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Commentary

Mechanisms of interferon-β effects on bone homeostasis

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ABSTRACT

Restoration of dysregulated bone homeostasis is a therapeutic goal in many diseases including osteoporosis, rheumatoid arthritis and metastatic cancer. The molecular pathways regulating bone remodeling are major therapeutic targets, and studies continue to reveal endogenous factors that may be pathologically up- or down-regulated and lead to an uncoupling of bone formation and resorption. The purpose of this commentary is to highlight new mechanisms of bone homeostatic regulation mediated through the induction of endogenous interferon-β (IFN-β). The receptor activator of nuclear factor-κB (RANK) ligand (RANKL) is an important factor in the bone resorption cascade, and the RANK–RANKL interaction has been shown to induce IFN-β and osteoclastogenesis via induction of the c-fos gene. Subsequent binding of IFN-β to its biological receptor initiates a signal transduction cascade through the classic JAK/STAT pathway, causing an inhibition of c-fos protein production and osteoclast proliferation and differentiation (negative feedback). Another mechanism pertinent to the anti-resorptive effect of IFN-β is the induction of nitric oxide which has been shown to inhibit osteoclast formation. The role of IFN-β in bone metabolism could warrant its systematic evaluation as a potential adjunct to therapeutic regimens of osteolytic diseases. Here we also provide discussion of the potential challenges to optimizing IFN-β pharmacotherapy for such purposes.

1. Introduction

The purpose of this commentary is to concisely review the signal transduction pathways implicated in the effects of interferon-β (IFN-β) on bone homeostasis and to provide perspective for the potential clinical use of IFN-β in the treatment of bone-related pathologies. Bone is often but erroneously viewed as a relatively static tissue whose primary function is to provide the structural support and to enable the biomechanical roles of the musculoskeletal system. To the contrary, bone is a dynamic organ system that is involved in many other critical functions including calcium ion homeostasis and hematopoeisis and is intricately regulated. Recent research has identified a host of paracrine, endocrine and cell–cell interactions between the immune system and bone and has led to an emerging field of research termed osteoimmunology.

The four major cellular components of bone are the osteoblasts (OBs), osteoclasts (OCs), bone lining cells, and osteocytes. The OBs arise from mesenchymal stem cells and are primarily responsible for bone formation. Osteocytes are bone cells surrounded by a mineralized matrix. The bone lining cells are flattened cells on the bone surface. Although their exact role is not yet known, it is speculated that they are precursors for OBs [1] or, in the presence of parathyroid hormone (PTH), secrete enzymes to aid the resorptive activity of OCs [2]. Hematopoeitic stem cells give rise to OCs, which are responsible for bone resorption.

A host of endogenous agents are known to regulate bone remodeling. These include calcium ions (Ca^{2+}), parathyroid hormone (PTH), calcitriol, glucocorticoids, estrogen, cytokines such as interleukin-6 (IL-6), tumor necrosis factor (TNF)-α and -β, fibroblast growth factor (FGF), and calcitonin. These factors can act through ligand-receptor signaling, cell–cell interactions, release of factors in the bone microenvironment that induce differentiation of specific cell populations, or by enabling the production of extracellular (EC) matrix for subsequent deposition and mineralization in bone tissue [3].

Many endogenous bone-modulating agents are also employed as pharmacological agents (e.g., parathyroid hormone, estrogen

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Abbreviations: IFN-β, interferon-beta; iNOS, inducible NO synthase; MAPK, mitogen-activated protein kinase; NO, nitric oxide; OPG, osteoprotegrin; PD, pharmacodynamics; PK, pharmacokinetics; PTH, parathyroid hormone; RANK, receptor activator of nuclear factor-κB; RANKL, RANK ligand; SOCS, suppressors of cytokine signaling; TNF, tumor necrosis factor; TRAF, TNF receptor associated factor.

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and corticosteroids) or mediate pathophysiological states (e.g., IL-6 and TNF-α). Bone homeostasis is also dysregulated in many disease states including post-menopausal osteoporosis, osteopetrosis, rheumatoid arthritis, multiple sclerosis, inflammatory bowel disease, lead poisoning and metastatic cancer.

