Therapeutic Implications for Interferon-α in Arthritis: A Pilot Study

THOMAS WONG, BEATA MAJCHRZAK, EARL BOGOCH, EDWARD C. KEYSTONE, and ELEANOR N. FISH

ABSTRACT. ***Objective.*** To evaluate the therapeutic potential of interferon–α (IFN–α) in osteoarthritis (OA) and rheumatoid arthritis (RA) by examining regulation of cytokine antagonist expression.

Methods. Expression of interleukin 1 receptor antagonist (IL-1Ra) and soluble tumor necrosis factor receptor (sTNFR) was examined by ELISA in cells from freshly isolated synovial fluids (SF) and synovial tissues (ST) from patients with OA or RA, either left untreated or treated with IFN–α. Single (7) and paired (5) SF and ST cells from OA and RA patients were examined. As well, the ability of IFN–α to regulate gene expression levels for osteoprotegerin (OPG) and osteoprotegerin ligand (OPGL) was examined in freshly isolated SF cells from patients with RA, by reverse transcriptase polymerase chain reaction.

Results. IL-1Ra and sTNFR were found to be constitutively expressed in OA and RA SF and ST cells. IFN–α treatment resulted in an increase in both IL 1Ra and sTNFR production. Freshly isolated RA SF cells exhibited constitutive OPGL gene expression in both the non-T and T cell fractions of the SF. In contrast, OPG gene expression levels were undetectable or low. IFN–α treatment of RA SF cells resulted in upregulation of OPG gene expression in the T cell fraction of the RA SF cells, whereas OPGL gene expression remained unaffected.

Conclusion. These in vitro data suggest a therapeutic role for IFN–α in the treatment of arthritis through upregulation of critical cytokine antagonists. (J Rheumatol 2003;30:934–40)

Key Indexing Terms:
OSTEOARTHRITIS RHEUMATOID ARTHRITIS INTERFERON-α
SOLUBLE TUMOR NECROSIS FACTOR RECEPTOR OSTEOPROTEGERIN
OSTEOPROTEGERIN LIGAND INTERLEUKIN 1 RECEPTOR ANTAGONIST

Rheumatoid arthritis (RA), an autoimmune disease affecting 1% of the population, is characterized by recruitment of leukocytes, primarily CD4+ T cells and monocytes, from the vasculature into inflamed synovial tissue (ST) and synovial fluid (SF). Antigen activated CD4+ T cells stimulate macrophages and fibroblasts to secrete factors that mediate synovial cell proliferation, pannus formation, and bone and cartilage erosion, resulting in a chronic inflammatory and destructive joint disease. Numerous reports have implicated various distinct mediators in the pathogenesis of RA that include autoantibodies against IgG (rheumatoid factor) and cartilage collagen, immune complexes, complement activation products, oxygen radicals, matrix metalloproteinases, and cytokines. Indeed, cytokines play a pivotal role in this process, orchestrating the trafficking of leukocytes to sites of inflammation and mediating the pathophysiologic events in the affected joint. The pathophysiology of RA is primarily driven by proinflammatory cytokines, dominant among which are tumor necrosis factor–α (TNF–α), interleukin 1 (IL 1), and IL-6[1]. TNF–α and IL-1 are potent activators of synovial fibroblasts, osteoclasts, and chondrocytes, inducing other proinflammatory cytokines such as IL-6 and IL-8/CXCL8, and invoking the release of tissue destroying matrix metalloproteinases. Activated CD4+ T cells express osteoprotegerin ligand (OPGL), a critical factor that mediates osteoclastogenesis and joint damage, independently of IL-1 and TNF–α[2].

Given the fundamental roles of proinflammatory cytokines in the progression of joint damage in RA, considerable activity has focused on cytokine based intervention strategies. Etanercept, comprising 2 soluble TNF receptors (sTNFR) fused with the Fc portion of human IgG1[3], and antibodies against TNF–α — infliximab[4], adalimumab[5], and CDP870[6] — specifically inhibit the activity of TNF–α in RA. Recombinant IL-1 receptor antagonist (IL-1Ra) is effective in reducing both inflammation and joint destruction[7]. Despite clinically significant improvements effected by these cytokine inhibitor modalities, disease progression continues, albeit at a slower rate. The implications are that

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Supported by a grant from The Arthritis Society to Dr. E. Fish.

