

IFN-BETA IN RHEUMATOID ARTHRITIS

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1. ABSTRACT

IFN-beta is a cytokine with pleiotropic effects and is expressed in rheumatoid synovial tissue. Based on *in vitro* work and experiments in animal models of rheumatoid arthritis (RA), the effects are mainly anti-inflammatory. Of special interest is the ability of IFN-beta to reduce the secretion of TNF-alpha, IL-1 beta, and IL-6, which are all key players in the pathogenesis of RA. At the same time IFN-beta could enhance the production of anti-inflammatory mediators like IL-1 receptor antagonist (IL-1Ra) and IL-10. Treatment of mice and monkeys with collagen-induced arthritis with daily IFN-beta injections resulted in clinical improvement, decreased synovial inflammation, and protection against joint destruction. Similar data were obtained after IFN-beta gene therapy. However, treatment of RA patients with IFN-beta has been unsuccessful so far, presumably due to pharmacokinetic issues. Novel approaches leading to constitutive IFN-beta production at the site of inflammation may be required to induce clinical efficacy in patients.

2. INTRODUCTION

Rheumatoid arthritis (RA) is a chronic disease characterized by synovial inflammation, leading to destruction of cartilage and bone (1;2). Macrophages and fibroblasts-like synoviocytes (FLS) are especially involved as key effector cells. Pro-inflammatory cytokines, produced by fibroblast-like synoviocytes and leukocytes infiltrating the rheumatoid synovium, play a major role in the initiation and perpetuation of the chronic inflammatory process in RA. On the other hand anti-inflammatory cytokines with counterbalancing effects are also expressed in the RA synovium.

The interferons (IFN) constitute a family of naturally secreted cytokines with immunomodulatory functions. They enhance the ability of macrophages to destroy tumor cells, viruses, and bacteria. The IFNs are divided into two types. Type 1 IFN comprises IFN-alpha and beta (3), and type 2 IFNs consist of IFN-gamma alone. All type 1 IFNs are likely to have their origin from one

common ancestral gene and are secreted in humans (4). Under normal physiological conditions, type I IFNs are secreted by most human cells at low levels. IFN-beta and IFN-gamma seem to have opposed effects. IFN-gamma promotes inflammatory responses, whereas IFN-beta has mainly anti-inflammatory properties (5).

3. THE EXPRESSION OF IFN-BETA IN RHEUMATOID SYNOVIAL TISSUE

It has been previously suggested that the expression of IFN-beta is increased in synovial tissue of RA patients compared to patients with osteoarthritis (OA) (6). A recent study examined the expression of IFN-beta in the synovium of a larger number of RA patients as compared to synovial tissue from OA, reactive arthritis and psoriatic arthritis patients (7). Staining for IFN-beta was detected in all compartments of the synovium, but especially in the intimal lining layer (Figure 1). In addition, expression was observed in vascular endothelium. Examination of stained sections by digital image analysis demonstrated a statistically significant increase in IFN-beta expression in RA compared with disease controls. The difference between RA and disease controls could not be explained by increased cellularity in RA, since after correction for cell numbers the differences remained significant. The expression of IFN-beta was also confirmed at the mRNA level. Of interest, staining for IFN-beta was particularly found in fibroblast-like synoviocytes, and to a lesser extent in macrophages and dendritic cells. About 76-100% of the FLS, 6-25% of the macrophages, and 26-50% of the dendritic cells were positive for IFN-beta. Thus, IFN-beta is expressed in various forms of arthritis with a specific increase in RA (7).

