Osteoarthritis and Cartilage (2000) **8,** 474–482

doi:10.1053/joca.2000.0323, available online at http://www.idealibrary.com on IDEAL®

1063–4584/00/060474+09 $35.00/0

Osteoarthritis and Cartilage

Journal of the OsteoArthritis Research Society International

Modulation of human chondrocyte metabolism by recombinant human interferon

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Summary

Objectives: Interferon gamma (IFNγ) is found to be elevated in the synovial fluid of patients with rheumatoid arthritis and osteoarthritis, suggesting its implication in joint disease pathogenesis. In this study, we investigated the effects of IFNγ on the production of cytokines (IL-6, IL-8, IL-10), prostaglandin E_2 (PGE_2), proteoglycans (PG), nitric oxide (NO), interleukin-1 receptor antagonist (IL-1ra) and stromelysin by non-stimulated and IL-1β-treated human chondrocytes. The role played by NO in the responses of chondrocytes to IFNγ was also examined by incubation of chondrocytes with N^G-monomethyl-L-arginine (L-NMMA), a competitive inhibitor of NO synthase.

Methods: Enzymatically isolated human chondrocytes were cultured for 48 h in the absence or presence of IL-1β, IFNγ or N^G-monomethyl-L-arginine (L-NMMA) added solely or in combination. The productions of IL-6, IL-8, IL-10, IL-1ra and stromelysin were measured by enzyme amplified sensitivity immunoassays (EASIA). PG and PGE_2 were quantified by specific radioimmunoassays (RIA). Nitrite concentrations in the culture supernatants were determined by a spectrophotometric method based upon the Griess reaction.

Results: As expected, IL-1β highly stimulated NO, IL-6, IL-8, IL-10, IL-1ra, PGE_2 and stromelysin synthesis, but dramatically decreased PG production. NO, IL-6, IL-1ra and PGE_2 production by non-stimulated chondrocytes was dose-dependently increased by IFNγ while PG production was inhibited. In the absence of IL-1β, IL-10 was undetectable in the culture supernatants. At the doses of 10 and 100 U/ml, IFNγ markedly inhibited the constitutive and IL-1β-stimulated IL-8, IL-10 and stromelysin productions. Interestingly, IFNγ synergized with IL-1β to increase NO, IL-6, IL-1ra and to depress PG production. As previously reported, the inhibition of NO synthesis by the competitive inhibitor L-NMMA led to enhancement of IL-6, IL-8 and PGE_2 production by IL-1β treated chondrocytes, but did not significantly modify IL-10, PG and MMP-3 productions. Inhibition of NO synthase significantly inhibited the stimulating effect of IFNγ on IL-6 and IL-1ra but did not affect the inhibitory effect of IFNγ on IL-8, PG or stromelysin production.

Conclusions: These findings suggest that IFNγ and IL-1 synergistically stimulate the production of IL-6, IL-1ra, NO and PGE_2 and inhibit PG synthesis. By contrast, IL-1β and IFNγ have opposite effects on IL-8, IL-10 and stromelysin productions. These effects are not reversed by L-NMMA, suggesting that NO is not the principal mediator involved in responses of chondrocytes to IFNγ. © 2000 OsteoArthritis Research Society International

Key words: Interferon, Cytokines, Chondrocytes, Osteoarthritis.

Introduction

The role of interferon gamma (IFNγ) in the pathogenesis of inflammatory joint diseases and the accompanying destruction of cartilage and other joint tissues remains poorly defined. IFNγ was found to be elevated in synovial fluid and sera of patients with rheumatoid arthritis and osteoarthritis (OA), suggesting a direct implication of this cytokine in joint disease pathophysiology.[1,2] Moreover, the presence of activated T-cells and Th1 cytokine transcripts in the synovial membrane of patients with OA suggests that T-cells and, thus, IFNγ, contribute to chronic inflammation in a large proportion of these patients.[3]

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This work was supported by the Léon Frédéricq Foundation and by grant 3.4552.92 from the Funds for Medical Scientific Research, Belgium.

At this time, divergent *in vitro* and *in vivo* results have been reported in the literature. When administered by systemic injection in a polyarthritis model induced by injection of peptidoglycan-polysaccharide (PG-APS), IFNγ suppressed the severity of acute and chronic erosive polyarthritis.[4,5] This effect was associated with a marked reduction in the number of neutrophils and monocytes infiltrating the tissue, and correlated with a decrease in C5a receptors in the inflammatory cells. Conversely, intra-articular injection of IFNγ exacerbated arthritis in the joint.[5] This enhancement of arthritis could reflect the capacity of IFNγ to activate the production of proinflammatory cytokines such as IL-1 and TNFα by monocytes already recruited into the synovial tissue. Furthermore, the effects of IFNγ in the treatment of rheumatic disease are considered ambiguous.[6–8] In one double-blind, controlled study of 91 patients with rheumatoid arthritis,[6] a statistically significant improvement in clinical disease among the recombinant IFNγ-treated patients was reported. In two other studies,[7,9] which included 26 and 105 patients respectively, the IFNγ treated group showed some

clinical improvement, but this was not statistically significant when compared with the placebo group.