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Submitted April 29, 2002; revision accepted October 21, 2002.

multiple cytokine targets must be simultaneously inhibited for a sustained and complete cessation of disease progression. Accordingly, we explored the therapeutic potential of a counter-regulatory cytokine, interferon–α (IFN–α) — accumulating data suggest it has the potential to oppose the activities of proinflammatory cytokines. Paradoxical data that IFN–α may induce autoimmune disease[8] have been considered. The frequency of autoimmune disorders in patients undergoing IFN–α therapy ranges from 0.15% to 13%. Incidence of autoimmune disorders varies with the target population (hepatitis C virus, chronic myelogenous leukemia, carcinoid tumors), the treatment regimen, and duration. There is contradictory and accumulating evidence that interferons α and ß interfere with the synthesis or actions of the proinflammatory cytokines TNF-ß, IL-1, and IL-8[9-11] and that IFN-ß has immunomodulating effects on rheumatoid synovium, affecting the cytokine profile and expression of matrix metalloproteinases[12]. To determine whether IFN–α therapy might have beneficial effects in arthritis, we examined the effects of IFN–α treatment on cytokine antagonist expression in osteoarthritis (OA) and RA tissues. Our data provide evidence for IFN-dependent upregulation of sTNFR, IL-1Ra, and OPG.

MATERIALS AND METHODS

Patients and cell fractionation. SF and ST samples were collected from 11 adult RA and 6 adult OA patients seen at Mount Sinai and Wellesley Central Hospitals in Toronto who presented with seropositive RA or OA according to the American College of Rheumatology criteria. Patients with RA were receiving a nonsteroidal antiinflammatory drug (NSAID) and a remittive agent, while patients with OA were receiving analgesics or NSAID. ST obtained at the time of surgical synovectomy was treated for 1.5 h with DNAse and collagenase. Isolation of mononuclear cells from SF and ST was carried out by density gradient centrifugation on a Ficoll-Hypaque Plus gradient (2200 rpm for 35 min at room temperature). Mononuclear cells were separated into T cell and non-T cell populations (10^6 cells/ml) using a standard rosetting protocol[13]. Experimental procedures were approved by the Ethics Committee.

Determination of sTNFR, TNF–α, IL-1Ra, IL-1ß, OPG, and OPGL concentrations. sTNFR (p55), TNF–α, and IL-1Ra protein concentrations were measured using ELISA according to the manufacturers' instructions (Boehringer Mannheim/Roche Molecular Biochemicals, Laval, Quebec, and R&D Systems, Minneapolis, MN, USA). Briefly, mononuclear cells (10^6/ml), unfractionated or fractionated into T cell and non-T cell populations, were either maintained in culture medium (modified Eagle's medium) for 14 h, or treated with 10^4 U/ml IFN for varying times. Cell suspensions were then centrifuged at 13,000 rpm for 5 min, culture supernatants were collected and aliquoted, and either assayed immediately or stored frozen at –70°C. Each sample was assayed using ELISA in triplicate. Reproducibility of determinations was confirmed by replicate assays of randomly selected samples that were included in ELISA estimations for each experiment (data not shown).

IL-1ß, TNF–α, OPG, and OPGL gene expression were determined by reverse transcriptase-polymerase chain reaction (RT-PCR) (2) using 100 ng total RNA (RNeasy mini kit; Qiagen, Valencia, CA, USA). PCR primers were: ß actin 5': GGG ACC TGA CTG ACT ACC T; ß–actin 3': CTA GAA GCA TTT GCG GTG GA; IL-1ß 5': TTG AAG CTG ATG GCC CTA; IL-1ß 3': TGC TCA GGT CAT TCT CCT; TNF–α 5': AGG CGG TGC TTG TTC CTC A; TNF–α 3': GTT CGA GAA GAT GAT CTG ACT GCC; OPG 5': AAG GAG CTG CAG TAC GTC; OPG 3': ACC AAG ACA CTA AGC CAG T; OPGL 5': AAA TCC CAA GTT CTC ATA CCC; OPGL 3': TCT CAT AAG GTC AAC CCG TAA. IL-1ß, TNF–α, OPG, and OPGL cDNA were used as positive controls.