4. THE ROLE OF IFN-BETA IN SYNOVIAL INFLAMMATION

IFN-beta has pleiotropic effects and, therefore, it is difficult to predict the ultimate effects on synovial inflammation (5). Previous work suggested that IFN-beta

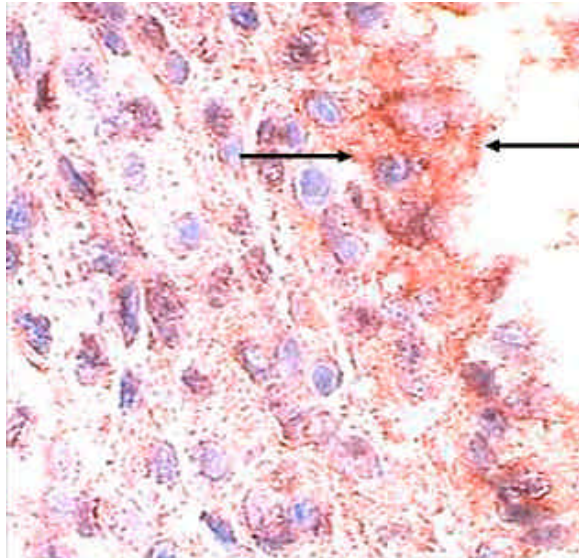


Figure 1. IFN-beta expression in rheumatoid synovial tissue. IFN-beta is represented by red-brown staining (Arrows). Single stain peroxidase technique; Mayer's Hämalaunlösung counterstained.

may inhibit the expression of proinflammatory cytokines like IL-1-beta and TNF-alpha and enhance IL-10 and IL-1 receptor antagonist (IL-1RA) production by monocytes (8-10). Consistent with these studies, IFN-beta was shown to have an inhibitory effect on the production of TNF-alpha by lipopolysaccharide (LPS)-stimulated mouse bone marrow-derived macrophages (11). Hence, these data suggest that IFN-beta could have an anti-inflammatory effect in rheumatoid synovial tissue. It should be noted, however, that the effects of IFN-beta on monocytes depend on the stimulus: IFN-beta inhibits the production of proinflammatory cytokines in human monocytes activated by cell contact with activated T cells, but not in monocytes activated by LPS. Therefore, the ultimate effect may be dependent on the pathogenetic mechanisms involved. In light of the literature showing the importance of T cell contact-activated monocytes/macrophages in RA (12;13), IFN-beta might predominantly act as an inhibitor of TNF-alpha and IL-1 beta production by macrophages in the inflamed synovium. Of note, the macrophages constitute the majority of the inflammatory cells in the synovial sublining in active RA (14). The importance of these cells is supported by clinical observations. There is for instance a strong correlation between scores for local disease activity and the numbers of macrophages and the expression of macrophage-derived cytokines in the synovium (15). Similar data were recently obtained in RA fibroblast-like synoviocytes (16). These cells secrete pro-inflammatory cytokines and matrix metalloproteinases (MMPs), promoting destruction of bone and cartilage and they may maintain macrophage activation. *In vitro* experiments revealed that IL-1beta induced production of IL-6, IL-8, and GM-CSF by these cells could be inhibited by IFN-beta. Preliminary data suggest that the reduced cytokine production may be explained in part by the inhibition of NF-kappaB activity by IFN-beta in RA fibroblast-like synoviocytes (16). Conversely, IFN-beta dose dependently

increased IL1Ra secretion by these cells (17). IFN-beta may also inhibit the production of MMP-1 and MMP-3 as well as prostaglandin E2 by RA fibroblast-like synoviocytes (18). Of interest, these effects were more consistent in RA fibroblast-like synoviocytes than in dermal fibroblasts. Together, these studies suggest an anti-inflammatory effect of IFN-beta on the major effector cell populations in rheumatoid synovial tissue, the macrophages and the fibroblast-like synoviocytes.

Interestingly, a marked proportion of dendritic cells expresses IFN-beta. These cells are derived from circulating immature precursors and may promote synovial inflammation. The observations in RA synovial tissue are in accordance with previous work showing that peripheral blood derived dendritic cell precursors are able to produce IFN-beta (19), which could activate them in an autocrine way (20). Mature dendritic cells may present antigens to T cells and in turn activate T cells leading to activation of fibroblast-like synoviocytes and macrophages. Thus, these observations suggest that IFN-beta could have pro-inflammatory effects more upstream in the disease process.