2. RANK–RANKL pathway in osteoclast differentiation

Osteoblast and osteoclast interactions regulate turnover of bone tissue through the release of specific factors such as the tumor necrosis factor (TNF) related cytokine "receptor activator of nuclear factor κB ligand" (RANKL) and osteoprotegrin (OPG) [4,5]. Such processes have been extensively reviewed [6–10]. The receptor for RANKL (RANK) is expressed on OCs and the RANK–RANKL interaction results in maturational activation of OCs. Pre-OCs are terminally differentiated into mature OCs when stimulated by RANKL [11] and undergo apoptosis after carrying out bone resorption. To balance resorptive activity, OPG is released from OBs, which acts as a decoy receptor for RANKL. Yasuda and coworkers were the first to clone OPG and relate its activity to inhibition of osteoclastogenesis *in vitro* [12], and binding of OPG to RANKL leads to a reduction in RANK receptor signaling. Although an alternate RANK-RANKL independent mechanism of osteoclastogenesis has been reported, which is mediated by TNF-α [13], the RANK–RANKL interaction is the primary regulator of osteoclastogenesis. Further differentiation of OCs involves the fusion of multiple cells to form a polykaryon containing roughly 5–8 nuclei. The resorptive capacity of activated polykaryons seems to be proportional to the number of nuclei present. This increase in activity has been attributed to an increase in cytoplasmic and brush border membrane area forming the resorption pits. RANKL and calcitriol have been found to be major determinants of cell fusion.

The OPG/RANK/RANKL axis is a primary regulator of OC differentiation, and its activation is critical in forming the terminal resorptive moiety [12,14]. This pathway has been extensively characterized in murine co-culture systems comprising of OBs and spleen cells. In the presence of osteotropic factors such as PTH and calcitriol, OBs are stimulated to produce membrane bound RANKL [9,10]. A known source of soluble RANKL (sRANKL) *in vivo* is the T lymphocyte, which under conditions of inflammatory bone disease, may contribute to excessive resorption [15]. Binding of RANKL to RANK, through cell–cell interaction of OBs and OCs, stimulates multiple intracellular signal transduction cascades, including: p38 mitogen-activated protein kinase (MAPK), nuclear factor of activated T cells c1 (NFATc1) through calcium signaling, NF-kB, c-fos, c-src, Ca^{2+}/calmodulin-dependent kinases (CaMKs), cAMP response element (CRE)-binding protein (CREB), and c-Jun N-terminus kinase (JNK) [16–18].

A major downstream transcription factor is the adaptor protein TNF receptor (TNFR) associated factor 6 (TRAF6) [19]. RANK exhibits multiple and distinct intracellular binding motifs (1, 2 and 3) capable of binding specific TRAF proteins [20]. Adaptor protein TRAF6 binds to motif 1 (amino acids 340–421), TRAF3 interacts with motif 2 (amino acids 571–573) and TRAF1, TRAF2 and TRAF5 bind to motif 3 (amino acids 609–610). Chimeric association studies with the different motifs of RANK have shown that different TRAFs have an important role in regulating OC differentiation, survival, and function [21]. The predominant step in RANK signaling for osteoclast survival is binding of TRAF6 to the cytoplasmic domain of RANK (i.e., motif 1). $TRAF6^{-}/^{-}$ mice exhibit an osteopetrotic phenotype similar to that seen in $c\text{-}src^{-}/^{-}$ mice. These mice had normal osteoclast numbers; however, results suggest that these cells were non-functional. Binding of TRAF6 leads to subsequent activation of two principal pathways: NF-κB and activator protein-1 (AP-1) transcription factors. These factors are the primary regulators directing OC-specific gene expression and enhanced osteoclastogenic activity.

2.1. The interferons

The interferons (IFN) are endogenous cytokines and are conventionally categorized into two major classes: the Type I interferon class consists of IFN-α, IFN-β and IFN-ω, and the Type II IFN class is comprised of IFNγ subtype [22,23]. IFN-β and IFN-γ are prominently involved in bone homeostasis. IFN-β is an anti-inflammatory, anti-viral and immunomodulatory glycoprotein (22.5 kDa and 166 amino acids) produced endogenously in humans by fibroblasts, epithelial cells, and macrophages in response to viral infection or nucleic acids [24].

