacteria such as *Streptococcus pneumoniae* and *Bacillus anthracis* (Gold et al. 2004; Weigent et al. 1986). In contrast, during *Listeria* infection, type I IFN synthesis increases the susceptibility of lymphocytes to infection (Carrero et al. 2006). IFN treatment also reduces host resistance to *L. monocytogenes* infection and has a negative impact on the survival of infected mice (Auerbuch et al. 2004).

Taken together, these data indicate that the effect of type I IFN on bacterial infection is complex. On one hand, it contributes to the clearance of pathogens; on the other, it can have harmful effects on the host.

3.3 Super IFN Producers: pDC

Although type I IFN can be produced essentially by any infected cell, most infected cells produce low levels of IFN that can act in an autocrine manner, or protect only cells localized in close proximity to the focus of infection. In human PBMCs, there is a rare type of cells, designated as natural interferon-producing cells, that produce very high levels of IFNα in response to viral infection and therefore can generate a systemic response (Fitzgeral-Bocarsly et al. 1988). Further characterization of these cells revealed that these cells are a CD123 and $CD4^+CD11c^+Lin^-$ subset of DCs referred to as plasmacytoid DCs (Siegal et al. 1999). Later, a pDC subset was also identified in mice; however, murine pDCs do not express CD123, but can be defined as CD11b-CD11c low $B220^+$ cells that also express Ly6C (Colonna et al. 2004). PDCs differ from the monocyte derived DCs (mDCs), not only by their phenotype but also by their migration pattern (Penna et al. 2002). PDCs are recruited to the site of inflammation, where they are activated, while immature mDCs in peripheral tissues migrate after maturation to lymphatic tissues (Jahnsen et al. 2002). mDCs and pDCs express a distinct set of TLRs and therefore recognize different pathogens. TLRs expressed in pDCs are those associated with recognition of viral or bacterial DNA and viral RNA, namely TLR7/8 and TLR9. The induction of the antiviral response is dependent on the co-adaptor MyD88 (Fig. 2). mDCs express relatively high levels of TLR3 and low levels of TLR4, the induction of the IFN response is through the adaptor TRIF, and it is MyD88-independent (see the chapter by Severa and Fitzgerald, this volume).

The IFNα subtypes and their relative level of expression induced in pDCs appear to be virus specific. While HSV-1 induced approximately 10- to 100-fold higher levels of IFNα in pDCs than in mDCs, the difference in the relative levels of IFNα induced by Sendai virus in pDCs and mDCs was much smaller. Since HSV-1 is recognized by TLR9 in pDCs, but by RIG-I in other cell types (Melchjorsen et al. 2005), the above observation indicates that the antiviral response induced by TLR9 is much stronger. Furthermore, the subtypes of IFNα induced in Sendai virus and HSV-1-infected cells were distinct (Izaguirre et al. 2003). The difference in the profile of IFNα subtypes expressed in pDC upon stimulation of TLR9 and TLR7 has not been yet determined. Both human and mouse pDCs also express high levels of IRF-8. While IRF-8 plays a critical role in pDC development its role in the activation of type I IFN is not yet clear (Tamura et al. 2005).