Other effects of IFN-beta may include enhancement of T cell cytotoxicity, inhibition of T cell proliferation and migration, enhancement of IL-2 production by T helper 1 (Th1) cells, and activation of natural killer cells (Table 1) (5). Recent studies have proposed that endogenous IFN-beta production in RA might enhance inflammation, as IFN-beta can promote T cell survival *in vitro* (21). The anti-apoptotic on IFN-beta effect might be counterbalanced, however, by decreased migration and proliferation of T cells in the synovial compartment. The ultimate effects on lymphocytes are difficult to predict on the basis of the available *in vitro* data. As described below, daily administration of exogenous IFN-beta starting at the onset of disease does not affect T cell numbers in the synovium in the murine collagen-induced arthritis model (16).

In vitro work has suggested that type I IFNs may also inhibit apoptosis in neutrophils in a phosphatidylinositol 3-kinase (PI3K) dependent way (22). Again, it is difficult to appreciate the relevance of this observation in the *in vivo* situation in view of the pleiotropic effects of IFN-beta. Treatment with IFN-beta for instance almost completely prevented neutrophil infiltration and attenuated blood-brain barrier damage in an animal model of brain inflammation (23).

IFN-beta may also have antiangiogenic effects (24-26). The systemic administration of IFN-beta can induce the regression of vascularized tumors through a mechanism associated with endothelial cell damage, leading to necrosis (27;28). Two pathways of angiogenesis have recently been identified, based on their dependence on the related but distinct integrins alphavbeta3 and alphavbeta5. Angiogenesis stimulated by basic fibroblast growth factor (bFGF) is dependent on alphavbeta3, whereas vascular endothelial growth factor (VEGF) enhanced angiogenesis requires alphavbeta5. IFN-beta has been shown to down-regulate bFGF at both the mRNA and

Table 1. Functions of IFN-beta

Stimulation	Inhibition
IL-1Ra	TNF-alpha
IL-10	IL-1 beta
IL-2	IL-6
TGF-beta 1	IL-8
TGF-beta RII	GM-CSF
MHC class I	MHC class II
Soluble adhesion molecules	Adhesion molecules
T cell cytotoxicity	T cell apoptosis
Natural killer cell activation	T cell proliferation
Dendritic cell activation	T cell migration
	Neutrophil migration
	Neutrophil apoptosis
	Prostaglandin E2
	Angiogenesis
	Matrix metalloproteinases
	Osteoclastogenesis

protein level (29). The inhibitory effects of IFN-beta on angiogenesis could also be relevant in RA (30). Recent work has demonstrated that the signal transducer and activator of transcription (STAT)-1 is essential for IFN-mediated inhibition of bFGF production (31). The total levels of STAT1 protein as well as its activated tyrosine and serine phosphorylated forms are increased in RA synovial tissue compared to disease controls (32). Moreover, STAT1 serine and tyrosine phosphorylation is rapidly induced in fibroblast-like synoviocytes upon stimulation with IFN-beta. In light of the abundant expression of IFN-beta and the activation of the STAT1 pathway, it is tempting to speculate that IFN-beta is a negative regulator of angiogenesis in RA synovium and, hence, synovial proliferation. IFN-beta is not only able to inhibit angiogenesis, but could also deactivate the endothelium resulting in decreased cell trafficking (33;34). However, whether this mechanism is operative in synovial inflammation remains to be shown.

5. EFFECTS OF IFN-BETA ON BONE

Recent work has shown that IFN-beta has inhibitory effects on osteoclastogenesis. The osteoclast, which is present in RA synovial tissue at the invasive front, plays a key role in the development of bone erosions (35). Under normal circumstances bone resorbing osteoclasts and bone forming osteoblasts maintain a balance between bone resorption and bone formation. When this balance is disrupted in favor of the osteoclasts, bone destruction as observed in RA may follow. The importance of these cells is illustrated by experiments in transgenic mice that express human TNF. When the mice were crossed with osteopetrotic, c-fos-deficient mice completely lacking osteoclasts, TNF-mediated arthritis was altered from destructive disease to a nondestructive arthritis (36). Osteoclast numbers and activity are dependent on the balance between the osteoclast promoting receptor activator of NF-kappaB ligand (RANKL) and osteoclast inhibiting osteoprotegerin (OPG).