IFN-β is an approved treatment for the relapsing forms of multiple sclerosis (MS), a neurodegenerative inflammatory condition. Treatment with IFN-β has been shown to alleviate conditions of RRMS and has an established safety profile [25]. Renewed interest in this cytokine was generated after Takayanagi and coworkers demonstrated that IFN-β is a major factor regulating bone turnover processes [26]. Understanding mechanisms of the osteoimmunogenic effects of IFN-β may provide insights into new therapeutic targets and the design and development of new drugs or drug combinations for the treatment of osteolytic diseases.

3. Mechanisms of action for IFN-β effects on bone homeostasis

The major mechanisms by which IFN-β influences bone metabolism are depicted in Fig. 1. Autocrine regulation via c-fos inhibition and NO induction are prominently featured pathways. Although not shown in the figure, a description of the 4-1BB ligand mediated regulation of bone homeostasis has also been included in this section.

3.1. Autocrine regulation of bone homeostasis by RANKL through IFN-β induction (c-fos mediated pathway)

The dimeric transcription factor Ap-1 includes the Fos (c-Fos, FosB, Fra-1 and Fra-2) and Jun (c-Jun, JunB and JunD) family of proteins. Pre- and mature OCs exhibit high expression levels of RANK, and activation of RANK by RANKL stimulates the production of c-fos through a sequence of phosphorylation cascades. C-fos is a major component of the AP-1 transcription factor complex and a critical regulator of OC differentiation. Mice deficient in c-fos develop osteopetrosis and the function of c-fos in osteoclast progenitor differentiation could not be substituted by other AP-1 family members [27,28]. During osteoclast formation, c-fos triggers a transcriptional regulatory cascade by inducing and cooperating with NFATc1 for activation of multiple target genes responsible for osteoclast function [29].

The pathway proposed by Takayanagi and coworkers involves the induction of IFN-β in response to OC activation by RANK-RANKL interaction [26]. Induction of IFN-β is mediated via a c-fos dependent mechanism that is present downstream of the RANK–RANKL signal transduction cascade. Activation of the c-fos gene upregulates expression of IFN-β in OCs. Elevated levels of c-fos protein bind to the promoter region of the IFN-β gene resulting in the observed upregulation. Increased production of IFN-β is in turn expected to inhibit c-fos activity resulting in the inhibition of osteoclastogenesis. This pathway presents a scenario wherein a critical component of the osteoclastogenesis cascade induces its own inhibitor and contributes to negative feedback regulation of osteoclastogenic action. Inhibition of c-fos by IFN-β is achieved through a post-translational mechanism, as downregulation of c-fos mRNA has not been detected. The importance IFN-β in regulation of osteoclastogenesis was evaluated in mice whose one

