

Interferon Research

A Brief History

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Summary

Interferons are the antiviral early inflammatory proteins produced in the cells in response to the infectious agents. The characterization of the interferon genes, their expression, and their function was advanced with the development of novel techniques in molecular and cellular biology. Using genetically modified mice revealed the critical role of the interferons in innate and acquired immune response. The critical steps and discovery that lead to the understanding of the interferon system and its role in the antiviral immune response are summarized in this chapter.

Key Words: Innate immunity; interferon; genes; receptors; clinical use; Toll receptors; viruses.

1. Introduction

Interferon (IFN) was described in 1957 by Isaacs and Lindenmann (*1*) as an antiviral protein synthesized by the cell in response to viral infection. The characterization of this protein, its expression, and its function has been closely linked to the availability of new methods and advances in cellular and molecular biology. Indeed, the isolation and detection of antiviral proteins synthesized by infected cells was dependent on the development of techniques enabling the cultivation of eukaryotic cells and the ability to use them for in vitro viral replication. Later, the availability of specific antibodies and molecular biology techniques made it possible to recognize that IFN is represented by a family of closely related, but distinct genes, to characterize IFN genes and to purify IFNs, as well as produce sufficient amounts for clinical studies.

From the onset, researchers held the hope that IFNs could be used as a general antiviral agent in the fight against viral infections, much like antibiotics are used to control bacterial infections, thanks to their ability to inhibit a variety of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) viruses. Unfortun-

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nately, the broad antiviral application has gone largely unfulfilled, mostly because of the pleiotropic effects that IFNs exert on the cells. Nevertheless, the critical role of IFNs in the antiviral immune response and cancer editing is just emerging from studies using the genetically modified mice, and IFNs have been used in the clinic for the treatment of selective viral infections and malignancies.

2. Purification and Characterization of IFNs

IFNs were initially identified as a group of proteins secreted by cells upon viral infection and able to inhibit the growth of a wide range of unrelated viruses. Whereas IFN did not appear to be virus-specific, it was recognized to be species-specific. Human white blood cells were shown to produce IFN upon infection, and they were regarded as a possible source of IFN for clinical purposes. A number of experiments using actinomycin D at doses that inhibited cell RNA synthesis, but not viral replication, demonstrated that IFN was a product of the cell genome. The use of protein synthesis inhibitors further suggested that IFN exerted its antiviral effect via the synthesis of one or more proteins, which were the actual antiviral effectors. Quickly, it was recognized as well that IFNs had properties able to regulate both cell growth and function. IFN preparations available at that time, however, contained a number of impurities, and the purification of small quantities of highly active IFN proved difficult. It was not until the advent of IFN-specific antibodies (2), which permitted the isolation of IFN to near purity by column chromatography, that the cell antigrowth effects of IFN could be confirmed.

The development of molecular biology techniques led to the detection messenger RNA (mRNA) and genomic DNA in cells. The translation of interferon mRNA in eukaryotic cell such as *Xenopus oocytes* and the high specific activity of IFN allowed for the detection of interferon proteins by their antiviral activity in cell cultures (3).

3. Identification and Cloning of the IFN Genes

Once a standard assay for IFN mRNAs was established, several laboratories nearly simultaneously cloned the IFN genes. The cloning of the IFN genes brought two unexpected findings. First, it became clear that IFN is represented by large number of cellular genes. These genes known as type I IFN, are represented by a family of 13 IFNA genes expressed in cells of lymphoid origin and one IFNB gene expressed in a majority of infected cells (4–6). Although it was believed for some time that there was at least one more IFN- β protein, IFN- β -2, this protein was shown to be identical to IL-6. A single IFNW gene (7), with sequence homology to IFNA, was found to be expressed in leucocytes, and recently one IFNk gene, with sequence homology to both IFNA and IFNB, was found to be expressed in keratinocytes and dendritic cells (8). Second, it

was found that all type I IFN genes are nonspliced genes, and although their expression shows cell specificity, all the genes are localized on the short arm of chromosome 9 in human cells and on chromosome 4 in the mouse. All type I IFNs are secreted proteins, although secretion of IFN- κ seems to be very inefficient. IFN- β is modified by glycosylation, whereas the majority of IFN- α are unglycosylated (9).