IFN-beta plays an important inhibitory role in osteoclast function by inhibiting c-Fos induction required for osteoclastogenesis (37;38). RANKL stimulation of osteoclast precursor cells, for instance by RANKL positive T cells and fibroblast-like synoviocytes in RA synovium (39;40), results in their differentiation into mature bone resorbing osteoclasts. RANKL stimulation at the same time induces c-Fos-dependent IFN-beta expression by osteoclasts. Subsequent IFN-beta signaling inhibits osteoclastogenesis, in part through negative feedback signaling to c-Fos. Consistent with these studies treatment with daily IFN-beta injections inhibits the development of bone erosions in the collagen-induced arthritis model of RA (see below) (16).

6. IFN-BETA THERAPY IN ANIMAL MODELS OF RHEUMATOID ARTHRITIS

IFN-beta treatment might be an interesting therapeutic strategy based on the inhibitory effects of IFN-beta on the production of pro-inflammatory cytokines like TNF-alpha, IL-1, and IL-6 combined with the stimulatory effects on IL-10 and IL-1Ra secretion (Table 1). Blockade of the effects of TNF-alpha (41), IL-1 (42), and IL-6 (43) is effective in RA. IL-10 could be a potent anti-inflammatory cytokine in combination with other anti-inflammatory cytokines (44). Moreover, IFN-beta might have a protective effect on bone by inhibiting c-Fos induction required for osteoclastogenesis (38).

Therefore, we examined the effects of IFN-beta therapy in the murine collagen-induced arthritis model (16). Mice were given daily intra-peritoneal injections of IFN-beta or saline for 7 consecutive days, starting on the day that clinical arthritis was first detected. Mice treated with IFN-beta showed a 50% decrease in arthritis score and an approximately 70% decrease in paw swelling, confirming the therapeutic potential. Histologic analysis of synovial tissue revealed a marked reduction in the number of inflammatory cells after IFN-beta treatment. There was an approximately 50% reduction in the number of macrophages, but the total numbers of infiltrating T and B lymphocytes were unaltered. In addition, a marked decrease in the number of granulocytes was observed after high-dose IFN-beta treatment. Although this study did not specifically address the effects of exogenous IFN-beta on survival of T cells and granulocytes in the joint, we did not observe an increase in these cells, and any potential anti-apoptotic effect did not prevent a beneficial therapeutic effect of IFN-beta. In addition, the expression of IL-6 as well as TNF-alpha in the inflamed synovium was decreased after IFN-beta treatment (16). IL-18 and IL-1beta expression also tended to be lower in IFN-beta treated animals. As expected, IL-10 expression was increased after IFN-beta treatment. Thus, these data support the view that the *in vivo* effects of treatment with the pleiotropic cytokine IFN-beta are mainly anti-inflammatory.

Of importance, there was also a protective effect on both cartilage and bone. In mice treated with the highest dose of IFN-beta scores for proteoglycan depletion in cartilage were significantly decreased. Consistent with the

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presumed mechanism of action, the mean scores for both c-Fos expression and histologic bone erosions were markedly lower in mice treated with IFN-beta than in control mice. However, this effect was not observed when low dosages of IFN-beta were given (16).

IFN-beta therapy has also been evaluated in collagen-induced arthritis in rhesus monkeys (45). The monkeys were susceptible to collagen type II induced arthritis because of the lack of the MHC class I allele A-26. Three out of four monkeys had active polyarthritis at the time of treatment initiation. The fourth monkey was in a preclinical phase of the disease, as shown by elevation of serum acute phase reactants and synovial inflammation by arthroscopy. The monkeys were treated with a high dosage of recombinant IFN- β 1a subcutaneously daily for one week. Two monkeys with established arthritis exhibited clear clinical improvement after treatment and the monkey with preclinical synovitis never developed signs of arthritis. During IFN-beta treatment all animals had a marked decrease in serum CRP levels.

Hence, these data confirm the work in the mouse model showing the therapeutic potential of daily IFN-beta injections for the treatment of arthritis.