Although IFNγ has primarily immunomodulatory as well as antiviral and antiproliferative properties, a number of recent studies has shown that IFNγ is also able to modulate the metabolism of connective tissue cells. IFNγ induces the production of reactive oxygen species and PGE_2[10–12] and the expression of ICAM-1 and major histocompatibility complex class II antigens by human isolated chondrocytes and synovial fibroblasts.[13–16] IFNγ also inhibits the synthesis of cartilage-specific collagens, fibronectin and proteoglycans by chondrocytes.[10,11,17,18] On the other hand, IFNγ inhibits TNFα and IL-1β-induced collagenase and stromelysin by chondrocytes, as well as IL-1 or TNF-stimulated proteoglycan degradation *in vitro*.[11,19] IFNγ has both proliferative and antiproliferative effects on synovial fibroblasts and stimulates chondrocyte proliferation. In addition, IFNγ is a potent inhibitor of bone resorption. In bone explants, it inhibits osteoclast differentiation,[20] and reduces the activity of existing cells in a manner similar to calcitonin.[21]

These observations suggest that IFNγ could be involved in cartilage degradation occurring in joint diseases, not only by acting on cartilage itself but also on neighboring tissues. Moreover, potent synergism and antagonism between IL-1β and IFNγ have been reported with reference to their actions on the metabolic function of chondrocytes. These findings led us to hypothesize that IFNγ could be a modulator of the IL-1β bioactivity. IL-1β modifies regulation of the metabolic functions of chondrocyte, synovial and bone cells leading to joint destruction.[22,23] It stimulates metalloproteases and reactive oxygen species production by chondrocytes and drastically inhibits cartilage matrix component synthesis.[24,25] Furthermore, it stimulates the proliferation of fibroblasts and their production of type I collagen and fibronectin. Moreover, IL-1β also induced the production by fibroblasts and chondrocytes of soluble mediators (e.g. IL-6, IL-8, NO, etc.) involved in the pathogenesis of joint diseases.[26,27] Some previous studies have reported that the regulation of IL-1 production and activity directly modulates cartilage resorption. IL-1β production is inhibited by IL-4, IL-10 and IL-13 but stimulated by TNFα.[28–30] IL-1β-induced cartilage resorption is down-regulated by two types of inhibitors: the IL-1 receptor antagonist (IL-1ra) which competitively binds to the receptor of IL-1 without inducing signal transduction and the soluble receptors (IL-1sR), which bind IL-1 and diminish the free concentration of soluble cytokines susceptible to binding the cell surface receptor. *In vitro*, IL-1β-stimulating effects on cartilage degradation are counterbalanced by TGFβ and osteogenic protein 1 (OP1), and partially reversed by NO synthase inhibitors.[25,31–33]

These findings prompted us to investigate the effect of IFNγ on NO, IL-6, IL-8, IL-10, IL-1ra, PGE_2, proteoglycans and stromelysin production by normal non-stimulated and IL-1β-stimulated human chondrocytes. At the present time, little is known about the regulatory functions of IFNγ on chondrocytes, or about its role in the pathophysiology of joint disease.

Materials and methods

HUMAN CHONDROCYTES IN CULTURE

Chondrocytes were cultured at high density for 48 h in order to keep their phenotype. Cartilage was obtained from the knee of cadavers immediately after death via excision from the superficial and medium layer avoiding the calcified layer. Upon dissection, femoral and patellar articular surfaces were evaluated for gross pathological cartilage modifications. Cartilage fragments were sequentially treated by hyaluronidase (EC: 3.2.1.35; Sigma Chemie, Bornem, Belgium) dissolved in Dulbecco's modified Eagle's medium (DMEM, Biowhittaker, Brussels, Belgium) (0.5 mg/ml–3 g of cartilage per 10 ml of enzyme solution) for 30 min at 37°C and under constant agitation (200 rpm). Cartilage fragments were then put into pronase solution (1 mg/ ml in DMEM-3 g of cartilage per 10 ml of enzymatic solution) (EC: 3.4.24.4; Merck-Belgolabo, Overijse, Belgium) and incubated with this enzyme for 1 h at 37°C, subsequently re-incubated under constant agitation (200 rpm) with collagenase (EC: 3.4.24.3; Sigma Chemie) (1 mg/ml–3 g/10 ml of enzyme solution) dissolved in DMEM containing 1% of Ultroser (Gibco, Gent, Belgium) for 20 h at 37°C. The cells were then filtered through nylon mesh with pore of 70 μm, washed three times, counted and re-suspended at 0.5 10^6 cells/ml of adequate culture medium. Nutrient medium was DMEM without red phenol containing 2 mM of glutamine and 1% of ITS^+. ITS^+ is a well-defined culture supplement containing 0.625 mg/ml of insulin, 0.625 mg/ml of transferrin, 0.625 μg/ml of selenium acid, 125 mg/ml of bovine serum albumin (BSA) and 0.530 mg/ml of linoleic acid. Cells were kept in this culture medium for 48 h to eliminate *in-vivo* contamination with drugs. After this washout period, cells were seeded into 24-well plates and maintained in culture for 48 h at 37°C in a 95%–5% oxygen–CO_2 environment. Cells and supernatant were separated by centrifugation (160 ***g***; 5 min). The cells recovered after collagenase digestion of the tissue were 95% viable as demonstrated by the vital stain with trypan blue. Cell pellets were washed twice with HBSS (Hank's buffer saline solution, Gibco, Merelbeke, Belgium) and then homogenized in phosphate buffer saline (PBS) pH 7.5 by ultrasonic dissociation (10 s pulses, power 50 W/cm^2). Culture media were directly assayed for NO, IL-6, IL-8, IL-10, prostaglandin E_2 (PGE_2), stromelysin, proteoglycan (PG) and IL-1ra. All the chemicals for which the source was not mentioned were of the purest grade available commercially.

