Review Article

New aspects of IFN-α/β signalling in immunity, oncogenesis and bone metabolism

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Although interferons (IFNs) were originally identified as humoral factors that confer an antiviral state upon cells, they have been demonstrated to be multifunctional in a variety of biological systems. The IFN-α/β system modulates not only the cellular immune response to viral and bacterial infections, but also the oncogenic process and bone metabolism. Further studies have revealed additional unique facets of the IFN-α/β system. A weak signal by constitutively produced IFN-α/β is critical not only for the regulation of cellular amplification of IFN-α/β production upon viral infection or the enhancement of signalling by other cytokines, but also for the regulation of adaptive immune responses, such as the enhancement of CD8⁺ T cell activation. Furthermore, IFN-β signalling is critical for the regulation of the bone-resorbing osteoclasts. In this review, we focus on the newly discovered roles of the IFN-α/β system in host defense and bone remodeling, particularly on the functions of the weak IFN-α/β signalling in the context of what we refer to as the "revving-up" model. (Cancer Sci 2003; 94: 405–411)

Biological systems have acquired adaptability to, and robustness against rapid environmental changes, and one typical example is the immune system, which eradicates invading pathogens such as viruses. Type I interferons, i.e., IFN-α and IFN-β, the production of which is induced upon viral infection, are essential components of this system.[1,2] Production is induced *en masse* in many types of cells upon infection by a variety of viruses, and this induction is primarily controlled at the transcriptional level. As well as eliciting strong antiviral activities in target cells, IFN-α/β also activates effector cells of the innate immune system, such as natural killer (NK) cells and macrophages.[1–4] Given the essential function of IFN-α/β as mediators of innate immune responses against viruses, it is interesting that there are many reports on IFN-α/β production occurring in the absence of viral infection, albeit at very low levels, both *in vitro* and *in vivo*.[1,5,6] However, in this article, we focus on other unique facets of the IFN-α/β system. A weak IFN-α/β signal, transmitted independently of viral infection, is critical for priming cells to enhance their response to other stimuli. In fact, the weak signal renders cells "ready-to-go," by stimulating amplified IFN-α/β production in response to viral infection, and enhances responses to other cytokines.[7,8] Spontaneous IFN-α/β signalling is also critical for enhancing the activation of CD8⁺ T cells, but it needs to be properly attenuated to maintain homeostatic CD8⁺ T-cell responses.[9] These observations offer interesting examples of how weak signals elicited by a given stimulus contribute to eliciting strong cellular responses to other stimuli. The role of continuous, weak IFN-α/β signalling, described here in the context of our newly proposed "revving-up model," may point to a broad operation of similar mechanisms in other biological systems. On the other hand, even a weak dysregulation of such a signalling system can also be the basis for disease development.

IFN-β has also recently been shown to be an essential regulator of osteoclastogenesis, wherein the IFN-β signalling makes a balance with RANKL (receptor activator of nuclear factor κB ligand) signalling through a unique signalling crosstalk mechanism to properly regulate the differentiation of osteoclasts.[10]

IFN-α/β system in viral immunity

1) *IFN-α/β* gene induction by viral infection: operation of a positive feedback regulation for robust IFN production. Type I IFNs are produced by a variety of cells in response to viral infections. The type I IFNs comprise a number of species of IFN-α and one IFN-β. All IFN-α/β species interact with the same receptor complex, termed the IFN-α/β receptor (IFNAR), which consists of at least two subunits, IFNAR-1 and IFNAR-2 (Fig. 1). The intracellular domains of these two subunits. IFNAR-1 and IFNAR-2, are associated with Jak PTKs (Janus protein tyrosine kinases), Tyk2 and Jak1, respectively. Binding of IFN-α/β to IFNAR results in cross-activation of these Jak PTKs, which then phosphorylate their downstream substrates, two members of the family of signal transducers and activators of transcription (Stats), namely, Stat1 and Stat2.[11–14] The tyrosine phosphorylation of these Stats leads to formation of two transcriptional activator complexes, IFN-α-activated factor (AAF) and IFN-stimulated gene factor 3 (ISGF3) (Fig. 1). AAF is a homodimer of tyrosine-phosphorylated Stat1, whereas ISGF3 is a heterotrimeric complex of tyrosine-phosphorylated Stat1, Stat2 and another transcription factor member, IFN-regulatory factor (IRF)-9/p48/ISGF3γ (Fig. 1). These complexes translocate into the nucleus; subsequently, AAF and ISGF3 bind to their specific DNA sequences containing each common motif, namely, the IFN-γ-activated sequence (GAS) and the IFN-stimulated response element (ISRE), respectively. IFN stimulation of promoters containing ISRE and GAS results in the transcriptional induction of a large number of target genes (ISGs; IFN-stimulated genes) to evoke antiviral activity.