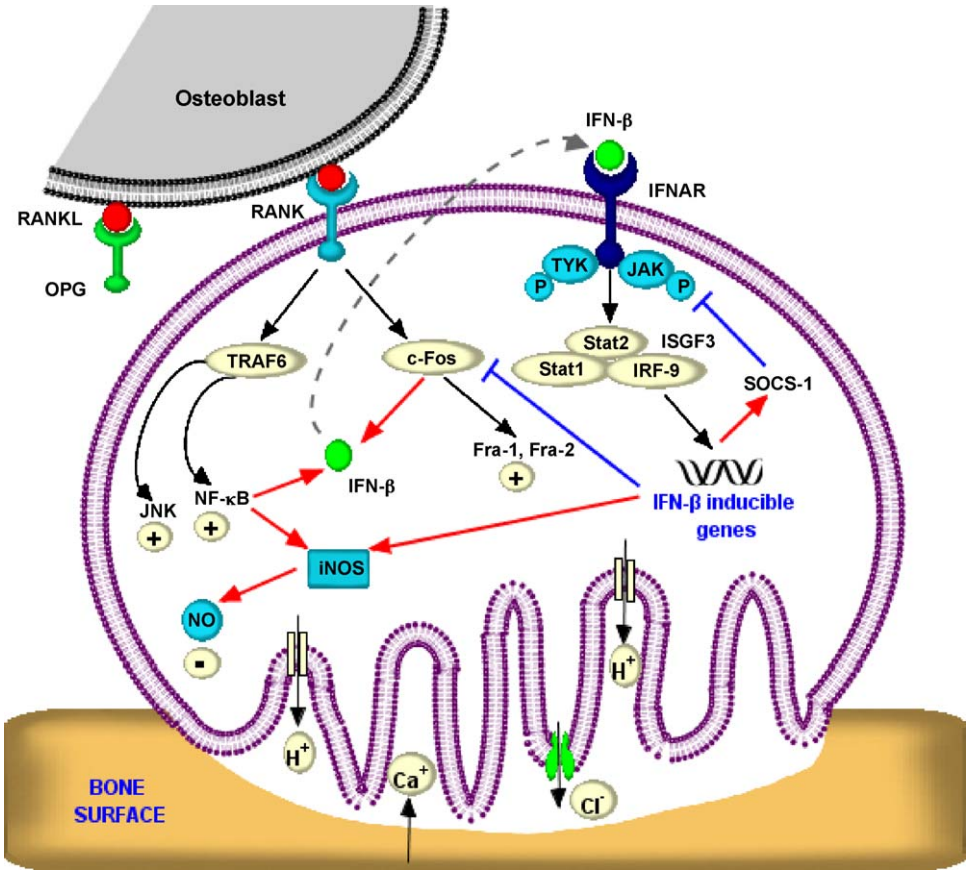

Fig. 1. Signaling pathways of osteoclastic bone resorption. The diagram shows signaling crosstalk between RANK–RANKL, IFN-β and iNOS. Interaction of RANKL expressed on the membrane of OB with RANK on the surface of OC results in the activation of the osteoclastic resorption cascade. A decoy receptor for RANKL, i.e., OPG is released by OB to maintain homeostasis and prevent excessive resorption. The intracellular signaling pathway activated by RANK/RANKL includes TRAF6 and c-Fos. Induction of NF-κB by RANKL is achieved through the TRAF6 intermediate. Downstream signaling activity of c-fos is mediated through Fra-1 and Fra-2 and is responsible for enhanced osteoclastogenesis. Both NF-κB and c-fos is expected to stimulate the production of IFN-β as a part of their negative feedback regulation. Signaling by IFN-β through the IFNAR receptor includes a series of phosphorylation events followed by gene transcription events that inhibit c-fos protein activity. Inhibition of osteoclastogenesis is also achieved through the induction of iNOS by IFN-β as well as direct binding of NF-κB to the promoter region of the iNOS gene. NO released as a result of iNOS stimulation is expected to inhibit osteoclast activity. Attenuation of IFN-β activity may also occur due to induction of SOCS-1.

subunit of the IFN Type I receptor was knocked out (IFNAR1$^{-/-}$). Characteristic reduction in trabecular bone mass and volume, along with a significant increase in multinucleated OCs was seen; indicative of increased resorption. Also, mice devoid of IFN-β (IFN-β$^{-/-}$) develop a similar osteopenic phenotype. The effects of IFN-β are primarily mediated through Jak-Stat pathway, although several other signaling pathways, including the phosphoinositide-3-kinase (PI3K) pathway, may be involved [30]. Inhibitory effects related to osteoclastogenesis may be regulated through the interferon stimulated gene factor 3 (ISGF3) complex comprising of signal transducers and activators of transcription 1 (STAT1) and STAT2 and interferon regulatory factor 9 (IRF9) [26]. Bone marrow macrophages (BMM) derived from mice lacking either STAT1 or IRF9 demonstrated a lack of inhibition of OC differentiation. This finding suggests that the IFN-β mediated OC inhibition is driven by ISGF3 binding with the interferon stimulated response element (ISRE) to initiate subsequent gene mediated transcription events. Although the exact mechanism of inhibition of c-fos protein inhibition is not known, ISGF3-inducible dsRNA-activated protein kinase (PKR) has been implicated. A potential candidate for gene induction by IFN-β is the chemokine CXCL11 (also known as I-TAC or SCYB11), which was found to be differentially upregulated *in vitro* by IFN-β [31]. The differentiation of CD14^{+} monocytes from peripheral human blood into mature OC in the presence of M-CSF and RANKL was potently inhibited by IFN-β. The number of TRAP positive cells was significantly lower in the presence of IFN-β. Additionally in the absence of IFN-β, direct administration of CXCL11 was able to inhibit osteoclast differentiation via an as yet unknown receptor. Induction of CXCL11 by IFN-β is mediated through ISGF3 as well as activation of the NF-κB transcription factor [32]. Activation of NF-κB is achieved by phosphorylation of the p65 subunit of NF-κB through a phosphoinositide-3-kinase (PI3K) dependent pathway with Akt kinase being the downstream target of PI3K. Although not clear yet, CXCL11 may be a key intermediary in the inhibition of c-fos by IFN-β.