CULTURE TREATMENT

Human normal chondrocytes (±0.5 10^6 cells/ml) were cultured for 48 h in the absence or in the presence of human recombinant IFNγ (rhIFNγ, Roche, Brussels, Belgium) (0–100 U corresponding to 0.05–5 ng/ml) added solely or in combination with interleukin-1β (IL-1β; 2.10^{-10} M, Roche, Brussels, Belgium) and/or N^G-monomethyl-L-arginine (L-NMMA; 0.5 mM). Four culture flasks were used for each concentration of cytokines as well as for the corresponding control. Each culture was run with chondrocytes from the knee joint of a single patient. Each culture condition was tested at least three times with cartilage samples coming from three different young (<35 years) donors. Cell viability was superior to 95% in all culture treatments.

PROTEOGLYCAN (PG) RADIOIMMUNOASSAY

PG released into culture medium was assayed as described previously.[34] Briefly, human cartilage PG was extracted according to the methods of Roughley *et al*.[35]

and Bayliss and Venn.[36] Antiserum against human cartilage PG was raised in rabbits according to the method of Vaitukaitis *et al*.[37] Antibodies were directed against the core protein of human cartilage PG. No cross-reaction was observed with glycosaminoglycans such as chondroitin sulfate or keratan sulfate nor with PG treated with various proteases. Only treatment with proteolytic enzymes (papain, trypsin and chymotrypsin) abolished or diminished immunoreactivity as opposed to treatment with chondroitinase ABC and neuraminidase. Antiserum did not cross-react with other cartilage matrix constituents such as type II, type IX, and type XI collagens. The lack of cross-reaction with bone PG or skin, cornea, liver, heart or lung tissue extracts, showed that the antiserum used in the study contains only specific aggrecan antibodies.

The analytical sensitivity of the RIA was 0.6 ng/tube. The intra- and interassay coefficients of variation were <10 and 20%, respectively, for the linear part of the curve.

IMMUNOASSAY FOR STROMELYSIN

Total stromelysin production was assayed using a EASIA (enzyme amplified sensitivity immunoassay) provided from Biosource Europe (Nivelles, Belgium). In this immunoassay, monoclonal antibodies are directed against prostromelysin, activated-stromelysin and stromelysin bound to TIMP-1 and TIMP-2. Antibodies do not cross-react with stromelysin bound with α2-macroglobulin, collagenase or gelatinase. The method was linear between 5 and 20 ng/ml, with a limit of detection of 2.5 ng/ml.

NO ASSAY

Nitrite is a stable end product of nitric oxide. Nitrite concentrations in conditioned media were determined by a spectrophotometric method based upon the Griess reaction.[38] Briefly, 200 µl of supernatant was added to 50 µl 6.5 M HCL and 50 µl 37.5 mM sulfanilic acid. After 10 min at room temperature, 50 µl of 12.5 mM N-(1-naphtyl)-ethylene diamine (Merck, Darmstadt, Germany) was added. The absorption was measured at 540 nm. Sodium nitrite ($NaNO_2$) was used for calibration.

PGE_2 RADIOIMMUNOASSAY

PGE_2 was assayed in conditioned culture medium without extraction according to a previously described radioimmunoassay (RIA).[39] Polyclonal antiserum, obtained from rabbit, did not cross-react with other prostanoids (TxB2, 6-keto-PGF-1α, PGA_2) or with fatty acids (arachidonic, linoleic, oleic acids). Intra- and interassay coefficients of variation are 6 and 10%, respectively. The recovery of known amounts of PGE_2 added to the culture medium was satisfactory (90–97% for the amounts of PGE_2 ranging from 2500 to 312 pg/ml). The limit of detection of the RIA was 20 pg/ml.

IMMUNOASSAYS FOR CYTOKINES AND IL-1 RECEPTOR ANTAGONIST

IL-6, IL-8, IL-10 and IL1-ra released by chondrocytes in the culture media were assayed by four EASIA (Cytosets) designed by Biosource Europe (Nivelles, Belgium). The assays were based on the oligonal system in which several monoclonal antibodies were directed against distinct epitopes of the cytokines.

DNA ASSAY

Chondrocyte DNA content was measured according to the fluorimetric method of Labarca and Paigen.[40]

STATISTICAL ANALYSIS

The results (mean±S.D.) were expressed per µg of DNA for proteoglycan, stromelysin, IL-1ra, PGE_2, cytokines and NO measured in the culture medium. Comparison of mean values was performed for each culture individually using the unpaired Student's *t*-test with a limit of significance at $P<0.05$. Multiple comparisons between treated groups were also achieved by analysis of variance test. Correlations between the variables were investigated by linear regression analysis and one-way analysis of variance was performed (*F*-test).

