A High Interleukin 1 Receptor Antagonist/IL-1ß Ratio Occurs Naturally in Knee Osteoarthritis

PASCAL RICHETTE, MATHIAS FRANÇOIS, ERIC VICAUT, CATHERINE FITTING, THOMAS BARDIN, MAÏTÉ CORVOL, JEAN-FRANÇOIS SAVOURET, and FRANÇOIS RANNOU

ABSTRACT.   *Objective.* To assess the interleukin 1 receptor antagonist (IL-1Ra)/IL-1ß ratio in synovial fluid (SF) of patients with knee osteoarthritis (OA) or rheumatoid arthritis (RA) to determine a possible relation between cytokine level and disease activity.

Methods. IL-1ß and IL-1Ra concentrations were measured by ELISA in knee SF from patients with OA (n = 42) or RA (n = 11). For OA patients, pain and disability were assessed by a visual analog scale (VAS) and the Lequesne index. RA disease activity was assessed using the Disease Activity Score 28 Joint Count (DAS28).

Results. Patients with OA showed lower median levels of IL-1ß and IL-1Ra in SF than patients with RA (p < 0.001) but a higher IL-1Ra/IL-1ß ratio: 1793 (584–6221) versus 773.5 (187.64–1570.5) (p = 0.05). For patients with OA, the IL-1Ra/IL-1ß ratio was not associated with pain or disability. For patients with RA, the IL-1Ra/IL-1ß ratio and IL-1Ra and IL-1ß levels were related to SF white blood cell count.

Conclusion. High endogenous IL-1Ra/IL-1ß ratio occurs in SF from knee OA and does not correlate with pain or Lequesne index. Our results suggest that intraarticular injection of IL-1Ra might be self-limited in patients with knee OA and a naturally high SF ratio. (First Release July 1 2008; J Rheumatol 2008;35:1650–4)

Key Indexing Terms:

OSTEOARTHRITIS	SYNOVIAL FLUID	INTERLEUKIN 1
INTERLEUKIN 1 RECEPTOR ANTAGONIST		PAIN

The proinflammatory cytokine interleukin 1ß (IL-1ß) plays a central role in cartilage extracellular matrix degradation in both osteoarthritis (OA) and rheumatoid arthritis (RA)[1]. Activation of cells by IL-1ß occurs through association of the cytokine with a specific cell-surface receptor, IL-1R. Two IL-1R are described: the biologically active IL-1R1 and inert IL-1R2, which functions as a decoy receptor[2]. A third ligand in the IL-1 family is the IL-1 receptor antagonist (IL-1Ra), a structural variant of IL-1ß that binds to both IL-1R with near equal avidity but fails to activate cells[2]. IL-1Ra is produced as 4 different isoforms, 1 secreted (sIL-1Ra) and 3 intracellular (icIL-1Ra1, 2, 3), derived from the same gene[1]. Little is known about the regulation of sIL-1Ra, the main isoform secreted by cartilage cells[3,4]. Several *in vitro* studies have shown that natural inflammatory mediators such as IL-1ß or IL-6[4,5], as well as synthetic compounds such as rosiglitazone[6] and clofibrate[7] or interferon-ß[3], activate IL-1Ra transcription in chondrocytes and macrophages.

In vitro, a 10- to 100-fold excess of IL-1Ra is required to efficiently inhibit IL-1ß activity in chondrocytes[8]. Many *in vivo* animal models of arthritis have revealed that an imbalance between IL-1Ra and IL-1ß may predispose to local tissue destruction (as reviewed[2]). Therefore, novel therapeutic strategies in OA were designed to reinforce local IL-1Ra concentration.