The mechanism for the induction of *IFN-α/β* gene transcription in virus-infected cells has been extensively studied (reviewed in Refs. 15, 16). The promoter region of the *IFN-β* gene contains at least four regulatory *cis*-elements; the positive regulatory domains (PRDs) I, II, III and IV (Fig. 2). Analysis of the promoter region revealed that the transcriptional activators NF-κB and ATF-2/c-Jun bind to PRD II and PRD IV elements, respectively, and activate, in cooperation with PRD I and PRD III, the *IFN-β* gene in virus-infected cells.[17] On the other hand, PRD I and PRD III elements, which members of the IRF family

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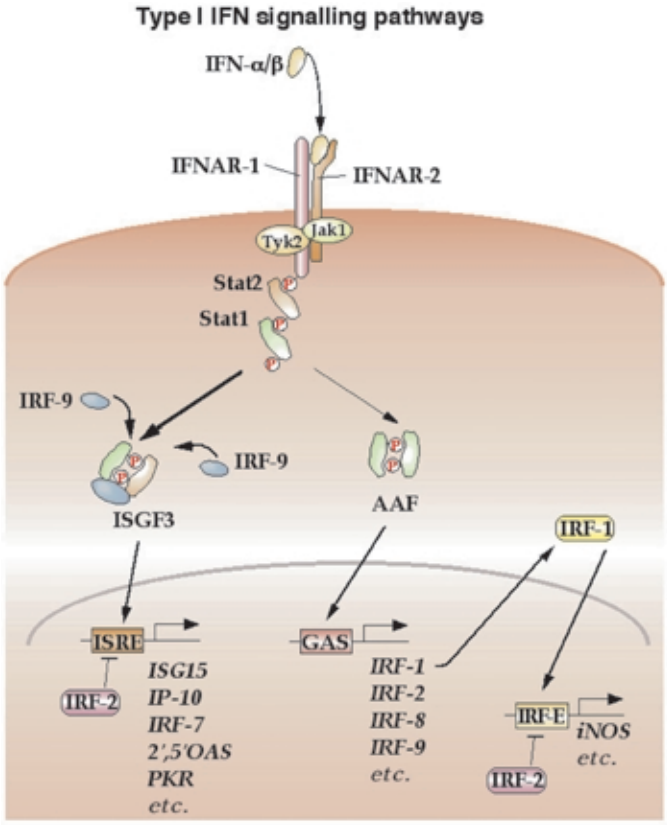

Fig. 1. Cardinal features of the IFN-α/β signalling pathway. IFN-α/β transmits signals via its homologous receptor complex, IFNAR.[13, 14] IFNAR is composed of at least two subcomponents, IFNAR-1 and IFNAR-2, of which IFNAR-2 is the major ligand-binding subunit. Ligand binding results in the formation of a signalling complex with IFNAR-1 and IFNAR-2, leading to activation of the receptor-associated Tyk2 and Jak1, respectively.[11–14] This is followed by the tyrosine phosphorylations of Stat1 and Stat2, leading to the formation of the heterotrimeric ISGF3 transcription factor together with an IRF-family member of transcription factors, IRF-9 (also referred to as p48). In addition, a Stat1 homodimer, termed AAF, is formed. Subsequently, these transcriptional activator complexes translocate into the nucleus and activate the interferon-stimulated response element (ISRE) or the IFN-γ-activated site (GAS). The human IFNAR-1 subunit contains within the cytoplasmic region four tyrosine residues, of which the tyrosine residue 466 (Y466) provides the main docking site for Stat2/Stat1. Of note in this review is the *IRF-7* gene, the expression of which is totally dependent on ISGF3. On the other hand, another IRF-family member, IRF-2, is a nuclear factor that also binds to ISRE, and this factor interferes with the ISGF3 action, thereby functioning as a transcriptional attenuator.[9] Although not depicted in this figure, it has been suggested that Stat1 and Stat2 are associated with IFNAR-2 before stimulation.[14]

can recognize, are essential as virus-inducible enhancers of IFN-β induction.[18–21] As for the *IFN-α* genes, it was reported that PRD-like elements (PRD-LEs), which are similar to the PRD I and PRD III elements in the IFN-β promoter, are found within IFN-α promoters.[22]