Results

EFFECTS OF IFNγ ON NON-STIMULATED NORMAL CHONDROCYTES

The effects of IFNγ were investigated on human chondrocytes isolated from young cartilage at concentrations ranged between 0.1 to 100 units/ml. These concentrations corresponded to the levels of IFNγ found in the synovial fluid of patients with OA or RA (values between 0.6 and 23.7 U/ml;[1,2]. As shown in Fig. 1, IFNγ at concentrations of 1, 10 and 100 U/ml significantly stimulated NO production ($0.05<P<0.001$). Furthermore, IFNγ dose-dependently increased IL-6, IL-1ra and PGE_2 production ($R=0.85-0.95$; $P<0.001$) but strongly inhibited IL-8. IL-8 production was depressed by 25 and 55% at the IFNγ concentrations of 10 and 100 U/ml, respectively. On the other hand, PG production was dose-dependently inhibited by IFNγ ($R=0.82$; $P<0.01$) whereas stromelysin was slightly but significantly decreased by IFNγ only at the highest concentration of 100 U/ml ($P=0.02$). IL-10 was undetectable in non-stimulated human chondrocyte cultures.

EFFECTS OF IFNγ ON IL-1β-STIMULATED HUMAN CHONDROCYTES

As previously reported,[26,27] IL-1β at the concentration of 2.10^{-10} M significantly stimulated NO, IL-6, IL-8, PGE_2, IL-10, IL-1ra and stromelysin production but drastically depressed PG synthesis ($P<0.05$, Table I). Addition of rhIFNγ at the concentrations of 10 and 100 U/ml significantly increased IL-1β-stimulated NO, IL-6 and IL-1ra production but strongly depressed the stimulatory effect of IL-1β on IL-8 and IL-10 productions, whereas IL-1β-induced stromelysin production was inhibited at the concentrations of 1, 10 and 100 U/ml of IFNγ. Finally, IFNγ acted synergically with IL-1β to depress PG production by human chondrocytes but did not significantly modify IL-1-stimulated PGE_2 production (Table I).

ROLE OF NO IN CHONDROCYTE RESPONSES TO IFNγ

To evaluate the role played by NO in the response of chondrocytes to IFNγ, NO synthesis was blocked

