EXTENDED REPORT

Interferon β stimulates interleukin 1 receptor antagonist production in human articular chondrocytes and synovial fibroblasts

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Background: Interferon (IFN) β displays anti-inflammatory and immunosuppressive activity and has been considered for the treatment of rheumatoid arthritis (RA). Information about the effects of this molecule on joint cells is scarce, however.
Objective: To investigate the effects of IFNβ on the production of interleukin-1 receptor antagonist (IL1Ra) in human articular chondrocytes and synovial fibroblasts.
Methods: Chondrocytes and synovial fibroblasts were stimulated with IFNβ alone or in combination with interleukin (IL) 1β. IL1Ra concentrations in culture supernatants and cell lysates were determined by ELISA. Expression of mRNA encoding the secreted sIL1Ra or the intracellular icIL1Ra1 isoforms was quantified by real time reverse transcriptase-polymerase chain reaction.
Results: In chondrocytes, IFNβ alone had no effect, but dose dependently enhanced the secretion of IL1Ra induced by IL1β. Chondrocyte cell lysates contained undetectable or low levels of IL1Ra, even after stimulation with IL1β and IFNβ. Consistently, IL1β and IFNβ induced sIL1Ra mRNA expression in chondrocytes, while expression of icIL1Ra1 was not detectable. Human articular chondrocytes thus mainly produce secreted IL1Ra. In synovial fibroblasts, IFNβ alone dose dependently increased IL1Ra secretion. In addition, IFNβ enhanced the stimulatory effect of IL1β on IL1Ra production. In synovial cell lysates, IFNβ and IL1β also increased IL1Ra levels. Consistently, IFNβ and IL1β induced the expression of both sIL1Ra and icIL1Ra1 mRNA in synovial fibroblasts.
Conclusion: IFNβ increases IL1Ra production in joint cells, which may be beneficial in cartilage damaging diseases such as RA or osteoarthritis.

Interferon (IFN) β displays anti-inflammatory and immunosuppressive activity, and IFNβ is emerging as a potentially effective form of treatment in immune mediated conditions such as multiple sclerosis. IFNβ also demonstrated effects in animal models of arthritis, as well as in human rheumatoid arthritis (RA).[1] However, little information is available about the effects of IFNβ on joint cells. Anti-inflammatory effects triggered by IFNβ include decreased expression of proinflammatory cytokines, such as interleukin (IL) 1 and tumour necrosis factor α, and increased expression of anti-inflammatory cytokines, such as IL10 and IL1 receptor antagonist (IL1Ra).[1] Such effects, by being chondroprotective, might also prove beneficial in osteoarthritis (OA).

The precise cause of OA is still incompletely resolved, but evidence suggests that an imbalance between catabolic cytokines and protective factors plays a part in cartilage alteration.[2 3] In this regard, IL1 and its natural inhibitor IL1Ra seem to have an important role. These cytokines are indeed present in the synovial fluid and therapeutic interventions aimed at correcting the IL1/IL1Ra dysbalance proved effective in animal models of OA.[2–4]

IL1Ra is a member of the IL1 family that binds to IL1 receptors but does not induce any intracellular response. IL1Ra prevents the interaction between IL1 and its cell surface receptors and thus, competitively inhibits its biological effects.[5–7] IL1Ra is produced as four different isoforms, one secreted (sIL1Ra) and three intracellular (icIL1Ra1, 2, 3), derived from the same gene.[7] The sIL1Ra and icIL1Ra1 mRNAs are transcribed from different promoters and contain isoform-specific 5′ sequences owing to alternative splicing.[8 9] The mRNA for icIL1Ra2 is transcribed from the icIL1Ra1 promoter, but contains an additional exon.[10] Finally, icIL1Ra3 is produced by alternative translation initiation from the sIL1Ra transcript.[11] Expression of the various IL1Ra isoforms is cell type and stimulus-specific (see review by Arend *et al*[7]).

IFNβ has been reported to increase IL1Ra production in various cell types.[12–14] However, the effects of IFNβ on the production of this anti-inflammatory cytokine have not been investigated in joint cells. Biological effects of IFNβ were previously reported in a human chondrosarcoma derived cell line, as well as in human fibroblast-like synoviocytes, indicating that both chondrocytic cells and synovial fibroblasts might respond to IFNβ.[15 16] Thus this study aimed at examining the potential effects of IFNβ on IL1Ra production in human articular chondrocytes and synovial fibroblasts.