RESULTS

On receipt, OA or RA SF and ST cell suspensions were immediately processed to collect mononuclear cells. Patient samples were then divided into 2 equal aliquots, to be assessed for basal and IFN-inducible expression of sTNFR and IL-1Ra, respectively. The available sample sizes for ST samples precluded fractionation into T and non-T cell populations. Whenever possible, SF samples were fractionated, and where yields permitted, both T and non-T cell populations were examined. In several instances, the non-T cell numbers were < 10^6/ml, so ELISA determinations were not performed. For all patient samples, duplicate mononuclear cell aliquots, either unfractionated or fractionated, at 10^6 cells/ml were either incubated in culture medium for 14 h or treated with 10^4 U/ml IFN-Con (Infergen, a human recombinant consensus IFN–α; provided by Amgen Inc., Thousand Oaks, CA, USA) for varying times. sTNFR and IL-1Ra levels present in the culture supernatants were then measured by ELISA. Figure 1 shows results for sTNFR expression. For OA patients numbered 1 and 2, and RA patients numbered 7, 8, and 9, where paired SF and ST samples were examined, constitutive/basal levels of sTNFR were higher in the ST samples than in the SF T cell populations. We observed an increase in sTNFR expression in only 2 of the 8 SF samples treated with IFN–α, compared with 7 of the 8 ST samples. There is no obvious distinction between OA and RA samples in the context of either constitutive expression levels or IFN-inducible levels of sTNFR.

As for sTNFR expression, we observed that constitutive/basal expression of IL-1Ra was higher in ST compared with SF, for the paired SF and ST samples from patients numbered 1 and 2 (OA) and 7, 8, and 9 (RA) (Figure 2). Treatment with IFN for 14 h resulted in induction of IL-1Ra in all 8 of the ST samples, and in 7 of the 9 SF T cell samples, but none of the 4 SF non-T cell samples examined. Our data do not suggest a difference in constitutive/basal expression or IFN-inducible expression of IL-1Ra between OA and RA patient samples.

Although both basal and IFN-inducible levels for sTNFR and IL-1Ra appear higher in the ST compared with the SF patient samples, ST sample measurements were performed on culture supernatants from unfractionated mononuclear cells, i.e., mixed T and non-T cell populations, compared with the fractionated SF sample measurements. Certainly, inherent T/non-T cell interactions in unfractionated mononuclear cell cultures might effectively enhance the activation state of the cells with regard to at least IL-1Ra and sTNFR protein production. However, the phenotype of the T and non-T cell subsets in the SF and ST are likely quite distinct, e.g., in regard to CD4, CD8, CD28, CD134, and