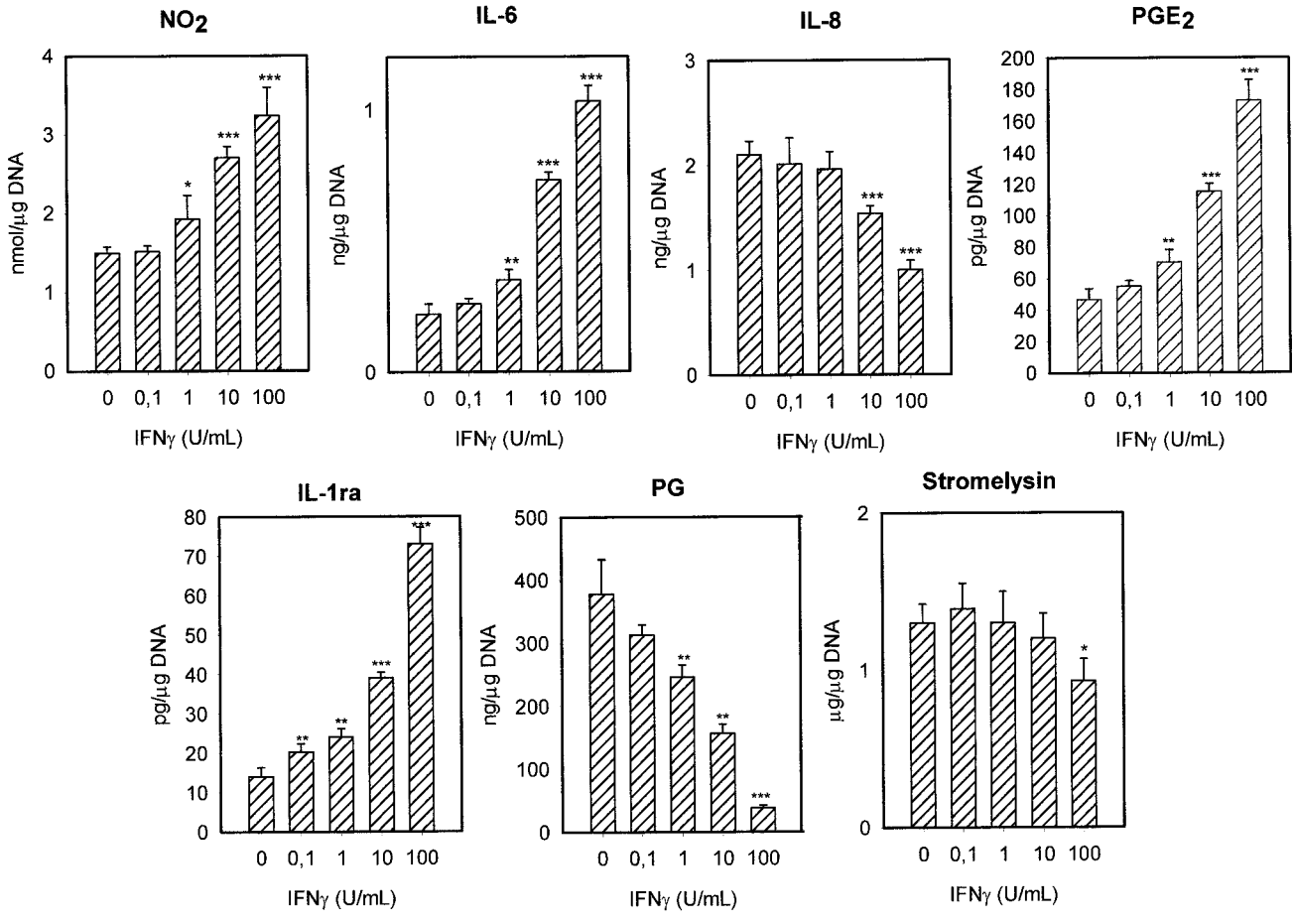

Fig. 1. Effects of increasing doses of IFNγ on the production of NO, IL-6, IL-8, PGE_2, IL-1ra, PG and stromelysin in the culture medium of normal human chondrocytes (5.10^4 cells/ml). Data are the mean and S.D. of triplicate determinations and representative of three separate experiments (total $N=9$).

Table I
Effects of increased concentrations of IFNγ on cytokines, PG, PGE_2 and stromelysin production by human articular chondrocytes

	NO_2 nmol/µg DNA	IL-6 ng/µg DNA	IL-8 ng/µg DNA	PGE_2 pg/µg DNA	IL-10 pg/µg DNA	IL-1ra pg/µg DNA	PG ng/µg DNA	MMP-3 µg/µg DNA
Controls	1.5±0.08a	0.22±0.04a	2.01±0.14a	47±7a	0.13±0.01a	14±2.3a	378±55a	1.3±0.1a
IL-1β 2.10^{-10} M	2.9±0.09b	19±2b	97±7b	1401±244b	71±3b	124±5.2b	41±4b	2.8±0.1b
IL-1β+IFNγ 0.1 U/ml	2.8±0.1b	18±1.5b	96±9.8b	1668±341b	*62±3c*	131±15b	*34±3.8c*	2.8±0.3b
IL-1β+IFNγ 1 U/ml	3±2b	20.1±2.3b	97±8b	1731±160b	*60±5c*	*174±30c*	*33±3.2c*	*1.8±0.3c*
IL-1β+IFNγ 10 U/ml	*3.7±0.3*	*24±1.4c*	*81±11c*	1454±280b	*39±3c*	*254±20d*	*35±3c*	*1.3±1.0d*
IL-1β+IFNγ 100 u/ml	*4.2±0.1c*	*29±5.3d*	*51±8d*	1534±382b	*23±1c*	*429±33e*	*32±5c*	*1.1±0.1d*

Chondrocytes were treated or were not treated with IL-1β (2.10^{-10} M). Data are the mean and S.D. of triplicate determinations and representative of three separate experiments. Letters indicate significant statistical differences between treatments. a≠b≠c≠d, with $P<0.05$ (Student's *t*-test).

by 0.5 mM of N^G-monomethyl-L-arginine (L-NMMA), a competitive inhibitor of NO synthase.

Non-stimulated NO synthesis was significantly inhibited by 0.5 mM of L-NMMA, whereas IL-1β-induced NO synthesis was reduced below the control value even in the presence of high amount of IFNγ (Table II and Fig. 2). As previously reported, addition of L-NMMA (0.5 mM) significantly enhanced IL-6, IL-8 and PGE_2 production over the IL-1β production, suggesting that NO exerts a negative feedback on PGE_2, IL-6 and IL-8 synthesis by human chondrocytes (Table II). Furthermore, here, we demonstrated that L-NMMA did not modify the stimulatory effect of IL-1β on IL-10, IL-1ra and stromelysin and did not affect the IL-1β-induced inhibition of PG production. However, the inhibition of inducible NO synthase by L-NMMA significantly reduced IFNγ-stimulated production of IL-6 and IL-1ra, but did not modify the effects of IFNγ on IL-8, PG, PGE_2 or stromelysin (Fig. 2). In the presence of IL-1β, addition of L-NMMA, partially reversed the inhibitory effect of 10 and 100 U/ml of IFNγ on IL-10 and stromelysin (Fig. 3).

Table II
Effect of NO biosynthesis on cytokines, PGE_2, PG and stromelysin (MMP-3) production by human chondrocytes

	NO_2 nmol/μg DNA	IL-6 ng/μg DNA	IL-8 ng/μg DNA	PGE_2 pg/μg DNA	IL-10 pg/μg DNA	IL-1ra pg/μg DNA	PG ng/μg DNA	MMP-3 μg/μg DNA
Controls	1.5±0.08a	0.22±0.04a	2.01±0.14a	47±7a	ND	14±2.3a	378±55a	1.3±0.1a
L-NMMA	0.5±0.05b	0.18±0.02a	1.94±0.22a	54±7a	ND	14.3±2.3a	330±23a	1.4±0.11a
IL-1β	2.9±0.06c	19±2c	97±7b	1401±244c	71±3a	124±5b	41±4c	2.8±0.08b
IL-1+L-NMMA	0.7±0.05b	25±2.1d	125±8c	5732±901d	72±1a	97±11c	44±0.6c	2.6±0.25b

Letters indicate significant statistical differences between treatments. a≠b≠c≠d, with $P<0.05$ (Student's *t*-test).

