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# Interferons α and β as Immune Regulators—A New Look

Minireview

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The type 1 interferons, α and β (IFN-α/β), are comprised of the products of multiple (up to 12) IFN-α genes and a single IFN-β gene. These factors use a common heterodimeric receptor, broadly expressed on most cells. The major pathway of intracellular signaling used by IFN-α/β and their receptors accesses the tyrosine kinases, Jak 1 and Tyk 2, activating the signal transducer and activators of transcription (STAT) 1 and STAT2 to form a STAT1/STAT2 heterodimer. Other pathways, however, are also induced. STAT1/STAT2 complexes associate with a p48 protein, identified as the interferon responsive factor (IRF) 9, to form the interferon-stimulated gene factor-3 (ISGF-3). ISGF-3 induces transcription as a result of recognizing interferon stimulated response elements (ISREs) in promoter regions of interferon responsive genes (Biron and Sen, 2001).

Although first characterized based on potent antiviral functions, IFN-α/β also mediate a variety of immunoregulatory effects. The immune modulating functions suggest that type 1 IFNs may be important links between innate and adaptive immune responses. IFN-α/β induction of MHC class I expression and activation of natural killer (NK) cell cytotoxicity has long been appreciated. The last few years of research have not only advanced the characterization of these classic IFN activities but have also revealed a number of surprises concerning other biologically important immunoregulatory functions. The strongest evidence is for IFN-α/β enhancement of induction of other IFN-α/β cytokines and IL-15; the apparently contrasting inhibition of IL-12 expression but induction of a high-affinity form of the IL-12 receptor; the shaping of NK cell responses; the complex positive and negative effects on IFN-γ expression; and effects on dendritic cell (DC) maturation and function. These are reviewed below.

### *IFN-α/β Regulation of IFN-α/β Expression*

High levels of type 1 IFN are elicited during certain viral infections. The major pathways to IFN-α/β induction have been thought to be cytoplasmic receptors activated by the presence of viral products. The best characterized recognize double stranded RNA (dsRNA), not generally observed in uninfected cells but often generated during viral replication. Not all viruses are good inducers of IFN-α/β in infected cells, however, and other known pathways to IFN induction are linked to virus binding to extracellular receptors and exposure to products of nonviral agents. Indeed, the large number of IFN-α/β genes is likely to be in place to allow access to these factors in response to a variety of stimuli. A remarkable newly identified regulatory function of IFN-α/β is the facilitation of expression of other IFN genes. Particular IFN genes, i.e., IFN-β and/or IFN-α4, are induced in the first cell targets of viral infection because IRF-3, broadly and constitutively expressed at a low level, is activated to promote their transcription (Juang et al., 1998; Marie et al., 1998). A consequence of expression of the first IFN gene targets is that their products are released to act through the IFN-α/β receptors and STAT1 for the induction of IRF-7 in neighboring cells. Upon infection with virus, cells expressing IRF-7 are induced to express other IFN-α genes, i.e., non α4 subtypes. This positive feedback for type 1 IFN expression is in effect an IFN-α/β to IFN-α/β induction cascade with the virus as a cofactor.

### *IL-12 Expression and Function*

Type 1 IFN can clearly influence the expression and function of a variety of cytokines. In contrast to the IFN-γ promotion of IL-12 expression, IFN-α/β can negatively regulate IL-12 expression. This was first demonstrated in the mouse in culture systems using a potent replication-independent stimulus, fixed *staphylococcus aureous* Cowan strain (SAC), and in vivo during infections of mice with lymphocytic choriomeningitis virus (LCMV) (Cousens et al., 1997). The effect requires high, but physiologically relevant, concentrations of IFN-α/β and is observed as decreases in both the inducible p40 chain and the biologically active p70 heterodimer comprised of the p40 and p35 chains. The negative effect of IFN-α/β on IL-12 expression has now been observed with human DCs and monocytes. In the case of DCs, addition of IFN-β inhibits the expression of IL-12 p40 in response to stimulation with activated CD4 T cells (McRae et al., 1998). In the case of monocytes, cells pretreated with IFN-α or IFN-β are inhibited in their p40 and p70 IL-12 responses to lipopolysaccharide (LPS) or SAC alone (Hermann et al., 1998; Karp et al., 2000). The inhibitory effects in these systems also require relatively high concentrations of IFN-α/β.