Since IRF-1 was first discovered as a transcriptional activator that binds to virus-inducible enhancer-like elements of the human *IFN-β* gene,[19] other IRFs have been found to constitute a family of transcriptional factors, which now consists of nine members (IRF-1 to -9; reviewed in Refs. 16, 23, 24). In a series of studies on *IFN-α/β* gene regulation, IRF-1, IRF-3, IRF-7 and IRF-9 among the above IRFs, were shown to be implicated in *IFN-α/β* gene induction by viral infection.[7, 20, 21, 25] Studies of the role of the IRF family members in the IFN system should provide an insight into the unique features of the IFN induction mechanism. A recent study using the gene targeting strategy revealed that two structurally related members, IRF-3 and IRF-7, are not only essential factors for *IFN-α/β* gene induction in response to viruses, but also are nonredundant in their roles in the IFN-α/β induction mechanism.[7]

2) Roles of IRF-3 and IRF-7 in two-step induction of *IFN-α/β* genes. In the initial phase (Fig. 2 (1)), IRF-3, which is constitutively expressed in normally growing cells, plays a dominant part in the initial induction of *IFN-α/β* genes.[7, 26–28] In fact, virus infection results in the serine/threonine phosphorylation of IRF-3 at its carboxyl-terminal region by an unknown kinase(s) to become an active form (See "Added-in-proof"). The activated IRF-3 undergoes nuclear translocation, and binds to IRF-Es in the IFN-β promoter. Since, unlike IRF-3, IRF-7 expression is inducible by IFN-α/β stimulation,[29, 30] IRF-7, which is induced by IFN-α/β secreted in the initial phase, mainly participates in the late phase of *IFN-α/β* gene induction (Fig. 2 (2)), particularly in mouse embryonic fibroblasts expressing IRF-7 at a low level in the absence of infection. This indicates the two-step induction of *IFN-α/β* genes by virus infection, wherein each of IRF-3 and IRF-7 plays a distinctive role in regulation of virus-induced IFN production.[7] In addition, there is a unique positive feedback mechanism in the late phase, where signalling by *de novo* synthesized IFN-α/β amplifies IFN-α/β production through its enhancement of the intracellular expression level of IRF-7.[7, 29, 30] Interestingly, a further study showed that the expression level of IRF-7 before infection was critical for triggering the positive feedback system for efficient induction of *IFN-α/β* genes by viruses,[31] which we will discuss in detail below.

3) Spontaneous IFN-α/β production. IFN-α/β is known to be massively produced upon viral infection, and functions in innate immune responses, but evidence has also been presented that the expression of IFN-α/β is constitutive, albeit at a very low level, in the absence of infection.[1, 5, 6] Although there is evidence that these IFNs contribute to antiviral, anti-tumor activities and cell growth control[1, 5, 32] in a manner similar to the virus-induced IFNs, recent studies have revealed a unique molecular machinery of this system.[8, 31, 33, 34] In fact, the weak signal renders cells "ready-to-go" for the amplification or enhancement of cellular responses to rapid environmental changes, such as viral infection. This machinery is similar to a car engine revving up before the car starts moving and accelerating. The "revving up" of cells by a weak signal is implicated in eliciting robust and efficient cellular responses against infections (Fig. 3; Ref. 34). This "revving-up" system is a unique signal-dependent regulatory mechanism for the responsiveness, particularly in host defense. Recent evidence has been shown that a weak IFN-α/β signal by the constitutively produced IFN-α/β is critical for eliciting strong responses of cells to IFN-γ and interleukin (IL)-6, which are major cytokines involved in adaptive immune responses.[8, 33] In addition, evidence has also been presented for a role of weak IFN-α/β signalling in the absence of viral infection in the regulation of innate immune responses against viruses; this signalling was found to play an important role in efficient production of IFN-α/β upon viral infection.[31]