MATERIALS AND METHODS

Materials

Cell culture reagents were obtained from Gibco (Life Technologies AG, Basel, Switzerland). Commercially available IFNβ1a (Rebif; Serono International SA, Geneva, Switzerland; specific activity 250×10^6 U/mg) was used for these studies. Recombinant human IL1β was purchased from

Abbreviations: AMV-RT, avian myeloblastosis virus-reverse transcriptase; CIA, collagen induced arthritis; ELISA, enzyme linked immunosorbent assay; FCS, fetal calf serum; icIL1Ra, intracellular IL1 receptor antagonist; IFN, interferon; IL, interleukin; IL1Ra, IL1 receptor antagonist; LC, Light Cycler; MMP, matrix metalloproteinase; OA, osteoarthritis; PCR, polymerase chain reaction; RA, rheumatoid arthritis; RT-PCR, reverse transcription and polymerase chain reaction; sIL1Ra, secreted IL1 receptor antagonist; TIMP, tissue inhibitor of matrix metalloproteinase

Table 1 PCR primer location and sequence

Primer	Gene	GenBank	Location (bp)*	Sequence 5′-3′
A	IFNAR1	NM000629	505-524 (s)	GTGATACACATCTCTCCTGG
B	IFNAR1	NM000629	830-807 (as)	GTATAATCCCATTTAAGAACATAG
C	IFNAR2	NM000874	545-566 (s)	GAGTAAACCAGAAGATTTGAAG
D	IFNAR2	NM000874	938-918 (as)	CGTGTTTGGAATTAACTTGTC
E	IL1RN	U65590	16128-16147 (s)	TTATGGGCAGCAGCTCAGTT
F	IL1RN	U65590	25831-25851 (s)	TCCGCAGTCACCTAATCACTC
G	IL1RN	U65590	29134-29115 (as)	TTGACACAGGACAGGCACAT
H	*28S*	U13369	10661-10679 (s)	TTGAAAATCCGCGGGAGA
I	*28S*	U13369	10760-10741 (as)	ACATTGTTCCAACATGCCAG

* Sense (s) or antisense (as) orientation with respect to the corresponding gene is indicated.

Table 2 PCR primer combinations and conditions

cDNA	Forward primer	Reverse primer	Annealing temp. (°C)	Product length (bp)
IFNAR1	A	B	*55*	326
IFNAR2	C	D	*55*	394
icIL1Ra1	E	G	*56*	254
sIL1Ra	F	G	*57*	268
28 S rRNA	H	I	*54*	100

R&D Systems (Abingdon, United Kingdom). DNAse I, avian myeloblastosis virus-reverse transcriptase (AMV-RT) and random hexamer primers were obtained from Promega (Wallisellen, Switzerland). Light Cycler (LC) polymerase chain reaction (PCR) reagents were purchased from Roche Molecular Biochemicals (Rotkreuz, Switzerland).

Cell isolation and culture

Cartilage was obtained from patients undergoing joint replacement (knee or hip prosthetic surgery) for OA or broken femoral neck (normal adult cartilage) or from children undergoing spinal surgery for scoliosis (vertebral posterior joints—normal paediatric cartilage). Synovium was obtained from patients undergoing joint replacement (knee or hip prosthetic surgery) for OA or RA. Chondrocytes and synovial fibroblasts were isolated by collagenase digestion as reported previously[17 18] and cultured in Dulbecco's modified Eagle's medium supplemented with L-glutamine, streptomycin, penicillin, and 10% fetal calf serum (FCS). Primary chondrocytes were used directly after isolation from cartilage. Dedifferentiated chondrocytes were used for the various studies between passages 1 and 6, and synovial fibroblasts between passages 1 and 14. Cells were plated in 96 well plates for assessment of IL1Ra production (40 000 cells per well) and in 25 cm^2 flasks (10^6 cells per flask) for RNA extraction. To reduce the non-specific effects of agonists present in FCS, the cells were incubated in low serum (0.5% FCS) medium for 24 hours before stimulation with the various agents.

ELISA for human IL1Ra

After incubation of the cells with cytokines for the indicated times, the culture supernatants were collected and stored at −20°C. Cell lysates were obtained by adding fresh medium containing 1% NP-40 to the cells. The concentrations of IL1Ra in supernatants and lysates were measured by a sandwich enzyme linked immunosorbent assay (ELISA), as previously described.[19] This ELISA detects all IL1Ra isoforms. The detection limit of the assay is 78 pg/ml.

RNA isolation, reverse transcription and polymerase chain reaction (RT-PCR) analysis

Total cellular RNA was extracted using the Tripure reagent (Roche Molecular Biochemicals, Rotkreuz, Switzerland). Total RNA (3 μg) was digested with DNAse I and reverse transcribed using AMV-RT and random hexamer primers. For the detection of IFNAR1 and IFNAR2, PCR amplification (35 cycles) was performed using Taq DNA polymerase (Qiagen, Basel, Switzerland) with indicated primer pairs and conditions (tables 1 and 2). For detection of sIL1Ra and icIL1Ra1, quantitative real time PCR was performed with an LC (Roche Molecular Biochemicals) using the FastStart DNA Master SYBR Green I reaction mix, appropriate primer pairs, and conditions (tables 1 and 2). Expression of sIL1Ra or icIL1Ra1 mRNA was corrected for 28S ribosomal RNA (rRNA) levels, which were quantified by LC real time PCR using indicated primers and conditions (tables 1 and 2). The absence of DNA contamination in RNA preparations was tested by including RNA samples which had not been reverse transcribed. Distilled water was used as a negative control for PCR amplification. The identity of the amplified products was confirmed by DNA sequencing.