The negative regulation of IL-12 expression appears to contradict the IFN-α/β enhancing effects on expression of the heterodimeric high-affinity receptor for IL-12 comprised of β1 and β2 chains. This complex is upregulated on human T cells in response to either IFN-α/β or IFN-γ (Gollob et al., 1997; Rogge et al., 1997, 1998). In the mouse, IFN-γ plays a similar role by enhancing mRNA expression of the IL-12 receptor β2 chain (Szabo et al., 1997). An equivalent function for IFN-α/β in this species is more controversial. Protein expression of the IL-12Rβ1 chain is preserved and the IL-12β2 chain is induced on NK cells at times of high IFN-α/β induction during LCMV infections of mice (Nguyen et al., 2000). However, IFN-α fails to induce detectable upregulation of the IL-12Rβ2 mRNA in mouse T cell lines (Rogge et al., 1998) and inhibits the spontaneous induction of mRNA for the IL-12β1 but not the IL-12β2 chain during the culture of mouse peritoneal macrophages (Fantuzzi et al., 2000). Although the variations may be attributed to differences in cell populations or stimuli used, they

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might be biologically consistent. Under conditions of high concentrations of IFN-α/β, inhibition of IL-12 production would be dominant because expression of the receptor would not have a biological consequence if no ligand were available to engage it. On the other hand, induction of a high-affinity receptor for IL-12 by either IFN-α/β or IFN-γ might be biologically important in the context of low concentrations of IFN-α/β because there would still be significant IL-12 production. It should be pointed out, however, that the components of the IL-12 receptor may be regulated differently from IL-12 because they have additional functions. In particular, little is known about the induction and function of a cytokine identified as IL-23, comprised of the IL-12 p40 and a newly identified p19 molecule. This cytokine binds to the IL-12Rβ1 but not the IL-12β2 chain (Oppmann et al., 2000). Thus, there are likely to be a number of conditions under which regulation of IL-12 might diverge from regulation of its receptor.

***NK Cell Responses***

IFN-α/β promote NK cell-mediated cytotoxicity in culture and in vivo (Biron et al., 1999). Remarkably, exposure to these factors also is associated with NK cell blastogenesis and proliferation but not IFN-γ expression in vivo. In the context of a viral infection inducing both IFN-α/β and IL-12 in the mouse, murine cytomegalovirus (MCMV), there is a clear dichotomy of function with enhanced cytotoxicity and blastogenesis dependent upon IFN-α/β but IFN-γ induction dependent upon IL-12 (Orange and Biron, 1996). The IFN-α/β cytokines also block NK cell responsiveness to IL-12 for IFN-γ production (Nguyen et al., 2000). The effect on IL-12 responsiveness is dependent upon the presence of STAT1 and occurs despite expression of both IL-12 receptor constituents. Basal and IFN-α/β induction of NK cell cytotoxicity is dependent upon STAT1 (Lee et al., 2000). Remarkably, the STAT1-dependent effects on NK cell lytic function occur even though the mRNA levels for a number of molecules involved in the activation and delivery of lytic function, i.e., perforin, granzyme A, granzyme B, DAP10, and DAP12, are not reduced. These results suggest that there may be STAT1-independent expression of certain components of the lytic machinery but that expression of other key elements in the killing pathway must be STAT1 dependent. Because STAT1 is required for the induction of NK cell cytotoxicity and is contributing to the induction of an IL-12 refractory state in NK cells, the effects may be linked.