4) Two amplification mechanisms for efficient *IFN-α/β* gene induction by virus. Analysis of the roles of IRF-7 in the regulation of virus-induced IFN-α/β production revealed that there are two regulatory mechanisms for the efficient amplification of the IFN-α/β production system (Fig. 4). One is an IRF-7-mediated positive feedback mechanism that is triggered by IFN-α/β initially produced after infection (Fig.4 (1)). The other is a regulatory mechanism that is controlled by a weak signal by constitutively produced IFN-α/β before infection (Fig.4 (2)). The latter mechanism is also mediated by IRF-7. As mentioned above, both IRF-3 and IRF-7 are essential factors for virus-induced IFN-α/β production. On the other hand, the nonredun-

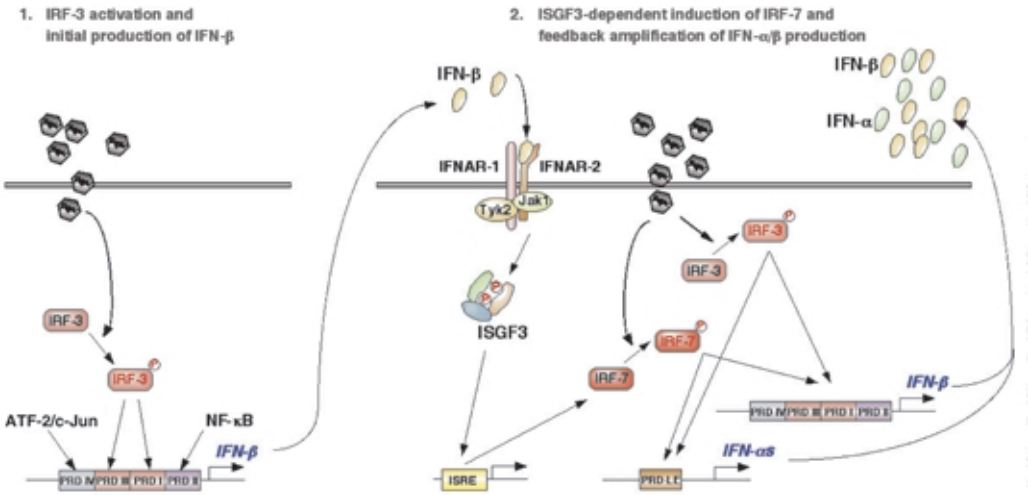

Fig. 2. Two-step induction of *IFN-α/β* genes upon viral infection. (1) The initial phase of induction *of IFN-α/β* genes, mediated by IRF-3 that mainly acts on the *IFN-β* gene. (2) The late phase of induction of *IFN-α/β* genes. The IFN-induced IRF-7 is activated and IRF-7 acts on both *IFN-α* and *-β* genes to amplify the IFN production. The latter step is therefore considered as a positive feedback regulation. See the text for further explanation.

dant roles of these two factors in IFN-α/β induction were clarified by a recent gene disruption/introduction study.[7] Unlike constitutively expressed IRF-3, IRF-7 is short-lived and its gene expression is dependent on IFN-α/β signalling[7, 29, 30]; hence, the IFN-α/β-dependency of IRF-7 provides an underlying mechanism for the autoamplification of IFN production, whereby induction of the *IRF-7* gene by *de novo* produced IFN-α/β during viral infection results in a positive feedback enhancement of *IFN-α/β* gene induction (Fig.4 (1)). In addition, there is another unique signal-amplification mechanism that is mediated by the weak IFN-α/β signalling in the absence of infection (Fig.4 (2)). This weak IFN-α/β signalling contributes to setting the IRF-7 expression level beyond an appropriate threshold for the activation of the positive-feedback loop upon infection.[31] In fact, in splenocytes, which express a relatively high level of IRF-7 even before infection, this induction mechanism is more effective than in fibroblasts with a much lower level of IRF-7 expression.[31] These expression levels of IRF-7 were found to be correlated with the expression level of the constitutive IFN-α/β. Thus, in advance of viral invasion, the constitutive, weak IFN-α/β signalling puts cells in a "revving up" state for the robust and efficient production of IFN-α/β upon viral infection.[34]

Furthermore, recent reports have shown that the activation of Toll-like receptors (TLRs) by several pathogen-associated molecular patterns (PAMPs) resulted in the induction of the *IFN-α/β* genes. Among these TLRs, TLR3 is known to bind to double-stranded RNA (dsRNA), leading to the induction of *IFN-α/β* genes.[35] Our recent data show that TLR3 itself is induced by type I IFNs in an ISGF3-dependent manner (unpublished data by Honda, K. *et al.*). In this regard, it may be suggested that TLR3 signalling is positively regulated through *Tlr3* gene induction by *de novo*-produced IFN-α/β, and that IFN-α/β-induced TLR3, as well as IRF-7, is one of the targets in the "IFN priming."[36] More interestingly, this may provide a possible explanation for a mechanism by which uninfected cells neighboring infected cells can get ready for the recognition of invading pathogens and for efficient cellular responses.