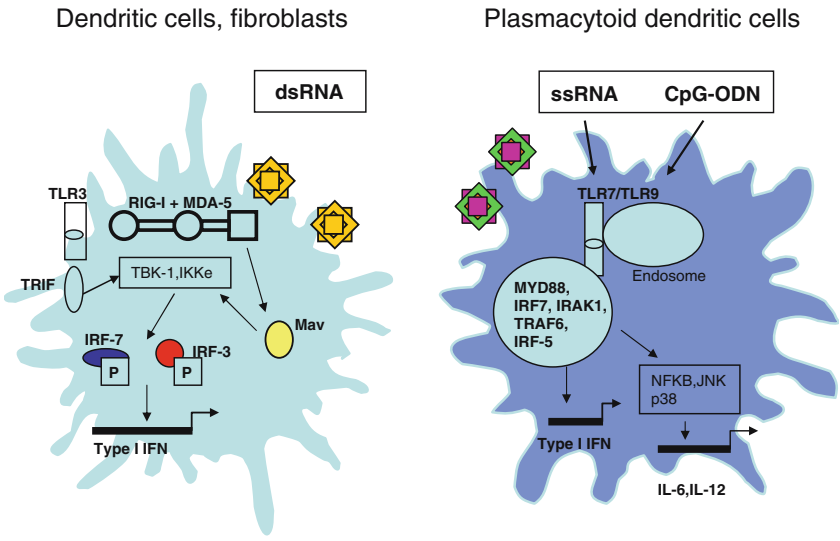

Fig. 2 The distinct difference in the induction of the antiviral response in pDCs and other cell types. In fibroblast and conventional DCs, ds viral RNA and viral transcripts are recognized by cytoplasmic RNA helicase RIG I (or MDA5) or TLR3. The TLR3 and RIG I pathways are mediated by cofactor TRIF or MAV, respectively. Both of these pathways activate TBK1 and IKKε and consequently IRF-3 and IRF-7. In pDCs, the antiviral pathway is mediated either by TLR7, which recognizes ss viral RNA, or TLR9 recognizing the unmethylated viral DNA. The activation of the respective TLRs leads to an assembly of multicomponent complex containing MyD88, IRF-7, IRAK 1, and TRAF-6 and activation of constitutively expressed IRF-7 and IRF-5

Several factors may contribute to the high production of IFNα in pDCs. In cells that constitutively express only IRF-3, an autocrine IFNβ feedback is required for an efficient production of IFNα (Marie et al. 1998; Prakash et al. 2005). However, in pDCs, which express relatively high levels of IRF-7, this autocrine feedback is not required (Dai et al. 2004; Izaguirre et al. 2003). The degradation of IRF-7 also seems to be attenuated in pDCs (Prakash et al. 2005). However, it has recently been suggested that the main reason for the high IFN production in pDCs is the distinct intracellular localization of TLR and TLR/MyD88 complexes in pDCs and other cell types. While in cells other than pDCs the TLR9-MyD88-TLR7 complex is rapidly translocated to lysosomes and degraded, in pDCs it is retained in the endosomal compartment for a longer period of time (Honda et al. 2005).

The specific impact of pDCs on innate and acquired immunity in vivo is virus-dependent. In MCMV or VSV infection, pDCs are the major producer of type I IFN (Dalod et al. 2003). In contrast, pDCs do not contribute to type I IFN synthesis in mice infected with LCMV or West Nile virus (Colonna et al. 2004; Dalod et al. 2003), and the cells producing type I IFN in either one of these viral infections have not yet been identified. Compared to other type I IFN-producing cells, pDCs have two unique functions: they can rapidly produce high levels of type I IFN and the induction of the antiviral response does not require direct viral infection. Since pDCs can respond to noninfectious viral particles or viral nucleic acid, the induction of the antiviral response in these cells is not subjected to viral mimicry (Hengel et al. 2005). The downside of this property is that the ability of pDCs to respond to exogenous nucleic acids or nucleic acid–protein complexes can result in the unregulated production of IFNα and inflammatory cytokines, such as that associated with autoimmune and inflammatory diseases (see the chapter by Crow, this volume).

4
The Good, the Bad, and the Promising

4.1
IFN in the Treatment of Viral Infections

IFNα/β has a long history of clinical use for the treatment of viral infections. Our understanding of the molecular mechanisms by which IFN exerts its remarkably pleiotropic effects is constantly being refined, from the intricate cascade of phosphorylations, which characterize its signaling pathways, to the identification of an ever-growing array of antiviral ISGs. The greater understanding of their virus-specific antiviral functions may provide a new approach to antiviral therapy.