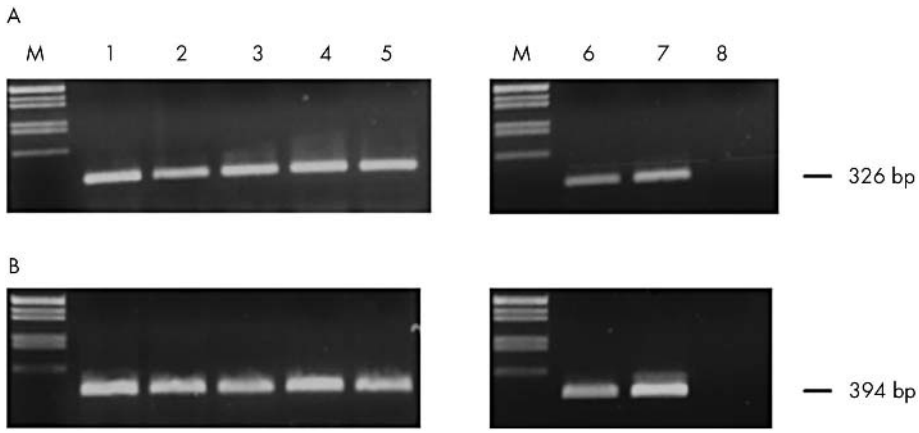

Figure 1 Expression of IFNAR1 and IFNAR2 mRNA in human articular chondrocytes and synovial fibroblasts. Expression of mRNA encoding (A) IFNAR1 and (B) IFNAR2 was assessed by RT-PCR using total RNA from freshly isolated (lanes 1, 6, 7) or dedifferentiated (passage 1; lanes 4 and 5) human articular chondrocytes or from human synovial fibroblasts (passage 3; lanes 2 and 3). Cells were left unstimulated (lanes 1, 2, 4, 6) or stimulated for 24 hours with 1 ng/ml IL1β (lanes 3, 5, 7). M: DNA molecular weight marker. Lane 8: distilled water was used as a negative control for PCR amplification.

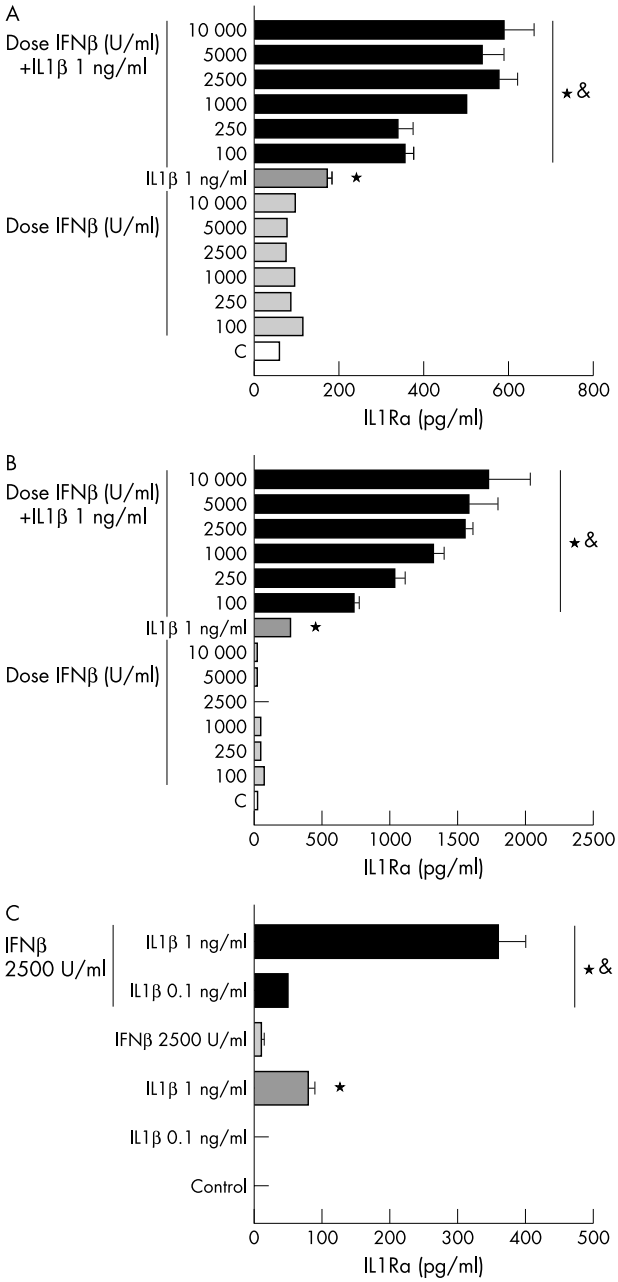

Figure 2 Dose dependent effects of IFNβ and IL1β on IL1Ra secretion by human articular chondrocytes. (A) Human articular chondrocytes freshly isolated from OA knee joint cartilage or (B) dedifferentiated human articular chondrocytes (passage 4, OA femoral head) were left unstimulated (control; white bars) or stimulated with indicated doses of IFNβ (grey bars; 250 U/ml corresponds to 1 ng/ml), 1 ng/ml IL1β (dark grey bars), or the combination of 1 ng/ml IL1β and indicated doses of IFNβ (black bars) for 72 hours. (C) Freshly isolated human articular chondrocytes (OA knee) were left unstimulated (control; white bars) or stimulated with indicated doses of IL1β (dark grey bars), 2500 U/ml (10 ng/ml) IFNβ (grey bar) or the combination of 2500 U/ml (10 ng/ml) IFNβ and indicated doses of IL1β (black bars) for 72 hours. IL1Ra concentrations in culture supernatants were measured by ELISA. Each bar represents the mean (SEM) of three determinations in a representative experiment. * $p<0.05$ as compared with control; & $p<0.01$ as compared with cells treated with the same dose of IL1β alone.