These observations offer an interesting example of how weak signals by a given stimulus contribute to eliciting strong cellular responses to other stimuli. Thus, the role of constitutive, weak IFN-α/β signalling, described here in the context of the "revving-up model," may point to a broad operation of similar mechanisms in other biological systems.

5) Role of the weak IFN-α/β-mediated signals for efficient CD8⁺ T cell activation upon T-cell receptor (TCR) stimulation. Recently, interesting data have shown that spontaneous IFN-α/β expression was only marginally augmented upon stimulation of CD8⁺ T cells by a mixed lymphocyte reaction.[37] In fact, different from the case of viral infections, induction of IFN-α/β mRNAs upon TCR stimulation is not efficient (less than 1/200 of the level of gene induction by viral infection.[31] In the context of the rev-

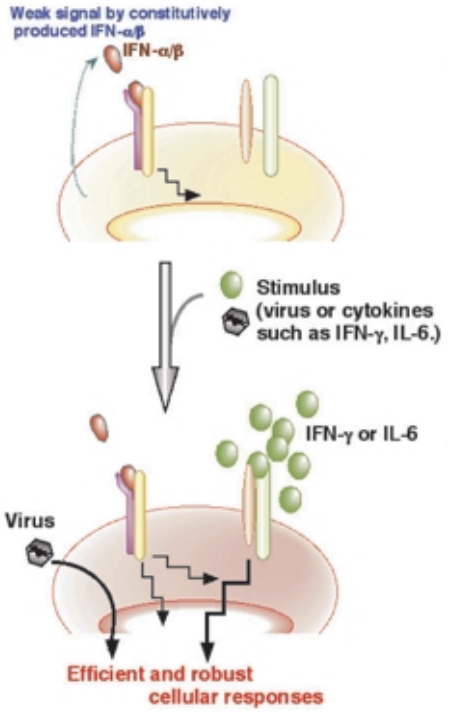

Fig. 3. "Revving up the cellular engine" by spontaneously produced IFN-α/β. A weak IFN-α/β signal by constitutively produced IFN-α/β plays an important role in the efficient and robust responses to IFN-γ, IL-6 or viral infection.[8, 31, 33] The IFN-α/β continuously transmit weak signals to elicit from cells strong responses to these other cytokines. Therefore, somewhat analogous to revving up the engine in car racing, this signalling may provide the foundation for efficient cellular responses in the immune system.

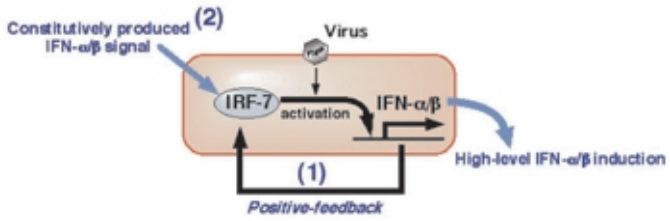

Fig. 4. Two amplification mechanisms for efficient *IFN-α/β* gene induction upon viral infection. There are two regulatory mechanisms for the amplification of virus-induced IFN-α/β production, both of which are dependent upon the expression of IRF-7. One is a postinfection positive feedback mechanism (1). The other is a preinfection regulatory mechanism that is controlled by a weak signal of constitutively produced IFN-α/β (2).