An *in vitro* study evaluated STAT1 dependent IFN-β action on the modulation of OC activity in response to "toll-like receptor 5" (TLR5) activation by a specific microbial ligand flagellin [33]. Contrasting results were observed in BMM and co-cultures of bone cells. However, the reduction of differentiated OC from BMM through RANKL induction was achieved due to lower c-fos protein expression. The lower expression of c-fos protein was due to the induction of endogenous IFN-β production by RANK activation.

3.2. Regulation of bone homeostasis through RANKL induction of nitric oxide (NO)

The induction of IFN-β by RANKL has been linked to inducible NO synthase (iNOS) [34]. Synthesis of NO is controlled by the NOS enzymes (three isoforms: neuronal, endothelial and inducible) from molecular oxygen and L-arginine. OCs express inducible NOS (iNOS) and release NO, which further inhibits OC formation and

bone resorption [35]. NO generated through this pathway serves as a negative feedback signal to limit RANKL stimulated osteoclastogenesis. In the murine monocytic cell line RAW264.7 and murine primary bone marrow cells, RANKL upregulates the expression of iNOS mRNA, iNOS protein expression, and NO in a dose-dependent manner. Conversely, in iNOS deficient ($iNOS^{-/-}$) marrow cells, RANKL induced NO production was suppressed, resulting in elevated numbers of terminally differentiated OCs. The link between IFN-β and iNOS/NO was confirmed using an NF-κB inhibitor as well as determining the effect on iNOS/NO by direct administration of IFN-β in RAW264.7 cells. The NF-κB inhibitor reduced the induction of iNOS in a dose-dependent manner and IFN-β was able to stimulate the expression of iNOS in the absence of RANKL. These results suggest that IFN-β may be a key mediator for RANKL induction of iNOS derived NO in developing OCs.

3.3. 4-1BB ligand (4-1BBL) mediated regulation

Another interaction involved in bone homeostasis regulation by IFN-β is that of 4-1BBL with 4-1BB [36]. Similar to RANK, the 4-1BB receptor (4-1BB) is a member of the TNF family and has been found to be constitutively expressed on T cells, natural killer cells (NKC), and myeloid cells. Upon activation, antigen presenting cells (APC), such as dendritic cells (DC), B cells, and macrophages, express 4-1BBL [37]. The signaling cascade of 4-1BB is initiated by the association of TRAF proteins (especially TRAF2). Interestingly, studies in HEK mammalian cells have shown that NF-κB is activated as a downstream mediator of its actions in T-cells [38].

BMM from C57BL/6J mice were incubated with M-CSF and RANKL, 4-1BBL mRNA was found to be upregulated [36]. In the presence of immobilized recombinant 4-1BB (4-1BB-Fc), the number of TRAP positive multinucleated cells that were expressed was significantly lower. This inhibition of OC differentiation was reversed by antibodies to 4-1BBL. Furthermore, IFN-β mRNA levels were quantified in the presence and absence of 4-1BB-Fc and were found to be higher in the presence of 4-1BB-Fc. Neutralizing antibodies to IFN-β partially reversed the inhibition of OC maturation. This study also suggested that higher levels of IFN-β were induced in the presence of both, RANKL and 4-1BB-Fc compared to RANKL exposure alone [36]. It is not clear yet as to which factors are stimulated or repressed by the interaction of 4-1BBL and 4-1BB leading to the inhibition of osteoclastogenesis. Binding activity of IRF-3 DNA was found to be induced by 4-1BB-Fc. Stimulation of PI3K is essential for activation of IRF-3 [39]. In addition, studies suggest that PI3K inhibition induces IFN-β synthesis [40]. IRF-3 is activated through 4-1BB stimulation in conjunction with suppression of repressors of the IFN-β promoter via PI3K inhibition resulting in induction of IFN-β. Though not explicit, one cannot rule out that CXCL11 may also be involved, as NF-κB mediated induction of CXCL11 is PI3K dependent. However, activation of PI3K is essential for subsequent activation of NF-κB leading to the upregulation of CXCL11 [32]. The complex role of PI3K is intriguing, will require further research, and its action may be cell-type specific.