In hepatocytes, hepatitis C virus (HCV) triggers the induction of IRF-3 and NF-κB, via the signaling cascade initiated by HCV genomic RNA. It has been shown that TLR7 confers immunity against HCV via IFN-dependent and -independent pathways. Thus TLR7 agonists might present an alternative to IFN in the treatment of chronic HCV infection (Lee et al. 2006). Clinically, polyethylene glycol-modified IFNα2a in addition to ribavirin is currently the treatment of choice for chronic HCV infection, which leads to cirrhosis and hepatocellular carcinoma. IFNα has been shown to suppress HCV replication. In most patients, a sustained inhibition of HCV genotype 2 and 3 replication is achieved after 24 weeks of treatment (Dalgard and Mangia 2006).

In spite of the efficacy with which IFN inhibits HCV, chronic infection can be established in the liver, mainly because HCV has been remarkably successful

in evolving mechanisms to evade these defenses. The HCV-encoded NS3/4A protease is an effective antagonist of both the RIG-I and TLR3 signaling pathways that are induced by dsRNA regions of secondary structure in the ssRNA HCV genome. Not only does NS3/4A inhibit direct signaling for IFN secretion, but it also prevents IFN amplification via the autocrine and paracrine loops (Foy et al. 2003). HCV core protein induces in vitro expression of suppressor of cytokine signaling (SOC) proteins, which downregulate the JAK-STAT pathway (Bode et al. 2003). Lastly, because the HCV polymerase lacks a proofreading function, a number of viral variants can be generated during the course of a persistent infection, thus affording a great deal of viral complexity and variable sensitivity to IFN (Gale and Foy 2005). The understanding of the molecular strategies employed by the virus to evade immune surveillance will provide novel targets for therapeutic control of HCV (see the chapter by Loo and Gale, this volume).

IFNα was shown to inhibit angiogenesis in Kaposi sarcoma and reactivation of KSHV in primary effusion lymphoma cells (Albini et al. 2000; Marchisone et al. 1999) and, in combination with antiviral therapy, it was used in patients with AIDS-associated Kaposi sarcoma (KS) (Krown et al. 2006).

Another viral infection where IFN has been used therapeutically is respiratory papillomatosis associated with human papilloma virus (HPV) infection (Gerein et al. 2005). Interferon inducer imiquimod has also been used topically (Aldara cream) for treatment of genital warts caused by HPV (Slade et al. 1998).

However, the use of the recombinant IFN at therapeutically effective doses is generally associated with side effects and toxicity and thus a novel method of delivery or use of interferon analogs with higher specific activity that would allow a lower well-tolerated dose are being developed.

4.3 Role of IFN in Autoimmune Diseases

Constitutive production of IFN has been associated with the pathogenesis of some autoimmune diseases, whereas while in others, IFN treatment seems to be beneficiary. There is a preponderance of evidence for an association of IFN with the pathogenesis of systemic lupus erythematosus (SLE) and that of insulin-dependent diabetes mellitus (IDDM). In both diseases, serum levels of IFN are increased, but no initial inducer, be it endogenous or exogenous, has been identified so far. In addition, either disease may appear as an unintended consequence of IFN treatment for an unrelated condition (Devendra and Eisenbarth 2004).

The role of IFN on IDDM appears to be dependent on the stage of the disease. Initially, IFN might be responsible for an aberrant autoimmune response to a viral inducer with pancreatic tropism. As mentioned earlier, no such inducer has been identified to date, but pro-inflammatory products of damaged cells and secretion of other cytokines may induce local IFN secretion and

IFN-mediated pancreatic tissue damage. In later stages of IDDM, proliferation and survival of reactive T cells appears to be suppressed by IFN. Type 1 diabetes has been reported in association with IFN treatment of unrelated disorders, such as cancer and chronic hepatitis (Fabris et al. 2003) and in association with elevated IFN levels during coxsackievirus B infection (Chehadeh et al. 2000). In mice, transgenic expression of type I IFN in beta cells of the pancreas resulted in the destruction of the beta cells (Stewart et al. 1993). However, in NOD mice IFN had a beneficial effect (Sobel and Ahvazi 1998).