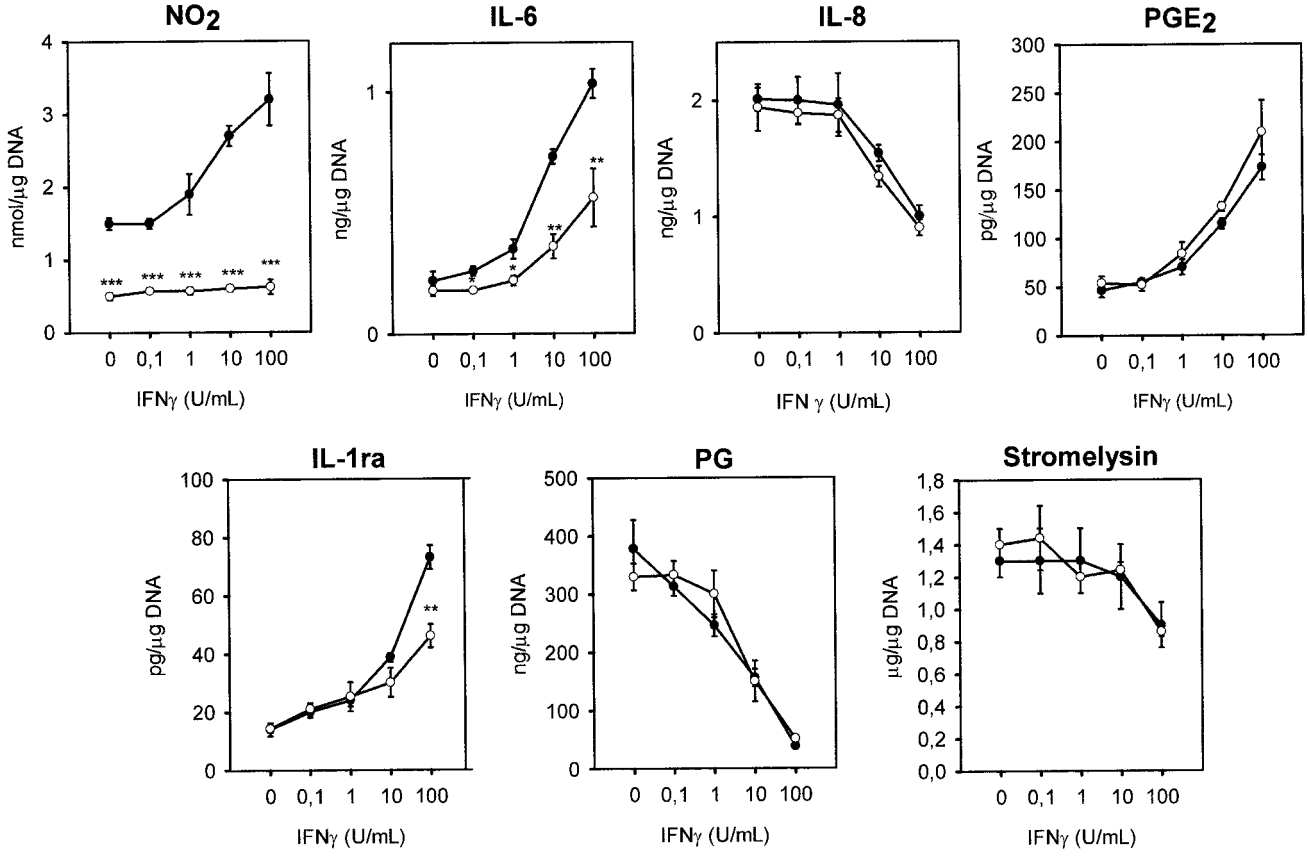

Fig. 2. Production of NO, IL-6, IL-8, IL-10, IL-1ra, PG and stromelysin by human chondrocytes treated with rhIFNγ, in the presence (—○—) or absence (—●—) of L-NMMA 0.5 mM. Results are the mean±S.D. values for four replicate wells and are representative of three similar experiments. *=$P<0.05$; **=$P<0.01$ and ***=$P<0.001$ as compared with control.

Discussion

Joint diseases are characterized by synovial membrane inflammation, cartilage degradation and subchondral bone remodeling. Cartilage plays a central role in the pathophysiology of joint diseases by producing a spectrum of soluble mediators able to regulate its own metabolic functions in an autocrine or paracrine manner as well as the metabolism of neighboring tissues such as bone or synovium. Inversely, chondrocyte functions are controlled by factors produced by synovium and bone cells. Among these factors, IFNγ is often described as a potent mediator of the cellular immune reaction with potential regulatory actions on connective tissues.[41] Although IFNγ was not expressed in cartilage,[42] IFNγ is found in OA and RA synovial membrane, suggesting that this cytokine could influence cartilage metabolism. In this work, we demonstrated that IFNγ synergized with IL-1β to stimulate NO, IL-6 and IL-1ra and to inhibit proteoglycans but antagonized the IL-1β stimulating effect on IL-8, IL-10 and stromelysin production. These observations were recorded at concentrations of IFNγ similar to those found in the synovial fluid of patients with OA or RA.