***IL-15 Expression***

IL-15 can be a product of nonlymphoid populations at early times following stimulation or challenge (Fehniger and Caligiuri, 2001). The high-affinity receptor for IL-15 utilizes the β and γ chains of the IL-2 receptor along with a unique α chain. Although there is some debate about the efficacy of type 1 IFNs as compared to other stimuli, there is growing evidence that IFN-α/β or their inducers can elicit expression of IL-15 at least at the mRNA level in mouse cell populations (Zhang et al., 1998; Durbin et al., 2000) and at both the mRNA and protein level in human DCs derived from peripheral blood monocytes cultured in type 1 IFN and GM-CSF (Santini et al., 2000). This immunoregulatory effect of IFN-α/β has been proposed to contribute to the induction of NK cell proliferation (Biron et al., 1999) and memory CD8 T cells (Tough et al., 1996; Zhang et al., 1998) at early times following viral infections.

***IFN-γ Expression***

Culture studies with human T cell populations provided the first indications that type 1 IFN could upregulate IFN-γ expression (Brinkmann et al., 1993). IFN-α/β mediate positive effects on T cell IFN-γ expression following stimulation with particular molecules, including the chemical analog for viral dsRNA, polyinosinic:polycytidylic acid (polyI:C) (Manetti et al., 1995; Sareneva et al., 2000). The factors also work in synergy with IL-18 to enhance IFN-γ production following stimulation with influenza virus (Sareneva et al., 1998). A modest IFN-α effect on IL-12 induction of IFN-γ has also been observed with mouse cells in culture (Wenner et al., 1996), and there is a more dramatic role for IFN-α/β in enhancing IL-12-independent CD8 T cell IFN-γ expression during LCMV infections of mice (Cousens et al., 1999). STAT4 is known to promote IFN-γ expression in the human and the mouse, and it has been clearly demonstrated that type 1 IFN can activate STAT4 in the human (Cho et al., 1996; Rogge et al., 1998). However, a minisatellite insertion into mouse STAT2 severely impairs STAT4 activation in this species (Farrar et al., 2000). Thus, there is a readily accessible and potent pathway from IFN-α/β to IFN-γ expression in the human that does not appear to be operational in the mouse.

Nevertheless, the complexities of IFN-α/β signaling in a physiologic context are only superficially understood. STAT4 may not be the only pathway to IFN-γ expression in either the human or the mouse. As one example, although stimulation through the T cell receptor for antigen for CD4 T cell IFN-γ expression requires STAT4, equivalent stimulation of CD8 T cell IFN-γ responses does not (Carter and Murphy, 1999). Thus, there are STAT4-independent pathways to IFN-γ expression. The STAT4-dependent pathway may be a minor player in the context of other more dominant pathways elicited under challenge conditions. In this regard, it is interesting to note the recently described negative effects of STAT1. In addition to playing a positive role in association with STAT2 for gene activation in response to type 1 IFN, STAT1 homodimers are major intermediaries for signaling from the IFN-γ receptor (Biron and Sen, 2001). STAT1 has been recently shown to negatively regulate a number of effects including early NK and T cell IFN-γ expression in response to type 1 IFN exposure in the mouse (Nguyen et al., 2000) and c-myc expression in response to IFN-γ in the human (Ramana et al., 2000). These studies suggest that the effects of cytokine exposure may vary greatly depending upon the ratio of signaling molecules expressed in the cell cytoplasm. Furthermore, if the negative regulatory effects of STAT1 are dominant in both species, IFN-α/β activation of STAT4 might only occur, even within human cells, if the levels of STAT1 are downregulated.

***Expression by and Regulation of DCs***

IFN-α/β have the potential to indirectly influence adaptive immune responses by modifying maturation of DCs, e.g., drive DC1 populations promoting Th1, as compared to DC2 populations promoting Th2, CD4 T cell response. Alternatively, if the cytokines are expressed by the DCs themselves, they might directly influence the development of T cells responding to antigen presented by these