Statistical analysis

Significance of differences was calculated by analysis of variance. A difference between experimental groups was considered significant for a p value <0.05.

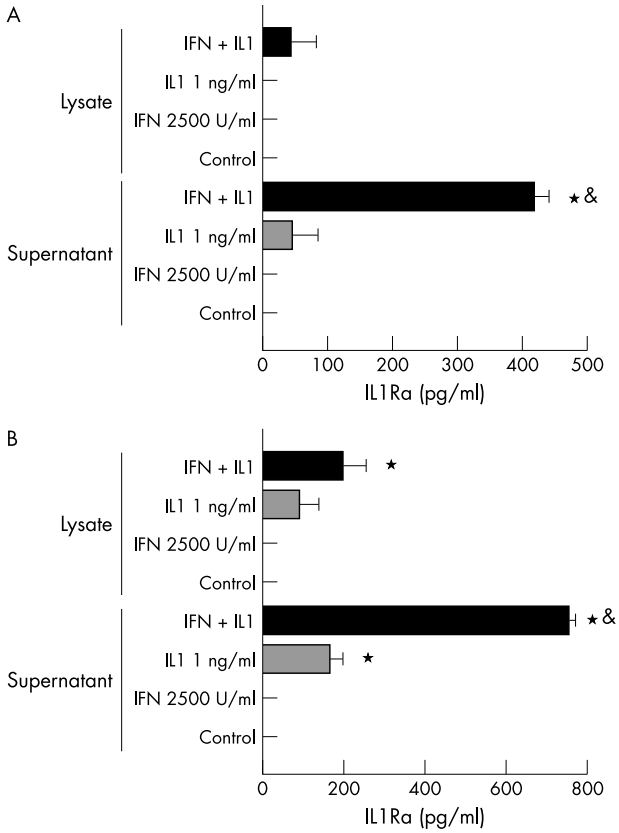

Figure 3 Effects of IFNβ and IL1β on the production of secreted and intracellular IL1Ra by in human articular chondrocytes. (A) Human articular chondrocytes freshly isolated from normal adult femoral head cartilage or (B) dedifferentiated human articular chondrocytes (passage 5, OA femoral head) were left unstimulated (control; white bars) or stimulated with 2500 U/ml (10 ng/ml) IFNβ (grey bars), 1 ng/ml IL1β (dark grey bars), or the combination of 2500 U/ml (10 ng/ml) IFNβ and1 ng/ml IL1β (black bars) for 72 hours. IL1Ra concentrations in culture supernatants and cell lysates were measured by ELISA. Each bar represents the mean (SEM) of three determinations in a representative experiment. * $p<0.05$ as compared with control; & $p<0.001$ as compared with cells treated with IL1β alone.

RESULTS

Expression of mRNA encoding type I IFN receptors in human joint cells

Type I interferons exert their cellular effects by binding to the IFNAR1 and IFNAR2 cell surface receptors.[20] We examined the expression of IFNAR1 and IFNAR2 mRNA by RT-PCR in human articular chondrocytes and synovial fibroblasts. IFNAR1 and IFNAR2 transcripts were detected in primary and dedifferentiated chondrocytes, as well as in synovial fibroblasts (fig 1). Furthermore, IFNAR1 and IFNAR2 expression was seen both in unstimulated cells and after 24 hours' stimulation with 1 ng/ml IL1β. Human joint cells thus produce IFNAR1 and IFNAR2 transcripts.

Production of IL1Ra in response to IFNβ and IL1β in human articular chondrocytes

We next assessed the effect of IFNβ on IL1Ra production in human articular chondrocytes in the presence or absence of IL1β. IL1Ra was low in conditioned media of untreated cells, but IL1β (1 ng/ml) stimulated its production (fig 2), as recently described.[21] IFNβ had no effect on its own, but dose dependently enhanced the stimulatory effect of IL1β on IL1Ra production. The effect of IFNβ on IL1β induced IL1Ra secretion was maximal at 2500 U/ml IFNβ (figs 2A and B). A similar dose dependent effect of IFNβ was seen using cells

Table 3 IL1Ra secretion by OA articular chondrocytes

Samples	Condition	IL1Ra (% of maximum)
OA knee (n = 3)	Control	3.09 (3.09)
Primary cells	IFNβ 2500 U/ml	4.72 (3.64)
	IL1β 1 ng/ml	18.11 (7.71)
	IFNβ and IL1β*	100 (0)‡ §
OA femoral head (n = 5)	Control	1.49 (1.24)
Dedifferentiated cells	IFNβ	2.31 (2.31)
	IL1β	18.77 (5.62)‡
	IFNβ and IL1β†	100 (0)‡ §