ving-up system based on the constitutively produced IFN-α/β, one can postulate that the low levels of IFN-α/β expression, which are only slightly induced upon T cell activation, play a similar role to the more efficient TCR signalling in $CD8^+$ T cells. In addition, the augmentation of the local IFN-α/β concentration at the site of T-cell activation may contribute to a more efficient operation of this mechanism for the activation of TCR signalling in $CD8^+$ T cells. During viral infections, it is known that $CD8^+$ T cells can be activated in a $CD4^+$ T-cell-independent manner, concomitant with the massive production of IFN-α/β (Refs. 38–40). Our recent results obtained with ovalbumin (OVA)-specific activation assays show that exogenous treatment with recombinant IFN-β enhances the proliferation of $CD8^+$ T cells in a dose-dependent manner.[37] On the other hand, $CD8^+$ T cells lacking the IFN-α/β signalling are hyporesponsive to antigen stimulation. Further evidence showed that IFN-α/β-mediated signals are required for the induction of chemokines, IFN-γ-inducible protein (IP)-10/CXC chemokine ligand (CXCL) 10 and IFN-inducible T-cell α chemoattractant (I-TAC)/CXCL11, as well as their common receptor, CXC chemokines receptor (CXCR) 3; this in turn provides evidence that CXCR3-mediated signals subsequently contribute to the efficient induction of activation markers such as CD25 and CD69, thereby leading to the efficient proliferation of $CD8^+$ T cells.[37] These observations offer another indication of possible involvement of the constitutive, weak IFN-α/β signalling in the efficient activation of $CD8^+$ T cell upon the engagement of TCR with major histocompatibility complex (MHC)/peptide complexes on antigen-presenting cells.

6) Dysregulation of weak IFN-α/β signalling. As described above, it was found that the weak IFN-α/β signalling is critical for efficiently eliciting robust cellular responses in the immune system, and may point to a broad operation of similar mechanisms in other biological systems. One can infer that dysregulation of this weak signalling may give rise to potentially serious disruption of these biological systems. Recent studies on IRF-$2^{-/-}$ mice provided an interesting model for clarifying this issue.[9] IRF-2, a stable nuclear protein expressed in a variety of cells, is found to be a transcriptional attenuator of IFN-α/β signalling, which is known to function negatively on ISRE sites of IFN-inducible genes by competition with ISGF3 for these sites.[21] Accordingly, in the absence of IRF-2, the action of ISGF3 becomes dominant, leading to the continual, elevated expression of ISGF3-dependent IFN-inducible genes, such as *IRF-7*. Our recent report showed that IRF-$2^{-/-}$ mice spontaneously develop an inflammatory skin lesion, which is similar to lesions observed in patients with psoriasis.[9] It was also found that the development of this skin disease results from selective $CD8^+$ T-cell hyperactivation, with a remarkable upregulation of ISGF3-dependent IFN-α/β-stimulated genes. Other pathological conditions observed in IRF-2-null mice, such as impaired hematopoiesis and autoimmune-like pancreatitis, may be caused by dysregulated weak IFN-α/β-signalling. These abnormal findings in IRF-$2^{-/-}$ mice suggest that the constitutive, weak IFN-α/β signalling should be controlled by a proper regulatory mechanism for maintaining homeostasis in the host. In this context, IRF-2 is one of the essential regulators that properly set the level of constitutive, weak IFN-α/β signalling to balance the beneficial and harmful effects of this signalling. Thus, dysregulation of such a signalling system can also be the basis for disease development.

The IFN-α/β system in oncogenesis

Much evidence regarding anti-tumor activities of IFNs has been reported (reviewed in Refs. 41, 42). Most of their antitumor functions are explained in terms of modulatory actions on the immune system. At least hundreds of cellular genes are transcriptionally activated after IFN stimulation.[43] Several of these ISGs were shown to encode proteins that mediate tumor suppressor activities directly or indirectly: for example, IRF-1, dsRNA-dependent protein kinase (PKR), and 2′-5′oligoadenylate synthetase (OAS).[44–51] They modulate the functional activities of the immune system against tumors in various ways and affect cell proliferation and differentiation. Recent studies on the immunomodulatory activity of type I IFNs have shown that IFNs act on T-lymphocytes or dendritic cells to modulate their functions, which may explain IFN-induced tumor immunity (reviewed in Ref. 52).