Finally, IFN- γ , or type II IFN, is encoded by a spliced gene localized on chromosome 12 and has been shown to be synthesized selectively in cells of the immune system, such as natural killer cells, CD4 Th-1 cells, and CD8 suppressor cells (10,11). The ability to express IFN genes in bacterial expression systems, coupled with affinity purification, provided sufficient amounts of IFN proteins to study their specificity and ultimately provided sufficient amounts for clinical studies

4. IFN Gene Regulation

The optimization of DNA transfection into eukaryotic cells has facilitated the identification of the regulatory regions of the IFN genes. In this method, genomic fragments localized at the 5' or 3' end of an IFN gene are cloned in front of a reporter gene encoding an easily detectable protein, transfected into cultured cells, and then their ability to induce expression of the reporter gene in infected and uninfected cells is analyzed. These studies have identified a virus-regulated element (VRE) in the promoter region of IFNA and IFNB, which alone confer responsiveness to virus infection (12–14).

Studies of the molecular mechanism involved in the virus-mediated activation of type I IFN genes has brought about the discovery of IFN regulatory factors (IRFs), a new group of transcriptional factors (15). The IRFs play a critical role in the induction of type I IFN genes; chemokine genes; and genes mediating antiviral, antibacterial, and inflammatory responses. Three of these IRFs, IRF-3, IRF-5, and IRF-7, function as direct transducers of virus-mediated signaling (16–18). In uninfected cells, these IRFs are expressed in the cytoplasm, whereas in infected cells, they are activated by a C' terminal serine phosphorylation, which results in their translocation from cytoplasm to nucleus (19). Recently, an IKK kinase, TBK-1, was shown to be responsible for the phosphorylation and activation of IRF-3 and IRF-7 in infected cells, as well as cells treated with double-stranded RNA (dsRNA)-polyIC (20,21). The target of TBK-1 phosphorylation is a cluster of 4 serines in the carboxy terminus of the IRF-3 polypeptide (22). In infected cells, the ubiquitously expressed IRF-3 mediates the induction of IFNB (23,24). Activation of this gene involves co-operative assembly of several transcription factors: nuclear factor (NF) κ B, ATF-2/c-june, IRF-3, and IRF-7 on the VRE of the IFNB promoter (25). This complex-enhanceosome recruits two coactivators, acetyltransferase CBP/P300 and

holoenzyme polII (26), whereas in the uninfected cells the IFNB promoter is under a negative control (27). Most of the promoters of IFNA genes do not contain an NFkB site, and their activation depends not only on IRF-3 but also on IRF-5 or IRF-7, both of which were shown to be components of the IFNA enhanceosome assembled on the VRE of IFNA genes (19,28). The chromatin precipitation assay has permitted the detection of these enhanceosomes in living cells. IRF-5 or IRF-7 expression in infected cells, unable to express IFNA genes, restored the expression of a number of IFNA genes and IFN- α synthesis (18,29). In most of the cells, expression of IRF-7 can be induced by interferon induced transcriptional factor ISGF3 (30). Type I IFN genes can be therefore generally divided into two groups: immediate-response genes, represented by IFNB, which requires only IRF-3 for its induction and is therefore rapidly induced in most infected cells, and late IFNA genes, which require IFN-activated IRF-5 or IRF-7. The fact that IFNB-null mice are unable to synthesize IFN- α supports the dependence of IFNA expression on IFNB and the hypothesis of a positive feedback operation in interferon mediated antiviral response (31,32). However, the recently developed quantitative RT-PCR analysis of RNA transcripts, as well as the sensitive detection of proteins by intracellular immune staining, have shown that the high IFN- α -producing pDC2 cells, considered to be natural IFN-producing cells, express high levels of IRF-7 constitutively in the absence of IFN synthesis (33). Thus the requirement for IFN- β synthesis may not apply to these cells.