The effects of IFN-beta were also investigated by IFN-beta gene therapy in collagen-induced arthritis in mice (11). Fibroblasts from DBA/1 mice were infected with a retrovirus expressing murine IFN-beta, and injected intraperitoneally into mice with collagen-induced arthritis. This approach results in a constant level of IFN-beta production by syngeneic fibroblasts. A significant decrease in inflammation was observed after IFN-beta gene therapy when treatment was initiated before as well as after the onset of disease. Histologic analysis revealed a significant reduction in joint destruction, consistent with findings after exogenous IFN-beta treatment (16). In addition, a modest decrease in total anticollagen IgG levels was observed. The IgG1:IgG2a ratio was increased in IFN-beta treated mice, suggesting a beneficial effect on the Th1/Th2 balance (11). Taken together, the different animal models consistently suggest an anti-inflammatory effect of daily injections with IFN-beta protein or IFN-beta gene therapy, resulting in constitutive IFN-beta expression. The obvious question is whether these effects can also be reached in RA patients if IFN-beta is administered three times weekly only, according to the regular treatment regimen in patients with multiple sclerosis; more frequent injections would be less tolerable to the patients (46).

7. IFN-BETA THERAPY IN RA PATIENTS

Several case reports of patients who were treated with IFN-beta for an indication other than arthritis have been published with ambiguous results. One RA patient, who was treated for chronic hepatitis C virus infection, showed no change in arthritis activity during IFN-beta therapy (47). Another RA patient with multiple sclerosis experienced relief of symptoms of RA during IFN-beta treatment (48). In contrast, patients have been described

who developed arthritis during IFN-beta treatment for multiple sclerosis (49;50).

An open phase I study was performed in 12 patients with active RA who were treated with purified natural fibroblast IFN-beta for 12 weeks (45). They were treated with IFN-beta subcutaneously 3 times a week, which is the schedule used in multiple sclerosis patients, but which differs from the treatment schedule used in the collagen-induced arthritis models. Treatment was generally well tolerated and there was a trend toward clinical improvement. It should be noted, however, that this was an open study and the effects might be explained by placebo effects, regression to the mean, or expectation bias.

Recently, a double-blind, placebo-controlled trial that evaluated the efficacy of IFN-beta-1a was completed in RA patients (51). Two hundred nine patients with active RA, who were receiving methotrexate for at least 6 months prior to inclusion, were randomized in a double-blind fashion to receive 2.2 μ g or 44 μ g of IFN-beta, or placebo, administered in subcutaneous injections three times weekly for the duration of 24 weeks. Analysis of changes in radiological scores and clinical parameters revealed no changes in the groups that received IFN-beta or the placebo group, and no difference between groups. Microscopic analysis of serial synovial tissue samples showed no significant change in the scores for infiltration by inflammatory cells after IFN-beta treatment. In addition, urinary levels of collagen crosslinks were unchanged between the treatment groups.

Conceivably, the discrepancy between the clinical study in RA patients and previous animal work might be explained by the mode of administration and the difference in IFN-beta-1a dosages used. In the phase II study IFN-beta was administered three times weekly in a dose up to 44 μ g per injection/patient (51). The preclinical studies were performed with either gene therapy, resulting in constitutive IFN-beta release, or daily IFN-beta injections at a dosage of IFN-beta 2.5 μ g/mouse/day. The difference in treatment regimen may be relevant in light of the known short half-life of the compound. Previous work has shown that frequent dosing may be required to sustain the activity of intracellular molecular signaling pathways responsible for regulating IFN-beta-induced gene expression (46). It is possible that more frequent injections, higher dosages or the use of compounds with a longer half-life are required to induce clinically meaningful effects in patients with RA. However, more frequent injections may not be tolerable to the patients due to injection site reactions and flu-like symptoms.

8. CONCLUSION

Based on preclinical studies IFN-beta appears to have a beneficial effect of synovial inflammation and the integrity of the joint. So far it has not been possible to translate this into effective treatment for RA, presumably due to pharmacokinetic issues. Novel approaches allowing the constant delivery of IFN-beta at the site of

inflammation are required to answer the question whether IFN-beta has potential for the treatment of RA patients.

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