Osteoclast precursors have been reported to express 4-1BB and 4-1BBL upon exposure to RANKL [41]. $4\text{-}1BB^{-/-}$ knockout mice also show an increased bone mass compared to wild type animals. Reduced OC activity was attributed to the decreased expression of c-fos. Further studies are needed to determine the significance of this signaling pathway in bone physiology.

4. Proof of concept studies

Several *in vitro* and *in vivo* studies have been reported that seek to exploit the relatively new mechanisms of IFN-β action on bone resorption [26,31,42–50]. The effects of IFN-β were evaluated in a mouse model of endotoxin-induced inflammatory bone destruction [26]. Exogenously administered IFN-β reduced the number of mature OCs along with a significant decrease in bone resorption. The authors also reported that similar results were obtained in an ovariectomized osteoporosis mouse model; however, results from the osteoporosis model have not been published yet. The effects of IFN-β in collagen induced arthritis (CIA) mediated bone destruction have been evaluated in mice [50]. In this model, mice were treated intraperitoneally with IFN-β for 7 days before subsequent analysis of bone destruction. Histologic analysis of the paws revealed a decrease in the number of OCs among treated animals, leading to reduced bone destruction. A parallel decrease in the number of c-fos and RANKL positive cells was also reported.

In a clinical study evaluating synovial tissue of patients suffering from rheumatoid arthritis (RA), endogenous IFN-β protein expression was significantly higher compared to negative controls and could represent an *in vivo* anti-inflammatory mechanism [48]. However, a clinical phase II placebo controlled study of IFN-β-1a in rheumatoid arthritis patients (2.2 or 44 μg three times weekly for 24 weeks) failed to demonstrate an improvement in radiological scores or biomarkers of bone resorption [49]. Weinstock-Guttman and colleagues conducted an open label pharmacodynamic study in RRMS patients undergoing IFN-β therapy [46]. Peripheral blood samples were analyzed for bone turnover specific biomarkers over a 1-year period. Short-term changes from baseline levels of OPG and RANKL were monitored. Levels of OPG expression were maximal at roughly the same time when free RANKL expression was low. Although it was reported that OPG induction by IFN-β is partly responsible for decrease in free RANKL, the precise mechanisms for such an induction is not yet clear. Increases in OCN from pre-treatment levels after 1 year were also indicative of the protective effects of IFN-β on bone in MS patients. Unfortunately, BMD could not be assessed due to the lack of pre-treatment measurements in these patients. Patients with MS have been shown to have low bone mineral density [51–53], and the clinical study of Weinstock-Guttman and colleagues illustrates the challenges of selectively studying the osteoimmunological therapeutic effects of IFN-β in patient populations [46]. In many diseases that afflict bone homeostasis, the effects on bone are viewed as secondary to the causative pathological process. MS is used as relevant illustrative example of a disease with increased bone resorption. Clinical studies and the subsequent data analysis must necessarily control for effects of the adverse effects of the MS disease process on bone, as well as the beneficial effects of IFN-β on the disease process, in order to delineate IFN-β effects on bone. In practice, a host of other confounding factors that can affect bone mineral density measurements on their own also have to be considered. For example, MS patients may require corticosteroid therapy, have low vitamin D levels or have limited ambulation due to the disability caused by the disease. Finally, concordance between the doses needed for obtaining of IFN-β effects on bone and that in use for controlling the MS disease process has to be demonstrated for clinical acceptance.

5. Prospectus

Osteoimmunology is a term coined to emphasize the study of the functional interplay between the skeletal and immune systems [54], simultaneously considering the impact of various cytokines, chemokines, and various transcription factors affecting these systems [55–58]. These interactions operate in a micro-environment wherein components of each system are in close proximity. Osteoimmunology is central to the mechanisms of action of IFN-β, as its anti-resorptive properties are induced through the RANKL–RANK signal transduction cascade.

Bone pathologies result from an imbalance between the processes of bone formation and resorption, with a majority of them being driven by excessive OC activity. An imbalance in the RANK/RANKL system is implicated in these OC related pathologies [16] as well as the mechanisms of IFN-β pharmacodynamics. Inhibition of excessive osteoclastogenesis by IFN-β is an enticing proposition owing to the molecular properties reviewed here; however, several challenges must be addressed before utilizing IFN-β as an adjunct to the treatment of conditions such as rheumatoid arthritis, osteoporosis, multiple myeloma or other bone degenerative diseases.