*IL1Ra secretion in primary cells from three different donors, stimulated with 2500 U/ml IFNβ and 1 ng/ml IL1β, ranged from 360.1 to 573.1 ng/ml (mean (SEM) 488.5 (65.3)); †IL1Ra secretion in dedifferentiated cells (passages 4–6, five different donors) stimulated with 2500 U/ml IFNβ and 1 ng/ml IL1β ranged from 369.2 to 1573.7 ng/ml (mean (SEM) 771.4 (218.8)); ‡$p<0.05$ *v* control; §$p<0.01$ *v* IL1β or IFNβ alone.

from six different donors (two OA knee and four OA femoral head samples). The stimulation of IL1Ra production by IL1β, used either alone or in combination with IFNβ, was also dose dependent (fig 2C). A similar dose dependent effect of IL1β was observed using cells from three different donors (one OA knee and two OA femoral head samples). The response to IL1β and IFNβ was similar in freshly isolated chondrocytes (fig 2A, table 3) or in subcultured dedifferentiated cells after 1–6 passages (fig 2B, table 3). Furthermore, similar induction of IL1Ra secretion was seen using OA chondrocytes from femoral head or knee, normal adult femoral head chondrocytes, or normal paediatric samples (table 3, figs 2 and 3, and data not shown). Finally, IFNβ and IL1β also increased IL1Ra production in culture supernatants of cartilage explants (OA and normal adult femoral head, data not shown), indicating that this response is not affected by the procedure that we routinely use for chondrocyte isolation.

The effects of IFNβ and IL1β on IL1Ra production were time dependent and maximal after 48–72 hours of stimulation (data not shown). Furthermore, the combined effects of IFNβ and IL1β were optimal when both cytokines were added simultaneously; preincubation with IFNβ did not further enhance the response (data not shown).

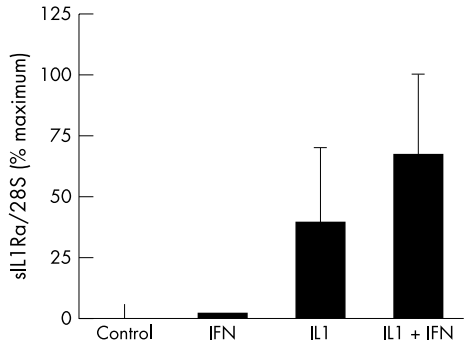

Figure 4 Effects of IFNβ and IL1β on the expression of sIL1Ra mRNA in human articular chondrocytes. Expression of mRNA encoding sIL1Ra was quantified by real time RT-PCR using total RNA from dedifferentiated human articular chondrocytes (passages 1–3). Cells were left unstimulated (control) or stimulated for 18 hours with 2500 U/ml (10 ng/ml) IFNβ (IFN), 1 ng/ml IL1β (IL1) or the combination of 2500 U/ml (10 ng/ml) IFNβ and 1 ng/ml IL1β (IL1 + IFN). The amount of 28S rRNA was monitored as an internal control. The expression of sIL1Ra was corrected for 28S rRNA levels and the IL1Ra/28S ratios were normalised to the maximal value observed in each experiment, which was set to 100%. The results shown represent the mean (SEM) of data obtained with three samples from different donors (one normal adult femoral head, one normal paediatric vertebra, and one OA femoral head).