In previous studies, similar opposite effects of IFNγ were reported on synoviocytes from RA and OA patients.[43] These observations assist in explaining that, relative to the circumstances, IFNγ may have inflammatory or anti-inflammatory activities. The antiinflammatory properties of IFNγ could be related to its effects on IL-8 and IL-1ra. The inhibition of IL-8 by IFNγ may lead not only to a reduction of the number of neutrophils and T-lymphocytes invading the joint cavity but also a decrease of neovascularization of the synovial membrane, as suggested by a recent paper on the properties of IL-8 as an angiogenic factor.[44] On the other hand, we have shown that IFNγ was a potent stimulator of IL-1ra synthesis by chondrocytes. IL-1ra is a

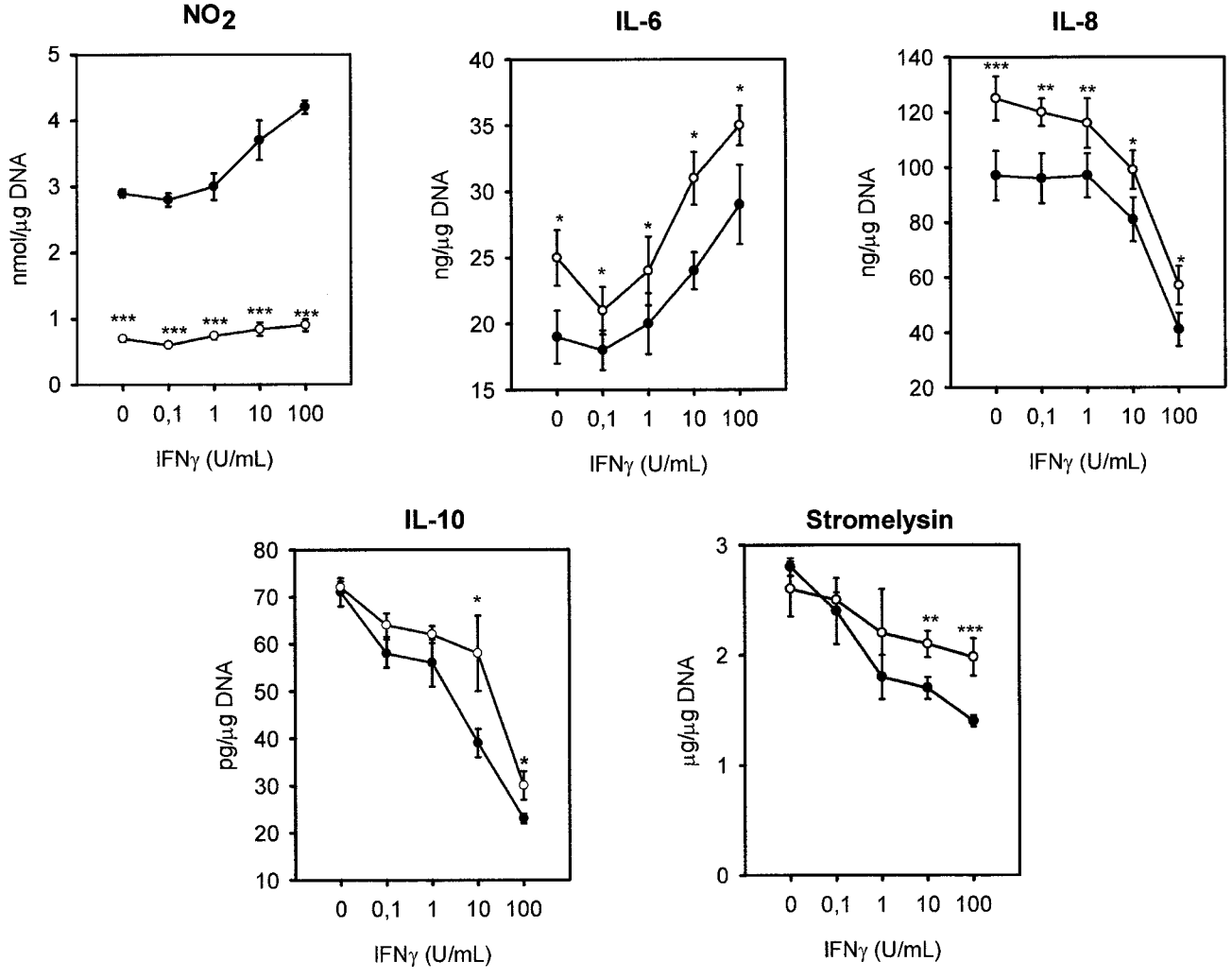

Fig. 3. Effects of increased amounts of IFNγ on NO, IL-6, IL-8, IL-10 and stromelysin synthesis by IL-1β-treated (2×10^{-10} M) human chondrocytes cultured for 48 h with (—○—) or without (—●—) L-NMMA 0.5 mM. Results are expressed as the mean±S.D. values for four replicate wells and are representative of three similar experiments. *=P<0.05; **=P<0.01; ***=P<0.001 compared with the control.

receptor-binding antagonist that blocks many of the effects observed during the pathological process of OA and RA, including prostaglandin synthesis by synovial cells, collagenase production by chondrocytes and cartilage matrix degradation.[45–49] Treatment of rats with IL-1ra can significantly suppress recurrence of swelling and severity of synoviotis and erosion induced by re-activation by i.v. injection of PG-APS (peptidoglycan-polysaccharide isolated from the cell wall of group A streptococci).[49] Furthermore, intraarticular injection of IL-1ra also prevents cartilage lesion appearance in rabbit OA model.[51] Therefore, our study provides novel mechanistic data to explain the antiinflammatory properties of IFNγ.