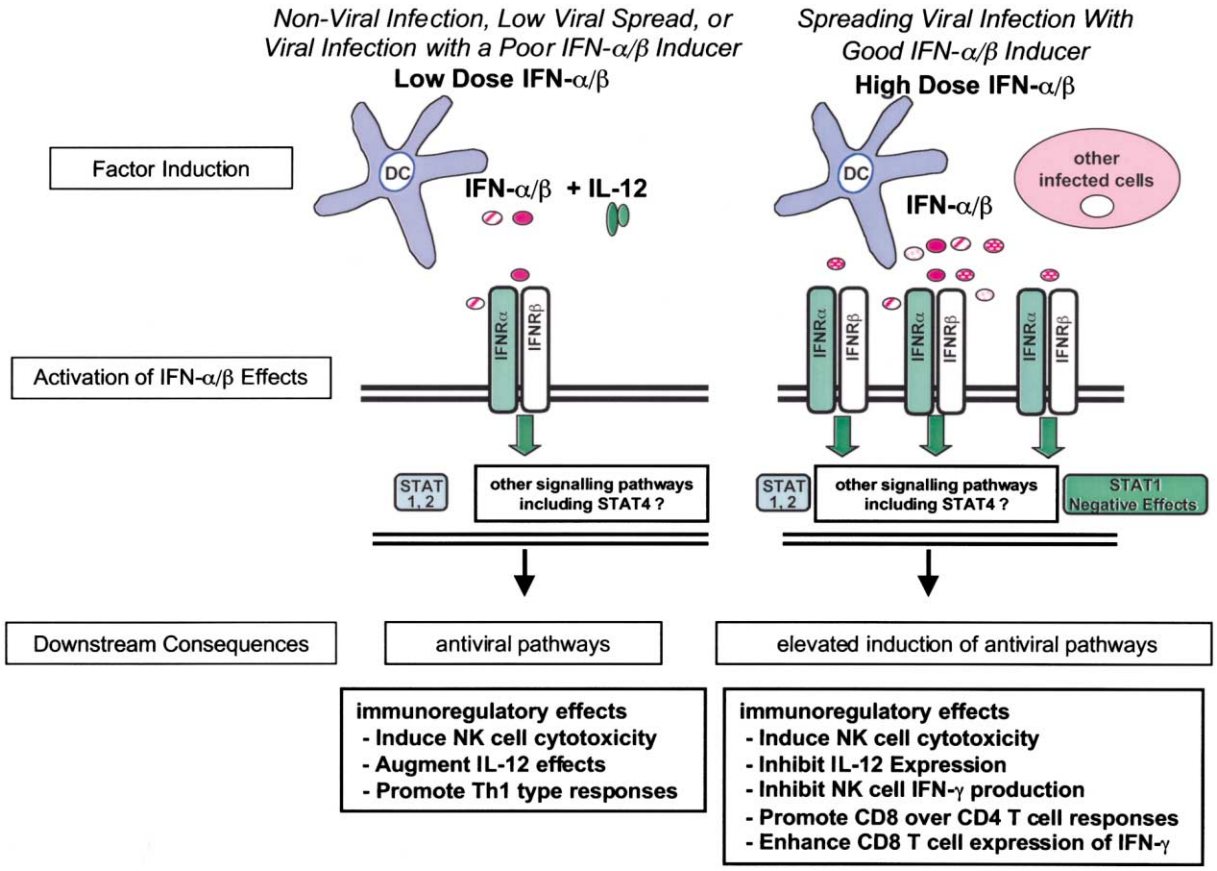


Figure 1. A Model for the Effects Mediated by IFN-α/β in the Context of Low- and High-Level Expression

The pathways proposed are based on the literature reviewed. The reader should consult the Integration and Summary section of the text for a discussion.

cells. Type 1 IFN are poor inducers of MHC class II but do enhance MHC class I expression. IFN-α/β do induce expression of MHC class I molecules by human DCs, but stimulation with influenza virus or polyI:C is more profound, with virus infection resulting in sustained expression of class I molecules presenting viral peptides (Cella et al., 1999b). Exposure to IFN-α/β may induce DC expression of the costimulatory molecules used for communication with T cells during activation, i.e., CD80, CD83, CD86, and/or CD40 (Gallucci et al., 1999; Ito et al., 2001; Santini et al., 2000). Interestingly, however, the responses of freshly isolated $CD11c^+$ and $CD11c^-$ human peripheral blood DCs are different with IFN-α only significantly inducing expression of costimulatory molecules on the former (Ito et al., 2001). Moreover, the effects of adding IFN-α to DC populations derived in culture from human blood monocytes are much less dramatic than those elicited by exposure to influenza virus or polyI:C (Cella et al., 1999b). IFN-α does induce DC expression of an antiviral protein, Mx, and increases resistance to influenza virus infection (Cella et al., 1999b).