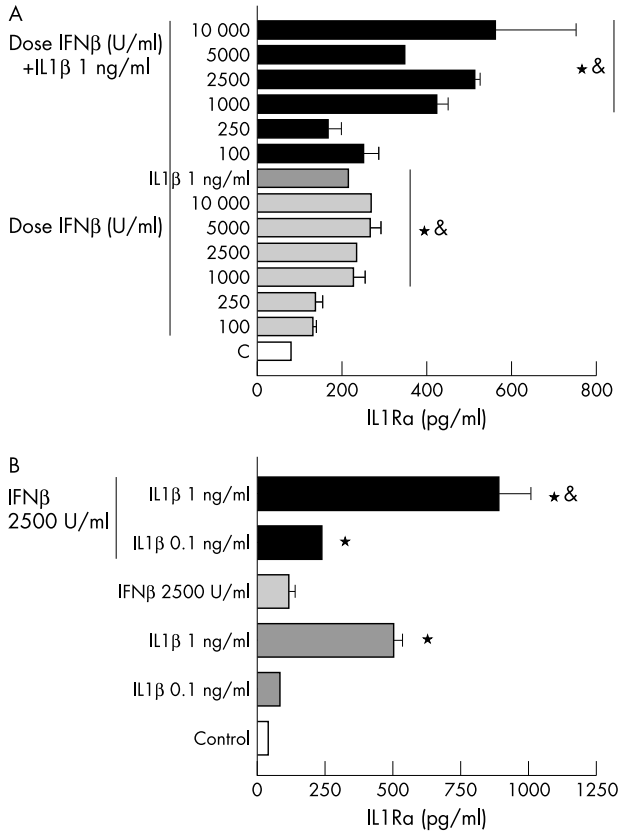

Figure 5 Dose dependent effects of IFNβ and IL1β on IL1Ra production in human synovial fibroblasts. (A) Human OA synovial fibroblasts (passage 4) were left unstimulated (control; white bars) or stimulated with indicated doses of IFNβ (grey bars; 250 U/ml corresponds to 1 ng/ml), 1 ng/ml IL1β (dark grey bar), or the combination of 1 ng/ml IL1β and indicated doses of IFNβ (black bars) for 72 hours. (B) Human OA synovial fibroblasts (passage 5) were left unstimulated (control; white bars) or stimulated with indicated doses of IL1β (dark grey bars), 2500 U/ml (10 ng/ml) IFNβ (grey bar), or the combination of 2500 U/ml (10 ng/ml) IFNβ and indicated doses of IL1β (black bars) for 72 hours. IL1Ra concentrations in culture supernatants were measured by ELISA. Each bar represents the mean (SEM) of three determinations in a representative experiment. * $p<0.05$ as compared with control; & $p<0.05$ as compared with cells treated with the same dose of IL1β alone.

In contrast with culture supernatants, cell lysates of primary human chondrocytes contained undetectable or low levels of IL1Ra, even after stimulation with IL1β and IFNβ (fig 3A). This observation suggests that primary articular chondrocytes produce essentially the secreted IL1Ra isoform. Similar results were obtained with cells from two different donors (normal adult femoral head and paediatric vertebra). Stimulated levels of IL1Ra in cell lysates increased somewhat with passages in dedifferentiated chondrocytes (fig 3B). This observation was confirmed using cells from three different donors (three OA femoral head samples).

Expression of sIL1Ra and icIL1Ra1 transcripts in human articular chondrocytes

To further characterise the IL1Ra isoforms expressed in articular chondrocytes in response to IL1β and IFNβ, we quantified the expression of sIL1Ra and icIL1Ra1 transcripts by real time RT-PCR using isoform-specific primers (tables 1 and 2). IL1β (1 ng/ml) induced the expression of sIL1Ra mRNA in human chondrocytes, as previously reported.[21] Expression of sIL1Ra mRNA tended to be further enhanced when IL1β was combined with IFNβ (2500 U/ml), although the difference in expression levels did not reach statistical

Table 4 IL1Ra secretion by human synovial fibroblasts

Samples	Condition	IL1Ra (% of maximum)
OA (n=7)	Control	12.69 (3.32)
	IFNβ	30.99 (7.60)‡
	IL1β	45.01 (5.14)‡
	IFNβ and IL1β*	100 (0)‡ §
RA (n=3)	Control	4.55 (4.55)
	IFNβ	15.75 (1.95)
	IL1β	46.02 (9.65‡
	IFNβ and IL1β†	100 (0)‡ §

*IL1Ra secretion in OA cells (passages 1 to 6, seven different donors) stimulated with 2500 U/ml IFNβ and 1 ng/ml IL1β ranged from 177.2 to 881.0 ng/ml (mean (SEM) 476.1 (90.5)); †IL1Ra secretion in RA cells (passages 2–12, three different donors) stimulated with 2500 U/ml IFNβ and 1 ng/ml IL1β ranged from 1126.7 to 1480.2 ng/ml (mean (SEM) 1327.6 (104.9)); ‡$p<0.05$ *v* control; §$p<0.001$ *v* IL1β or IFNβ alone.

significance (fig 4). The effects of IL1β and IFNβ on IL1Ra protein secretion, measured by ELISA (fig 2), were thus associated with increased sIL1Ra mRNA expression (fig 4). Expression of icIL1Ra1 mRNA was generally not detectable in these experiments (data not shown), which is consistent with the very small amounts of IL1Ra detected in cell lysates, confirming that human articular chondrocytes primarily produce sIL1Ra.[21]

Production of IL1Ra in response to IFNβ and IL1β in human synovial fibroblasts

While IFNβ alone failed to modulate IL1Ra production in chondrocytes, it increased the secretion of IL1Ra by human synovial fibroblasts in a dose dependent manner (fig 5A). In addition, like in chondrocytes, IFNβ dose dependently enhanced the stimulatory effect of IL1β on IL1Ra production by synovial fibroblasts. A maximal effect of IFNβ was observed with 1000 U/ml (fig 5A). A similar dose dependency was observed with cells from four different donors (three OA and one RA samples). In fact, the relative IL1Ra expression levels induced by IFNβ, IL1β, or the combination of the two cytokines were identical in OA and RA samples, although the absolute quantities of IL1Ra produced were higher in RA than in OA synovial fibroblasts (table 4, figs 5 and 6). The stimulation of IL1Ra production by IL1β, used either alone or