On the other hand, in the context of the antiviral actions of IFNs themselves, evidence points also to the potential benefits of IFNs against tumors of viral origin, such as hairy cell leukemia, Kaposi's sarcoma, hepatitis B or C virus-related hepatomas, HPV-related condylomata acuminatum and juvenile laryngeal papillomatosis, and others.[53, 54]

Different from the aspects mentioned above, we have recently found a novel aspect of the anti-tumor activities induced by IFN-α/β. As discussed above, low levels of IFN-α/β production in the absence of viral infection were previously observed in mouse embryonic fibroblasts (MEFs) and other cells, and IFN-α/β mRNAs can be detected in MEFs and other cells/tissues of the mouse by reverse transcriptase-polymerase chain reaction (RT-PCR) analysis. Evidence has also been provided that, very similar to virus-induced IFN-α/β, these low-level IFN-α/β expressions indeed contribute to anti-tumor activities and to the control of cell growth. Recently, we have made an interesting observation regarding the involvement of a weak signal by the constitutively produced IFN-α/β in the regulation of oncogenesis. Long-term cultures of IFNAR-1-deficient MEFs as well as IFN-β-deficient MEFs, both of which show little or no signalling by constitutively produced IFN-α/β, resulted in the formation of transformed colonies, albeit at a low incidence, which could form tumors in nude mice (Takaoka and Taniguchi, unpublished data). Moreover, IFNAR-1-deficient mice showed papilloma formation on the skin after chemical induction (Takaoka and Taniguchi, unpublished data). These findings suggest a novel biological significance of the weak signal by constitutively produced IFN-α/β, which may be implicated in homeostatic host defense mechanisms for preventing cells from developing tumors. Furthermore, it can be speculated that this weak IFN-α/β signalling might be involved in maintaining the constitutive expression level of some molecule(s) which might be a regulator(s) in the surveillance system against oncogenesis. Therefore, IFNs seem to be essential mediators not only for the host defense in the innate immune responses against microbial infections, but also for a host defense system against oncogenesis.

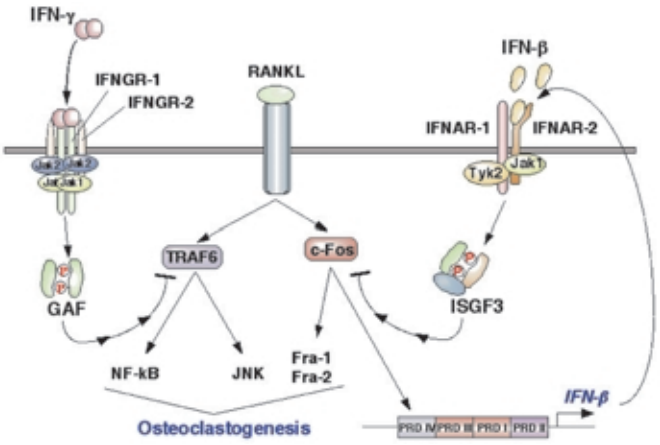

Fig. 5. Role of the IFN-β system in the negative regulation of osteoclastogenesis through crosstalk with RANKL signalling. During osteoclastogenesis, the IFN-β system is utilized in negative feedback regulation for shaping the RANKL signalling. IFN-β is induced by RANKL stimulation in a c-Fos-dependent manner. See the text for details.

The IFN-α/β system in bone metabolism

Several recent studies have revealed a novel facet of the IFN system in bone metabolism,[10, 55] which led to the development of a new field, termed osteoimmunology, that is, the overlapping of bone metabolism and the immune system.[56, 57] Maintenance of bone homeostasis is widely known to be regulated by a mechanism that balances bone formation and bone resorption, wherein osteoblasts and osteoclasts, respectively, are the essential effector cells.[58] In osteoclastogenesis, RANKL, a member of the TNF (tumor necrosis factor) family, is a critical cytokine for the differentiation of monocyte/macrophage precursors to osteoclasts.[59–61] It was demonstrated that osteoclastogenesis is strictly regulated by activated T-cell-derived IFN-γ via its counterbalancing action on RANKL signalling[55] (Fig. 5). In this regulatory system, IFN-γ negatively regulates RANKL signalling through the degradation of TRAF6 (TNF-receptor-associated factor 6), one of the major downstream molecules in RANKL signalling. Further extensive analyses revealed that IFN-β is also a critical mediator during osteoclast differentiation.[10] Both IFNAR-1-deficient mice and IFN-β-deficient mice showed a significant reduction in trabecular bone mass, with an increase in the number of osteoclasts. This observation indicates that type I IFN signalling is required for the negative regulation of osteoclastogenic bone resorption. Further analysis of the mechanism underlying the inhibitory action of type I IFN revealed that as yet unidentified protein(s) encoded by an IFN-stimulated gene(s) may posttranslationally suppress the expression of c-*fos*, one of the critical target genes for RANKL-induced osteoclast differentiation (Fig. 5). In bone marrow-derived macrophages (BMMs), which are known to be osteoclast precursor cells, RANKL stimulates the induction of the *IFN-β* gene, but not that of *IFN-α* genes, which suggests a unique signalling crosstalk wherein RANKL signalling finely regulates osteoclastogenesis by controlling the balance via the utilization of the IFN system with the RANKL-induced IFN-β. It is of interest that the mechanism of IFN-β induction by RANKL is totally different from that of the type I IFN induction by viral infection. The induction of the *IFN-β* gene by RANKL in BMMs is still detected even in the absence of two IRF transcriptional factors, IRF-3 and IRF-7, which are essential factors for the virus-mediated induction of IFN-α/β, as discussed above. However, the RANKL-induced IFN-β mRNA expression was no longer observed in cells lacking c-Fos. Actually, the (weak) induction of *IFN-β* gene by RANKL requires c-Fos, perhaps in the context of the AP-1 transcription factor complex that binds to the promoter. These results indicate a hitherto unrecognized new role of IFN-β, beyond its function in anti-viral immunity, whereby the IFN-β system is utilized for the negative regulation of RANKL signalling for osteoclastogenesis.