If appropriately activated, human DCs can produce high levels of type 1 IFN (Cella et al., 1999a, 1999b; Siegal et al., 1999). A variety of stimuli have been examined and shown to be active, including herpes simplex virus (HSV) infections, influenza virus infections, CD40L, and polyI:C. By comparison to the others, CD40L is a modest inducer of type 1 IFN but a good inducer of IL-12 p70 (Cella et al., 1996, 1999b). Certain nonviral products such as LPS from Gram-negative bacteria can also induce type 1 IFN production by DC. In the case of the response to HSV, the mannose receptor on the surface of DCs appears to play an important role in induction of IFN-α expression (Milone and Fitzgerald-Bocarsly, 1998). In the case of the response to polyI:C, the receptor for IFN induction may be protein kinase R (PKR) expressed in the cell cytoplasm (Cella et al., 1999b; Kumar et al., 1994). DC populations derived in the context of type 1 IFN, viral infections, and/or polyI:C appear to be potent inducers of human CD4 Th1 type responses with high levels of IFN-γ production (Cella et al., 1999a, 1999b; Ito et al., 2001). Surprisingly little has been done characterizing the consequences of DC maturation in the context of type 1 IFNs and/or DC expression of these cytokines for CD8 T cell responses. IFN-α pretreatments do inhibit the dramatic proliferative stimulation of a viral peptide-specific and MHC class I-restricted T cell clone, by immature DCs exposed to influenza virus (Cella et al., 1999b). The IFN-α/β effects on antigen presentation and T cell stimulation are not yet clear, however, because the treatment also renders the cells resistant to infection

and, as a consequence, reduces available antigen. Hence, DCs induced in the context of IFN-α/β and/or producing these factors appear to be poised to promote Th1 CD4 T cell responses, but the understanding of their functions for shaping of CD8 T cell responses is limited.

***Integration and Summary***

Much remains to be learned, but a general picture of how the IFN-α/β responses might act to shape adaptive immune responses under a variety of conditions can be proposed from the literature discussed above (Figure 1). If induced in response to nonviral agents requiring Th1 type responses for protection, infections with viruses that are poor inducers of IFN-α/β production, or controlled viral infections, expression of IFN-α/β may be limited to only the initially stimulated cells with DCs being a major source. As IFN-α/β can be detected along with IL-12 under these conditions, promote expression of the IL-12 receptor, and enhance IFN-γ production by CD4 and CD8 T cells, consequences of this lower type 1 IFN production would be to enhance CD4 Th1 type responses and, if antigen is presented on class I MHC, CD8 T cell IFN-γ production. In the context of a spreading viral infection directly inducing IFN-α/β and accessing the priming pathway for IFN-α/β production elicited by IFN-α/β exposure, high levels of IFN-α/β would be induced. This is because the factors could be made by the first targets of viral infection and then by neighboring cells primed to express a broader range of IFN-α/β genes. These conditions of high type 1 IFN exposure may direct the host to preferentially promote early expression of IFN-α/β over IFN-γ by delivering immunoregulatory functions to downregulate IL-12 and NK cell IFN-γ production. Although the effects may interfere with peak induction of Th1 CD4 T cell responses, they would continue to induce NK cell cytotoxicity and may help preferentially promote CD8 T cell responses particularly effective in antiviral defense, including IFN-γ production. The induction of IL-15 is likely to occur under both conditions to boost NK cell and memory CD8 T cell responses.

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