The discovery of toll receptors (TLRs) and their role in the innate immune response has brought further unexpected findings. Three of these TLRs, TLR-3, TLR-7, and TLR-9, are intracellular and double stranded RNA (dsRNA), single-stranded RNA (ssRNA), and CpGDNA, respectively, are their ligands. Furthermore, binding of the dsRNA to TLR-3 activates TBK-1 and results in phosphorylation of IRF-3 and IRF-7 and the induction of type I IFNs. In contrast, TLR7 and TLR9 activate IRF-5 and IRF-7 but not IRF-3 (60,61).

It is noteworthy that synthesis of IFN- β also can be induced by the binding of lipopolysaccharide to TLR-4 and that the induction proceeds through activation of TBK-1, and activation of IRF-3 and IRF-7 (34). These results indicate that although the initial recognition of the infectious entity may be distinct, the cellular response to bacterial or viral infection shows profound similarities. However, none of these mechanisms could have been unambiguously established, without the availability of genetically modified null mice with various components of the TLR-mediated signaling pathway deleted.

Experiments with genetically modified mice have also indicated a role for the members of the IRF family in the antiviral immune response. Thus, targeted disruption of IRF-1 results in an increased sensitivity to viral infection, a defect in the development of TH-1 responses and a resistance to apoptosis.

IRF-4-null mice have a defect in both T- and B-cell maturation and, consequently, defective immune functions (35). IRF-8-null mice show an increased sensitivity to viral infection and a defect in the development of myeloid cells and pDC2 subtype of dendritic cells that are high IFN-producing cells (36,37). IRF-5-null mice show a profound defect in CPG DNA mediated responses (62). Furthermore, because the IRF-5 is a component of the p53-mediated growth inhibitory and pro-apoptotic pathway (38) and, thus, a recently observed antiviral activity of p53 may be mediated by IRF-5 (39).

5. IFN Receptors

Cellular receptors for type I IFNs and IFN- γ belong to the class 2 cytokine receptor subfamily. In recent years, these receptors and the signaling pathways they induce have been elucidated (40–42).

With varying degrees of avidity, all type I IFNs bind to the same receptor made of two subunits, IFNAR1 and IFNAR2, of which there are a short and a long variant, the result of differential mRNA splicing. IFN- α or IFN- β induces the association of IFNAR1 with the long variant of IFNAR2 and initiate a signaling pathway involving the tyrosine kinases Tyk2, Jak1, and the ultimate migration of activated transcription activators signal transducer and activator of transcription (STAT)-1 and STAT-2 to the nucleus, where they bind together with IRF-9 to a specific sequences (i.e., IFN-stimulated response elements) within the promoters of IFN-stimulated genes (ISGs) and initiate their transcriptional activation (43,44). The IFN- γ receptor also comprises two subunits and the signaling pathway with which it is associated, involves Jak1, Jak2, and STAT-1. Activated STAT-1 homodimers translocate to the nucleus and bind to the γ -IFN activation sequence, culminating in the transcriptional activation of specific genes (11).

Infection of genetically modified mice in which type I IFNR or IFNgR receptor or critical component of the IFN signaling pathways had been deleted has shown a central but not redundant role for type I and II IFNs in the host response to infection. Thus, elimination of type I IFNR increases sensitivity to infection by number of RNA viruses, whereas these mice are still resistant to some bacterial infections (45,46). However, IFNGR-null mice show increased sensitivity to microbial infections, as well as infection with some DNA viruses such as HSV-1 and vaccinia (47).