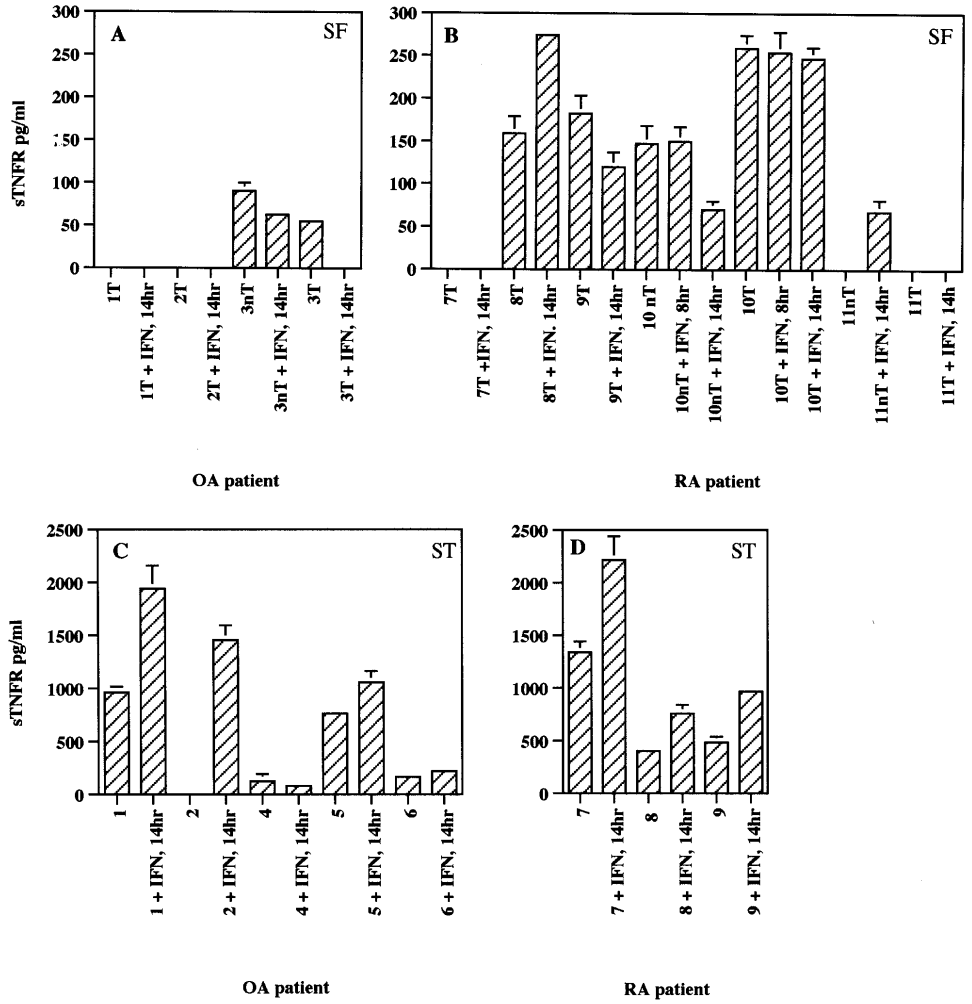

Figure 1. IFN regulation of sTNFR expression in OA and RA tissues. Mononuclear cells were isolated from patient (numbered 1–11) SF (A, B) and ST (C, D) samples and either left unfractionated, or were fractionated into T (T) and non-T (nT) cell populations; 10^6 mononuclear cells/ml were either left untreated for 14 h, or were treated with 10^4 U/ml IFN–α, for the times indicated. Culture supernatants were assayed by ELISA for sTNFR. Samples numbered 1 through 6 were derived from OA patients, samples 7 through 11 from RA patients. Values represent the mean and SD of triplicate assays for each sample.

CD45 expression in the T cells. Indeed, molecular fingerprinting has revealed heterogeneity of T cell populations in the peripheral blood, SF, and synovium compartments in patients with RA[14-16]. This phenotypic heterogeneity determines the activation state and consequently influences responsiveness and protein expression profiles[17]. Thus, in the absence of phenotypic characterization of the different mononuclear cell subsets derived from SF and ST, no assumption of identity or proportional similarities can be made.

In earlier gene expression studies, we found that T cells isolated from joints from all RA (n = 20) and all OA (n = 10) patients expressed OPGL[2]. We have accumulated additional RA patient samples and consistently find OPGL expression in the absence or paucity of OPG (data not shown). Accordingly, we examined the effect of IFN–α treatment on regulation of OPG and OPGL gene expression in RA SF cells, in time course studies. The results are shown in Figure 3. IFN–α treatment did not affect OPGL gene expression levels in all patient samples that we examined. By contrast, IFN treatment induced OPG gene expression in all RA patient SF samples that we examined, specifically in the T cell population. The data in Figure 3 show results from 3 of the RA patient SF samples analyzed. Our data would suggest that IFN regulation of OPG gene expression is rapid and was sustained for at least 14 h and diminished by 24 h.

As indicated above, quantitation of cytosolic protein concentrations for IL-1Ra and sTNFR by ELISA involved triplicate assays for each patient sample, fractionation of mononuclear cells into T and non-T populations whenever sample size permitted, and replicate assays for selected samples, again when sample size was sufficient. Because of