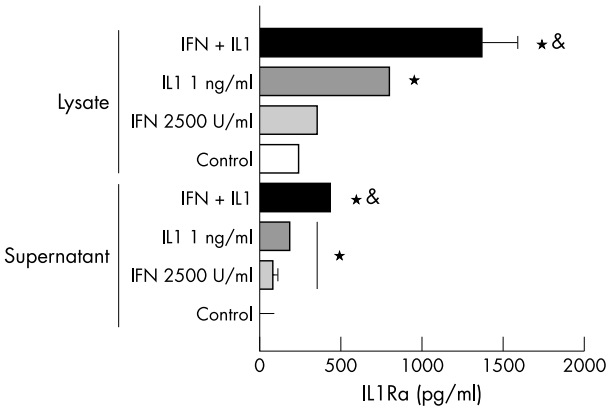

Figure 6 Effects of IFNβ and IL1β on the production of secreted and intracellular IL1Ra in human synovial fibroblasts. Human RA synovial fibroblasts (passage 5) were left unstimulated (control; white bars) or stimulated with 2500 U/ml (10 ng/ml) IFNβ (grey bars), 1 ng/ml IL1β (dark grey bars), or the combination of 2500 U/ml (10 ng/ml) IFNβ and 1 ng/ml IL1β (black bars) for 72 hours. IL1Ra concentrations in culture supernatants and cell lysates were measured by ELISA. Each bar represents the mean (SEM) of three determinations in a representative experiment. * $p<0.05$ as compared with control; & $p<0.05$ as compared with cells treated with IL1β alone.

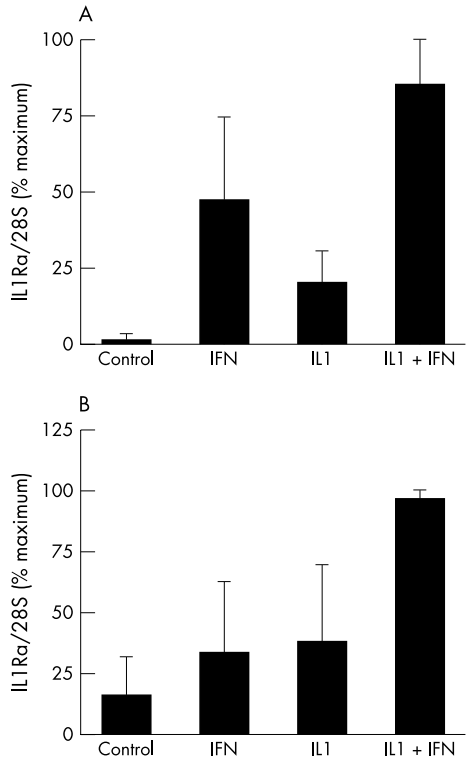

Figure 7 Effects of IFNβ and IL1β on the expression of sIL1Ra and icIL1Ra1 mRNA in human synovial fibroblasts. Expression levels of mRNA encoding (A) sIL1Ra and (B) icIL1Ra1 were quantified by real time RT-PCR using total RNA from human synovial fibroblasts (passages 2–14). Cells were left unstimulated (control) or stimulated for 18 hours with 2500 U/ml (10 ng/ml) IFNβ (IFN), 1 ng/ml IL1β (IL1) or the combination of 2500 U/ml (10 ng/ml) IFNβ and 1 ng/ml IL1β (IL1 + IFN). The amount of 28S rRNA was monitored as an internal control. The expression of sIL1Ra or icIL1Ra1 was corrected for 28S rRNA levels and the IL1Ra/28S ratios were normalised to the maximal value observed in each experiment, which was set to 100%. The results shown represent the mean (SEM) of data obtained with three samples from different donors (one OA and two RA samples).

in combination with IFNβ, was also dose dependent (fig 5B). Again, similar relative expression levels were observed with cells from two different donors (one OA and one RA sample).

The effects of IFNβ and IL1β on IL1Ra secretion by synovial fibroblasts were time dependent and maximal after 72 hours of stimulation (data not shown). In synovial cell lysates, IL1Ra levels were often detectable already in unstimulated cells and they increased in parallel with IL1Ra secretion upon stimulation with IFNβ and IL1β (fig 6). Similar data were obtained with synovial cells from three different donors (one OA and two RA samples).

Expression of sIL1Ra and icIL1Ra1 transcripts in human synovial fibroblasts

To further characterise the IL1Ra isoforms expressed in human synovial fibroblasts in response to IL1β and IFNβ, we quantified the expression of sIL1Ra and icIL1Ra1 transcripts by real time RT-PCR using isoform-specific primers (tables 1 and 2). IFNβ (2500 U/ml) and IL1β (1 ng/ml) alone induced the expression of sIL1Ra mRNA in human synovial fibroblasts and this effect tended to be further enhanced when both cytokines were added together, although the difference in expression levels did not reach statistical significance (fig 7A). Similarly, IL1β and IFNβ increased icIL1Ra1 mRNA expression in human synovial fibroblasts (fig 7B). The effects of IL1β and IFNβ on secreted and intracellular IL1Ra production, measured by ELISA (figs 5 and 6), were thus

paralleled by changes in steady state levels of sIL1Ra and icIL1Ra1 mRNA (fig 7).

DISCUSSION

In this study we described increased production of IL1Ra in human joint cells treated with IFNβ. IFNβ had been previously reported to increase IL1Ra production in various cell types, and raised serum IL1Ra levels are seen in patients with multiple sclerosis treated with IFNβ.[12–14 22 23] However, although IFNβ has been considered for the treatment of RA, the effects of IFNβ on the production of this anti-inflammatory cytokine had, to our knowledge, never been investigated in joint cells.