In spite of the conspicuous absence of a known inducer, a model has been proposed whereby SLE is the result of sustained activation of myeloid dendritic cells at the instigation of IFN secreted by pDCs in a predisposed background. It remains unclear whether a predisposing background is due to hypersensitivity to stimuli, a greater number of IFN-producing cells, or the existence of a particularly effective inducer (Theofilopoulos et al. 2005). Recently, global gene expression profiling of PBMCs from SLE patients has shown induction of ISG as a hallmark of SLE (Bennett et al. 2003). Moreover, two novel autoantigens have been identified in CD1 lupus mice and found to be IFNα-inducible (Hueber et al. 2004) (see the chapter by Crow, this volume).

The use of IFNβ in the treatment of multiple sclerosis (MS) is well established, although its mechanism of its action is mostly unknown. The beneficial effects of IFNβ in preventing relapsing episodes may be due to a combination of anti-inflammatory, antiproliferative, and pro-apoptotic responses (Hafler 2004). Both experimental rheumatoid arthritis and myasthenia gravis also appear to benefit from treatment with IFN (Deng et al. 1996).

Given its pleiotropic effect in both innate and adaptive immunity, it is not surprising that IFN would play a pivotal role in the pathogenesis of autoimmunity as well. By the same token, IFN provides both a privileged and vulnerable target for therapeutic intervention.

Type I IFN has been shown to have both positive and negative modulatory effects on autoimmune diseases, yet what needs to be established is the nature of the inducer in distinct autoimmune disease and which of the IFNα variants subtypes are induced.

5
Reflections and Considerations

Recent studies clearly established that type I IFN has not only a critical role in the innate antiviral response, but it also provides a stimulus for the adaptive immune response. It has also become clear that type I IFN can have a critical

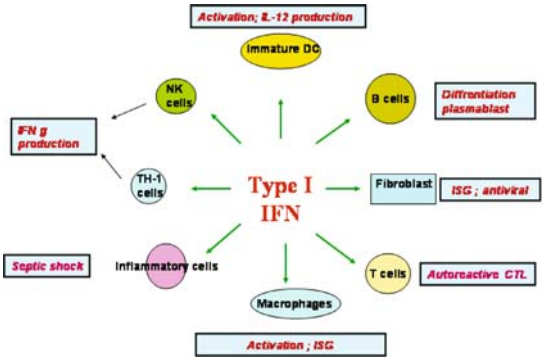

Fig. 3 Multiple functions of type I IFN

role in the pathogenicity of viral infection and in autoimmune disease (Fig. 3). However, a number of questions remain.

Thus the specificity and redundancy of the different type I IFN subtypes needs to be further clarified. In particular, the mechanisms involved in specific induction of distinct type I IFN in vitro have yet to be determined.

While it is easy to see the advantage of the redundancy of antiviral proteins in the defense against a wide variety of pathogens, it is not readily evident why IFN evolved to control so many cellular functions in addition to its role in immune responses. The ability to control the cellular life cycle of a pathogen and to initiate cell death is undoubtedly a powerful way to limit and ultimately eliminate infection, but it is puzzling why so much control over so many cellular mechanisms belongs to one small family of cytokines. One has to wonder then, whether the vast multiplicity of IFN-regulated genes mirrors the multiplicity of means by which pathogens are using the cellular machinery for their survival and propagation.

The felicitous exercise of IFN control over a vast array of cellular genes implies a need for tight regulation of the IFN signaling pathway to prevent unintended consequences. Autoimmune disorders are a glaring example of the deleterious consequences wrought on the organism, when IFN deregulation occurs. The identification of negative regulators of the type I IFN signaling pathway should facilitate the pharmacological manipulation of IFN function.

While the increased understanding of the interferon pathway and its cross-talk with the other inflammatory cytokine has revealed an ever-increasing degree of complexity in the mechanism of type I IFN action, the identification of IFN-stimulated proteins involved in the regulation of many cellular mechanisms and functions makes them very tempting agents for therapeutic

intervention. Future studies will no doubt expand the repertoire and function of these multiple proteins induced by the IFN signaling pathway even more.

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