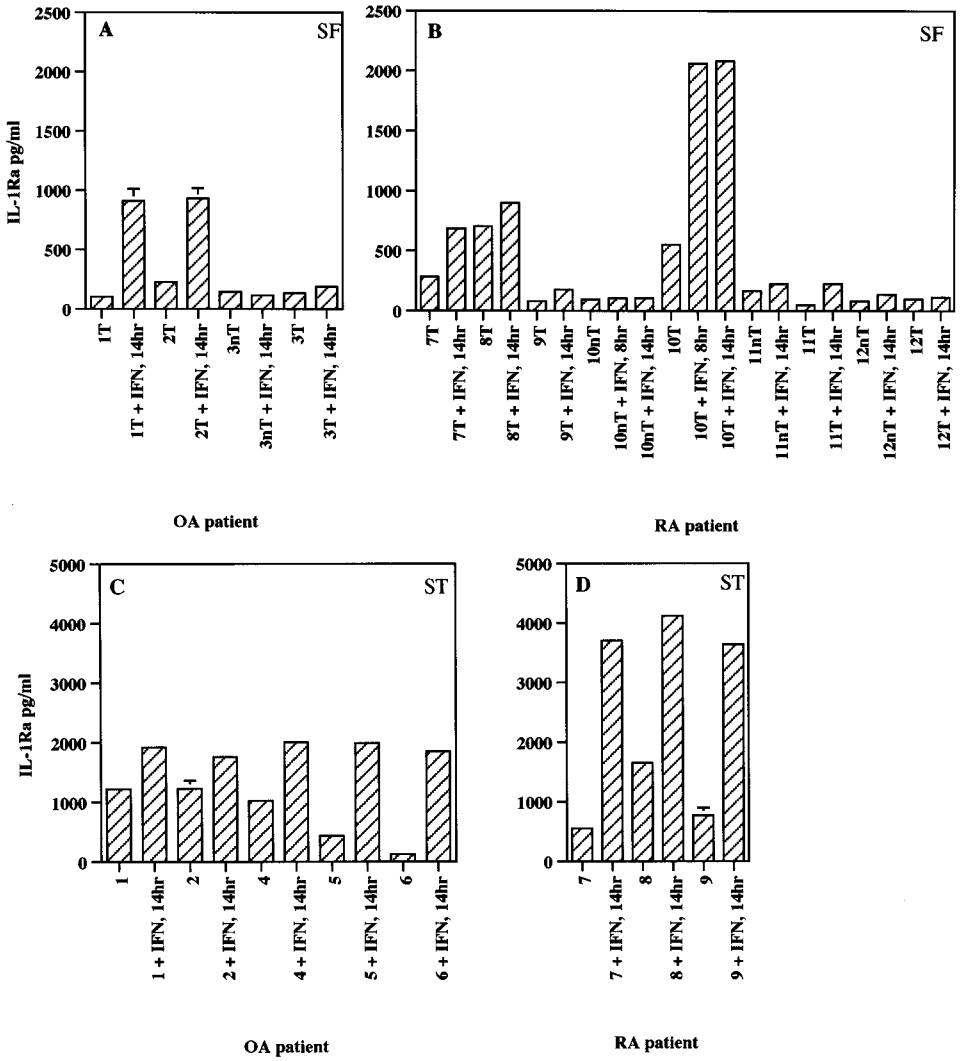

Figure 2. IFN regulation of IL-1Ra expression in OA and RA tissues. Mononuclear cells were isolated from patient (1–12) SF (A, B) and ST (C, D) samples and either left unfractionated, or were fractionated into T (T) and non-T (nT) cell populations; 10^6 mononuclear cells/ml were either left untreated for 14 h, or were treated with 104 U/ml IFN–α, for the times indicated. Culture supernatants were assayed by ELISA for IL-1Ra. Samples numbered 1 through 6 were derived from OA patients, samples 7 through 12 from RA patients. Values represent the mean and SD of triplicate assays for each sample.

limitations of the available amount of patient sample for protein estimation by ELISA, only a selected subset of RA SF samples were available for ELISA for TNF–α protein levels from untreated and IFN treated samples. The data reveal that IFN–α treatment of the different RA SF cells had a negligible effect on TNF–α protein levels (Figure 4A). Similar results were obtained when we examined the effect of IFN treatment on IL-1ß and TNF–α gene expression, using RT PCR (Figure 4B). Specifically, whether RA SF mononuclear cells were left untreated or treated with IFN–α for 15 min, 2 h, 8 h, or 14 h, gene expression levels for IL-1ß and TNF–α were indistinguishable. These results are consistent with our findings for OPGL, namely that IFN–α treatment of RA patient samples had no effect on the expression levels for the cytokine ligand, OPGL.

DISCUSSION

To control synovial inflammation and bone and cartilage destruction in OA and RA the activities of distinct cytokines must be blocked. Dominant among the proinflammatory cytokines are IL-1 and TNF–α. We had found that inhibition of OPGL through OPG can completely prevent bone and cartilage loss in a T cell-dependent rat arthritis model, even in the presence of severe inflammation[2]. In the present study we tested the hypothesis that a single counter-regulatory cytokine that exhibits pleiotropic effects, IFN–α, might