We previously observed that human articular chondrocytes produce sIL1Ra when stimulated with IL1β, alone or in combination with IL6.[21] A recent report described the biological effects of IFNβ in a human chondrosarcoma derived chondrocytic cell line, suggesting that chondrocytes are responsive to IFNβ.[15] We report that human chondrocytes indeed express mRNA encoding type I interferon receptors and that IFNβ modulates IL1β induced IL1Ra production in these cells. Although IFNβ displayed no stimulatory effect on its own, it markedly enhanced IL1Ra secretion in response to IL1β. In addition, the results of our study confirm that human articular chondrocytes essentially express the sIL1Ra isoform.[21]

Synovial fibroblasts themselves produce low amounts of IFNβ, as visualised by immunofluorescence on synovial specimens (Tak PP, personal communication). Large amounts of IFNβ, however, appear to be produced by fibroblastic cells only upon exposure to viral and, to a lesser extent, non-viral pathogen associated molecules.[24] Biological effects of IFNβ were previously seen in RA synovial fibroblasts and include inhibition of matrix metalloproteinase (MMP)-1, MMP-3, and prostaglandin E_2 production.[16] A potential effect of IFNβ on IL1Ra production had not been investigated. We observed that IFNβ alone increases IL1Ra secretion in synovial fibroblasts. In addition, IFNβ enhanced the stimulatory effect of IL1β on IL1Ra production. Synovial fibroblasts produced secreted and intracellular IL1Ra and the expression of both sIL1Ra and icIL1Ra1 isoforms was increased in response to IL1β and IFNβ.

The exact biological functions of the different IL1Ra isoforms are still not clear.[7 25 26] The major role of sIL1Ra is to block the effects of IL1 at the cell surface. The intracellular isoforms may be released from cells under some circumstances, but it has also been suggested that they perform important regulatory roles within cells. In a recent study, we observed that overexpression of either sIL1Ra or icIL1Ra1 similarly protected mice from collagen induced arthritis (CIA), blocking inflammation and joint damage.[27] The significance of the observed increase in IL1Ra levels induced by IFNβ can be questioned given that a significant excess of IL1RA is necessary to block the effects of IL1. High amounts of IL1 are needed to observe maximal catabolic effects in vitro. However, multiple evidence (see Chabaud *et al*,[28] among others) indicates that even very low levels of catabolic cytokines, including IL1, at suboptimal concentrations, can synergistically induce large effects. In vivo, most probably, a cocktail of cytokines, each present in low amounts, induces proinflammatory and catabolic effects synergistically. It is then likely sufficient to block lower amounts of IL1 to decrease synergisms in vivo. The increased production of both sIL1Ra and icIL1Ra1 isoforms seen in joint cells in response to IFNβ is thus potentially beneficial by preventing joint damage in inflammatory arthropathies such as RA. In this regard, treatment with IFNβ has been shown to limit the severity of CIA, decreasing, in particular, cartilage destruction.[1] The mechanisms of this protection may be multiple. However, a stimulatory effect on IL1Ra synthesis might be particularly relevant given the likely central role of IL1 in CIA.[29]

A phase I study with IFNβ in 12 patients with RA showed a significant clinical and immunohistological improvement: improved measures included swollen joint counts, as well as MMP-1 and tissue inhibitor of metalloproteinases (TIMP) expression.[30] These effects of IFNβ could likewise be beneficial in other conditions characterised by cartilage degradation, including OA. IL1 together with the MMP/TIMP ratio are indeed thought to play an important part in this disease.[2–4] The effects of IFNβ observed in the present study in OA chondrocytes and synoviocytes are thus interesting by indicating that not only these two major types of joint cells increase their synthesis of IL1Ra in response to IFNβ but also that this response is conserved during OA. At this time, no treatment compellingly prevents cartilage degradation in OA. IFNβ has been shown to display multiple effects, including a decrease in the synthesis of IL1 and MMPs, which might contribute to cartilage protection.[1] This study, showing that IFNβ also increases IL1Ra synthesis in human articular chondrocytes, further defines it as a molecule potentially available in treatment strategies for cartilage damaging diseases.

The plasma concentrations of IFNβ achieved in vivo during treatment by parenteral injections only range from 12 to 25 IU/ml. The concentrations present in the direct synoviocyte or chondrocyte environment during treatment are unknown, but IFNβ decreases very rapidly from the plasma after an injection; a significant fraction of the molecules is probably directed towards sites of inflammation and bound to receptors. In any event, our results provide information on the possible benefits of delivering IFNβ by intra-articular injections or gene therapy.

In conclusion, human articular chondrocytes, as well as synovial fibroblasts, respond to IFNβ, which increases their production of IL1Ra. In chondrocytes, the effect of IFNβ was seen only when IFNβ was added in combination with IL1β, while in synovial fibroblasts, IFNβ alone dose dependently increased IL1Ra production. These observations suggest that IFNβ may exert beneficial effects in cartilage damaging diseases, by increasing IL1Ra production in joint cells.

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