On the other hand, IFNγ can also inhibit IL-10 production by IL-1-stimulated human chondrocytes. *In vitro*, IL-10 is a cytokine with the capacity to down-regulate the synthesis of proinflammatory cytokines (IL-1 and TNFα) by mononuclear cells[51] and completely reverse inhibition of cartilage PG synthesis induced by stimulated RA mononuclear cells.[53] However, the role of this antiinflammatory cytokine in the pathogenesis of erosive joint diseases is not yet clearly demonstrated.

We also demonstrated that IFNγ may interfere with cartilage metabolism by inhibiting PG and stromelysin production. Our results are consistent with those found in the literature.[10,11,17,53,54] Destruction of cartilage in arthritis is partially attributable to metalloproteases released by the chondrocytes in response to IL-1. Here, we demonstrated that IFNγ counteracts the stimulating effect of IL-1β on stromelysin synthesis suggesting that IFNγ could modulate the progression of tissue degradation and remodeling in inflammatory conditions as observed in RA. Furthermore, it appeared that IFNγ inhibited PG synthesis at a concentration 100 times lower than the concentration required to inhibit stromelysin. This latter observation suggests that IFNγ could have different effects on cartilage metabolism according to its relative concentration which may vary during the course of the disease and at different sites within the joint. In addition, Quintavalla *et al*.[53] have demonstrated that subpopulations of chondrocytes within the joint may differ in their responsiveness to cytokines, including IFNγ. Collectively, these observations could explain some events occurring in joint diseases such as OA. During OA development, phases of destruction and

repair alternate. Therefore, we can hypothesize that in this disease, IFNγ contributes to repair reaction failure by inhibiting PG synthesis and has a protective role limiting cartilage degradation by metalloproteinases in inflammatory conditions. This hypothesis is supported by other work demonstrating that IFNγ partially counteracts the IL-1-induced cartilage degradation as measured by glycosaminoglycan released from cartilage fragments in culture, but acts synergically with TNFα to inhibit cartilage-specific type II, IX and XI collagens production by fetal and adult human chondrocytes.[10,11] The effects of IFNγ do not appear to be due to cell death or proliferation. No decrease in cell viability, as determinated by trypan blue exclusion, was evident in culture of chondrocytes incubated with up to 100 U/ml of IFNγ (data not shown) and DNA levels remained stable regardless of the concentrations of IFNγ employed.

Interestingly, we have observed that IFNγ stimulated NO production by chondrocytes, suggesting that several effects of IFNγ could be mediated by NO. To verify this hypothesis, L-NMMA, a NO synthase inhibitor, was added to the chondrocyte cultures. As previously described, we have observed that the inhibition of endogenously NO production resulted in a significant enhancement of the IL-1β-stimulated IL-6, IL-8, and PGE_2, suggesting that NO down-regulates the synthesis of some inflammatory mediators.[27] Inversely, the effects of IFNγ on IL-8 and PGE_2 synthesis, were not significantly affected by NO synthase inhibition whereas its stimulating effect on IL-6 and IL-1ra production was partially reversed. These data clearly show that IFNγ and IL-1β activate different signaling pathways in which NO plays different regulatory functions. On the other hand, neither IL-1β nor IFNγ effects on stromelysin and PG production were significantly modified by the addition of L-NMMA. These results differ from studies showing that L-NMMA partially reversed the effect of IL-1β on the synthesis of cartilage matrix molecules and on MMP activity.[33,56,57] This discrepancy can be explained by the difference in methodology. In our study, newly synthesized proteoglycans were quantified by RIA using an antiserum directed against the core protein of the aggrecans, whereas other studies revealed proteoglycans synthesis by the incorporation of $^{35}SO_4$ into glycosaminoglycans.[55,56] In our work, we have quantified stromelysin by an immunoassay in which monoclonal antibodies were directed against pro-stromelysin, activated-stromelysin and stromelysin bound with TIMP-1 and TIMP-2. In other studies, metalloprotease activity was measured by enzymatic assay using labeled substracts. Metalloprotease activity is the result of the efficiency of both activation and inhibition systems and not only the result of the enzyme synthesis.

In conclusion, these results show that IFNγ has a number of effects on articular chondrocytes *in vitro* and suggest that IFNγ may modulate joint inflammation by modulating mediator production by chondrocytes. Moreover, we clearly show that the role played by IFNγ in joint disease pathophysiopathology may differ according to its own concentration but also to the concentration of other cytokines such as IL-1β.

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