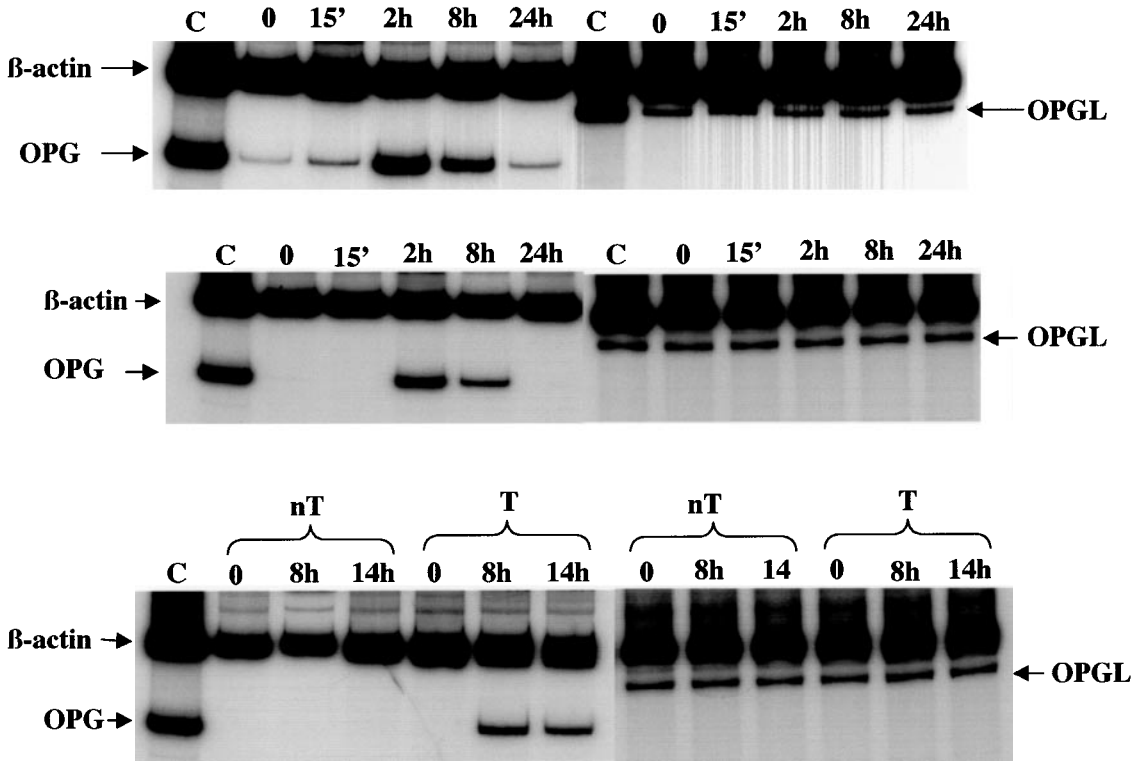

Figure 3. IFN–α induces OPG in RA SF. Mononuclear cells from RA SF samples were either left untreated, or were treated with 10^4 U/ml IFN–α for the times indicated. Cultures were either processed as unfractionated, or were fractionated into non-T (nT) and T cell populations, as shown. OPG and OPGL gene expression was quantitated by PCR amplification of cDNA derived from 100 ng of total RNA. cDNA for OPG and OPGL were used as positive controls (C) and ß-actin primers were included in all PCR to control for loading.

block the activities of several distinct cytokines through upregulation of antagonists. The Type 1 interferons, IFN–α/ß, are distinct from IFN-γ, both in terms of their immunoregulatory activities and the cognate receptors that they activate[18,19]. Although studies have advocated a therapeutic role for IFN-γ in RA, an increasing body of evidence suggests no longterm beneficial effects[20-25]. Indeed, different studies indicate that IFN-γ treatment may lead to exacerbated autoimmune disease[26,27], and that treatment with antibodies to IFN-γ may be a more appropriate therapeutic strategy in RA[28]. Our results indicate that IFN–α treatment of freshly harvested ex vivo SF or ST cells induces the expression of functional antagonists for IL-1, TNF–α, and OPGL, specifically IL-1Ra, sTNFR, and OPG. Moreover, we provide evidence that IFN–α treatment of the same patient samples has little or no effect on the expression levels of the corresponding cytokine ligands IL-1ß, TNF–α, and OPGL. Notably, constitutive levels of sTNFR and IL-1Ra are detectable in OA and RA synovial cells, but presumably insufficient to prevent the persistent inflammation in affected joints. These data are consistent with reports of an unbalanced IL-1Ra production relative to IL-1[29], and sTNFR relative to TNF–α[30]. It is intriguing to speculate that IFN–α treatment may raise the levels of IL-1Ra and sTNFR expression to a threshold such that effective antagonism of IL-1 and TNF–α occurs. With few exceptions, for both sTNFR and IL-1Ra, the synovial ST cells were more sensitive to the effects of IFN than the SF cells, in terms of pg levels induced. As discussed earlier, distinct clonally diverse mononuclear cells reside in the different tissue compartments in affected joints that are likely variably responsive to IFN–α-inducible IL-1Ra and sTNFR production.