IFN-β is relatively well tolerated clinically and has a relatively safe profile in humans. However, as with other protein drugs, patients develop neutralizing antibodies and efforts continue to focus on methods for developing formulations that render the protein less reactive to the host immune system. Some of these measures include formulation of a liposomal carrier for encapsulating the protein or site specific mono- or multiple-site pegylation [59,60]. The exposure-response relationship of IFN-β disposition and induction of neopterin (a classic endogenous biomarker) is itself characterized by complex non-linear drug distribution, elimination, and signal transduction processes [61,62]. Mathematical models of IFN-β pharmacokinetics and pharmacodynamics (PK/PD) have been based on a hypothesis that binding to the receptor influences disposition as well as dynamics, a phenomenon known as target-mediated drug disposition [63,64]. Improved PK properties of IFN-β as well as *in vivo* efficacy in a melanoma angiogenesis model were reported for the pegylated protein [60]. However, protein pegylation may not lead to a proportional increase in PD activity [65]. Lack of improvement in PD may be attributed to steric hindrance of the protein with its active site on the target receptor. The PK/PD properties of native and modified IFN-β are complex, and optimization of exposure and response profiles will require further experiments and modeling in relevant systems.

Efficient drug delivery to the bone microenvironment is another challenge for using IFN-β or other drugs in treating bone diseases. The bone microenvironment may be relevant in conditions like multiple myeloma (MM), which is characterized by osteolytic lesions. Studies in CIA have suggested that IFN-β is potent and smaller doses may be sufficient for bone resorptive diseases. Preclinical studies in an arthritic model have also shown IFN-β to be effective in reducing bone destruction compared to negative controls [50]. Alternatively, the clinical study reported by van Holten and colleagues evaluating IFN-β therapy in arthritic patients did not show improvement in inflammatory symptoms of the disease at the studied doses (2.2 or 44 μg SC three-times weekly) [49]. However, the study by Weinstock-Guttman and colleagues in patients with MS demonstrated complex changes in markers of bone formation and resorption with IFN-β treatment over a 1-year period [46]. These seemingly contradictory results are likely indicative of the complexities of bone regulation under pharmacological and pathological conditions and the challenge of achieving consistent therapeutic concentrations of IFN-β in bone tissue.

Interestingly, the effects of IFN-β are also subject to negative feedback inhibition. One such mechanism is the induction of suppressors of cytokine signaling 1 (SOCS-1) and SOCS-3 in response to stimulation by RANKL, which has been studied *in vitro* in OC progenitor cells [66]. Pre-treatment of progenitor cells with soluble RANKL induced SOCS-3 to an extent that significantly suppressed IFN-β signaling. Inhibition was shown to result from blockade of STAT-1 phosphorylation, which is responsible for IFN-β signal transduction via the ISGF3 complex. This represents a potential counteracting pathway to IFN-β mediated suppression of osteoclastogenesis and further studies are required to evaluate the significance of this system. Another mechanism for modulating the effect of IFN-β *in vivo* could be the biphasic action of NO in bone. Low concentrations of NO have been shown to induce OC activity [35], whereas high concentrations of NO in pathological conditions have been associated with decreased osteoclastogenesis [67].

The prospect of using IFN-β for treatment of bone disorders is still in its nascent stages of development. In contrast, the anti-resorptive therapeutic effect of bisphosphonates is well accepted for diseases such as osteoporosis. Interestingly, the addition of pamidronate was shown to increase biomarkers of bone formation in MM patients in a plateau phase under IFN-α maintenance therapy [68]. Whereas the role of bisphosphonates in osteolytic diseases is relatively clear, specific IFN intervention for bone restoration might prove useful in treating diseases in which IFN has shown beneficial effect in the control of systemic inflammatory processes that are most likely responsible for the bone loss. A clear understanding of the mechanisms of bone homeostasis coupled with the selection of an appropriate *in vivo* exposure profile of IFN-β delivered directly to the bone microenvironment may be required for effective therapy. Added to this, the counteracting roles of SOCS and bidirectional actions of NO may blunt the potent effects of IFN-β in a clinical setting. Current knowledge about the role of IFN-β in bone might represent a promising therapeutic agent in effectively treating bone diseases. However, due to the complex network of regulatory pathways involved, acceptance of IFN-β as an adjunct to therapeutic regimens for alleviation of excessive bone resorption will pose a significant developmental challenge.

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