6. IFN-Stimulated Genes

Although IFNs were initially identified by their antiviral properties, it was recognized early on that the actual effector was not IFN itself, but one or several proteins induced by IFN. Recently, microarray analysis of the cellular transcripts induced in cells treated with IFN has estimated that IFN stimulates more

than 300 ISGs with homology to genes involved in signaling, host defense, immune modulation, transcription, translation, apoptosis, cell adhesion, anti-viral and inflammatory responses, ubiquitination, and antigen processing (48,49).

Not surprisingly, the most studied ISGs have been those with antiviral properties. The enzymes of the 2,5-oligosynthetase family (OAS-1 and OAS-2) catalyze the synthesis of short oligoadenylates, which binds and activate RNaseL, an enzyme that cleaves viral and cellular RNAs, thus inhibiting protein synthesis (50). DsRNA-activated protein kinase (PKR) phosphorylates the translation initiation factor eIF2 α , also resulting in the inhibition of viral and cellular protein syntheses (51). More recently, PKR also was found to be required for the activation of the transcription factor NF κ B, a central actor in inflammatory cytokine induction, immune modulation, and apoptosis (52). Mx proteins are GTPases and this intrinsic activity is required for antiviral effect (53). Mx proteins inhibit the replication of RNA viruses by either preventing transport of viral particles within the cell, or transcription of viral RNA (54). Another very interesting ISG is the RNA-editing adenosine deaminase that converts adenosine to inosine, thus causing hypermutation of viral RNA genomes, such as those of VSV and measles virus (55,56).

A number of ISGs encode chemokines such as interleukin-8 and monokine induced by IFN- γ (Mig), which are involved in lymphocyte recruitment to the site of infection and inflammation and the expression of genes encoding adhesion molecules, such as ICAM-1 and CD-47, which are crucial for the ability of leukocytes to adhere to, infected cells. Other ISGs encode transcription factors, most of them activators of transcription. ISG-15 is an ubiquitin-like protein, conjugated to cellular proteins and has been shown to target Jak-1, STAT-1, and extracellular signal-regulated kinase-1 (57,58).

7. Clinical Uses of IFN

Recombinant IFN- α (rIFN- α ; Roferon A; Intron A) and recently its pegylated form (Pegasys), either alone or in combination with an antiviral agent, are used in the treatment of chronic hepatitis C virus infection. Because a number of ISGs are shown to have pro-apoptotic characteristics, there is also a renewed interest in using IFN in the clinic to control malignancies. In the past, Roferon A-rIFN α has been used in the treatment of malignant melanomas, Kaposi's sarcoma, genital warts, and hairy cell leukemia (59). Avonex (IFN- β), produced in hamster cells, remains an essential element in the treatment of multiple sclerosis (MS). Peripheral blood mononuclear cells isolated from patients with active MS show decreased sensitivity to type I IFNs, decreased ISG expression, and hypophosphorylation of STAT-1. In vitro treatment of these cells with IFN- β overcomes these defects, thus suggesting that IFN- β therapy may serve to restore normal levels of ISG expression in active MS.

One should be mindful to remember however, that IFN therapy is accompanied with burdensome side effects, presumably because of the large scope of biological processes influenced by IFN and that, at best, it has been able so far to only forestall but not halt the progression of the diseases mentioned here.

8. Conclusion

IFNs were the first early inflammatory proteins recognized to be produced in cells in the response to viral infection. The characterization of IFN genes, their regulation and functions, facilitated by the newly emerging techniques of molecular biology, opened a new insight into our understanding of the basic mechanisms involved in the virus cells interaction and in the innate antiviral response. The availability of genetically modified mice allowed them to study *in vivo* the role of the IFN system in the antiviral response. These studies have revealed the importance of the IFN system not only for the innate, but also for the acquired immunity and pointed out to the existence of the cross talk between interferon system and other cytokines. Furthermore, it has become obvious that the role of IFN is not limited to the antiviral response, but that the IFN system plays an important role in the regulation of cell growth, apoptosis, and maturation of lymphoid cells. Understanding the mechanisms of the cellular effects of IFNs and their interaction with other cytokines may also provide more realistic approach to the clinical use of IFNs.

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