Conclusions and future prospects

In this article, we reviewed new aspects of the IFN-α/β system, highlighting the functional implication of the weak signalling by constitutively produced IFN-α/β in the regulation of cellular responses to other extracellular stimuli.

As described in this article, it was found that the weak IFN-α/β signal provides unique signal regulatory mechanisms for its own IFN-α/β signalling system and signalling systems of other cytokines such as IFN-γ and IL-6, as well as the TCR signalling system. In this regulatory system, the weak IFN-α/β signalling by the constitutively produced IFN-α/β allows the cell to rev up for efficient and robust responses to various stimuli (the "revving-up" model[34]). In this regard, the weak IFN-α/β signalling in the absence of infection contributes to the control of the IRF-7 expression level beyond a certain threshold that is crucial for triggering the autoamplification mechanism for IFN-α/β production upon viral infection.[31] This IFN-α/β signal-mediated "revving-up" mechanism through IRF-7 expression enables cells to efficiently produce IFN-α/β to achieve a robust immune response to viral infections.

In the IFN-α/β signalling system described here, signalling molecules remain constantly activated, albeit weakly, and the expression of target genes is maintained, thereby providing a foundation for more efficient signalling, either in that pathway or in different pathways. Thus, consumption of cellular resources is a regulated "trade-off" to provide the cell with a greater dynamic range (signal-to-noise) in its response to stimuli. The IFN-α/β system may also provide an interesting illustration of the feedback loops required when such a function is operational. The advantages of highly efficient IFN-γ/IL-6 responses and the disadvantages of the failure of the IRF-2-mediated attenuation (autoimmune, psoriasis-like syndrome) indicate that the selective advantages of "revving up" the response can also be the basis of disease states when it is dysregulated. The possibility that this may be a key theme in the overall setting of host defense and autoimmunity opens exciting avenues for future research.

The host defense mechanism comprises two major defense systems: innate and adaptive immunities. Innate immunity initiates protection of the host organism against invasion and subsequent multiplication of microbes by recognition of the PAMPs.[62, 63] In this regard, several microbial pattern recognition receptors have been recently identified as TLRs, the mammalian homologues of the *Drosophila* Toll receptor (reviewed in Refs. 64, 65). Recently, much attention has been focused on the roles of the IFN and IRF systems in relation to TLRs. In fact, it was reported that several microbial products, such as dsRNA, lipopolysaccharide (LPS), and oligodeoxyribonucleotides containing unmethylated CpG motifs (CpG-DNA), activate the induction of *IFN-α/β* genes through their respective TLRs.[66–70] Recent studies led us to envisage the sequence of IFN-α/β-mediated events that operate during the maturation of dendritic cells (DCs) (Honda, K., unpublished data). The newly discovered role of IFN-α/β in DC maturation may provide practical approaches to an adjuvant for vaccines that enhance anti-tumor and anti-viral immunity.

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Added in proof: Interestingly, it has recently been reported that IκB kinase (IKK)-related kinases, IKKε and TANK-binding kinase 1, are components of the virus-activated kinase responsible for IRF-3/7 phosphorylation (Fitzgerald, K. A. *et al. Nat Immunol* 2003; **4**: 491–6, and Sharma, S. *et al. Science* 2003; Apr 17 [epub ahead of print]).