Our data do not allow us to discern which mononuclear cell types in ST are responsible for the IFN-dependent production of sTNFR or IL-1Ra but it is generally accepted that activated T cells will produce sTNFR and activated macrophages will secrete IL-1Ra. We infer from our findings that IFN–α treatment likely regulates gene expression in different synovial cell populations, critical targets being the T and macrophage cell populations. Notably, our data do not suggest that OA and RA synovial cells are differentially sensitive to the effects of IFN–α. Our most provocative results surround the OPG and OPGL data. Accumulating evidence indicates that activated T cells are responsible for the bone and cartilage destruction in arthritis, chiefly as a consequence of OPGL activity. Clinical data confirm that

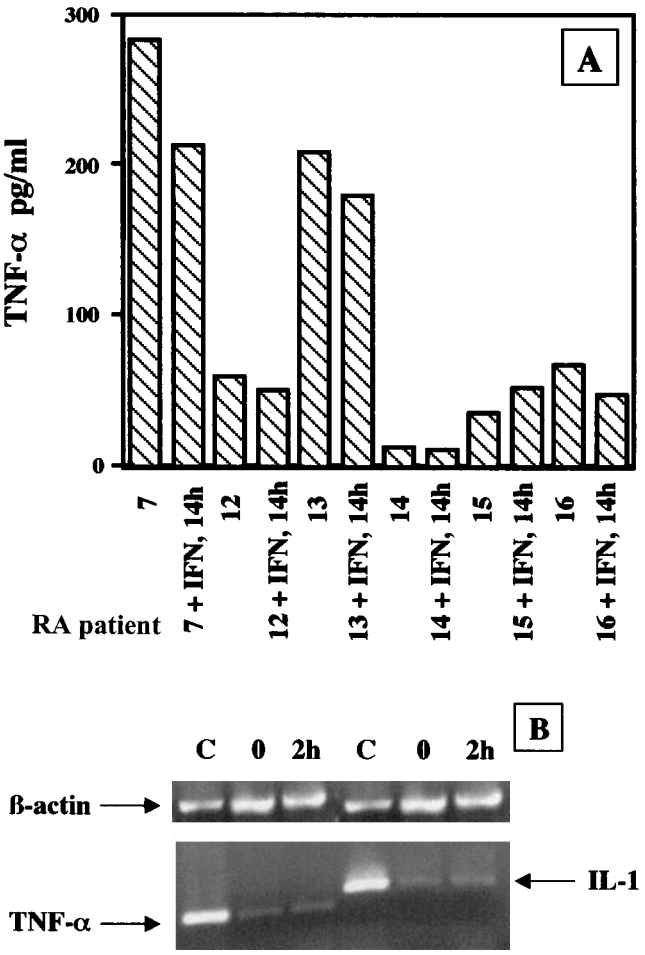

Figure 4. IFN treatment does not affect IL-1ß and TNF–α expression in RA SF. Mononuclear cells were isolated from SF from RA patients 7 and 12–16; 10^6 mononuclear cells/ml were either left untreated for 14 h, or were treated with 10^4 U/ml IFN–α for varying times up to 14 h. Culture supernatants were assayed by ELISA for TNF–α (A). IL-1ß and TNF–α gene expression was quantitated by PCR amplification of cDNA derived from 100 ng of total RNA. cDNA for IL-1ß and TNF–α were used as positive controls (C) and ß-actin primers were included in all PCR to control for loading. A single representative of multiple experiments is shown (B). IL-1ß and TNF–α gene expression levels were indistinguishable whether SF cells were left untreated or were treated with IFN for 15 min, 2 h, 8 h, or 14 h (data not shown).

inhibition of IL-1 and TNF–α activities does not shut down joint destruction. IFN targeting of OPGL activity through induction of the decoy receptor OPG has the potential to limit bone and cartilage damage. Together, our results suggest that a single biological agent, IFN–α, may effectively block both the inflammation and joint destruction in arthritic joints. We are exploring the therapeutic potential of IFN–α in RA in preclinical studies.

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