In OA animal models, intraarticular delivery of IL-1Ra slowed disease progression[9,10]. An open-label study in humans revealed the benefit of intraarticular injection of anakinra, a recombinant form of human IL-1Ra, in sympto-

matic knee OA[11]. However, a double-blind, placebo controlled study failed to demonstrate a positive analgesic effect of a single intraarticular injection of anakinra in knee OA at 12-week evaluation, but a significant reduction in pain was observed at Day 4[12]. A plausible explanation for this result could be the short biological half-life of IL-1Ra (4–6 h). Conversely, intraarticular inhibition of IL-1ß with IL-1Ra might not result in symptom improvement because of no association between level of IL-1ß or IL-1Ra or IL-1Ra/IL-1ß ratio in joints and pain during OA.

It has been reported that both IL-1ß and IL-1Ra were present in the synovial fluid (SF) of patients with OA or RA[13-15]. Surprisingly, the relation between the joint IL-1Ra/IL-1ß ratio and clinical outcomes has never been specifically investigated. We aimed to determine the IL-1Ra/IL-1ß ratio in SF of knees of patients with OA or RA and to relate the ratio and cytokine level with disease activity.

MATERIALS AND METHODS

Subjects. SF samples from knees of patients were obtained with appropriate consent from 42 patients with knee OA and 11 patients with RA, who were undergoing arthrocentesis for therapeutic or diagnostic purposes. Knee OA and RA were defined according to the classification of the American College of Rheumatology. For each individual, the following data were collected just before the arthrocentesis: age, sex, body mass index (BMI; kg/m^2), pain [on a visual analog scale (VAS) ranging from 0 to 100 mm], Lequesne index for patients with OA, and Disease Activity Score 28 (DAS28) for patients with RA. Weight-bearing anteroposterior radiographs of knees were obtained for each patient with OA, and were assigned a Kellgren and Lawrence (K&L) grade. Patients with definite chondrocalcinosis were excluded from our study. All patients with RA received low-dose corticosteroids and none was receiving anakinra. Patients with OA were receiving various drug regimens, including analgesics (acetaminophen or dextropropoxyphene) and nonsteroidal antiinflammatory drugs (NSAID). Because of the diversity of medications taken by patients from both groups, we did not attempt to correlate any of the different therapeutic regimens with the measures under study.

Preparation of SF samples for cytokine assays. Following arthrocentesis, SF samples were aliquoted and used to determine total leukocyte (white blood cell; WBC) count. Other samples were centrifuged at 12,000 g for 10 min to remove cells and debris and stored at –80°C for later use.

Measurement of IL-1Ra concentration in SF. As described[16], we used a sensitive and specific enzyme linked immunosorbent sandwich-type assay (ELISA) to measure IL-1Ra protein concentration in SF. We used the anti-IL-1Ra primary antibody IgG1 84-1, a mouse monoclonal antirecombinant human IL-1Ra (Synergen, Boulder, CO, USA). The peroxydase conjugated secondary anti-IL-1Ra antibody was a rabbit polyclonal antibody. The internal standard used was the recombinant human IL-1Ra from the National Institute for Biological Standards and Controls (Potters Bar, UK). Briefly, microtiter plates were coated overnight at 4°C with anti-IL-1Ra primary antibody. The saturation was performed during 1 h at 37°C by use of carbonate buffer with 2% bovine serum albumin. Diluted SF samples (1:10) were incubated for 2 h at 37°C. The secondary antibody was incubated for 90 min at 37°C. After each incubation, microtiter plates were washed 5 times with 0.1% phosphate buffered saline-Tween. Plates were assayed with ortho-phenyl diamine (1 mg/ml) and 0.06% H_2O_2, then stopped with 3 N HCl. The lower limit of sensitivity of the assay was 40 pg/ml. All measurements were performed in duplicate.

Measurement of IL-1ß concentrations in SF. For measurement of IL-1ß

concentrations, ELISA kit was used according to the manufacturer's instructions (Duoset, R & D Systems, Abingdon, UK). The lower limit of detection was 0.1 pg/ml. All measurements were performed in duplicate.

Statistical analysis. Data are means ± SD or medians and quartiles [(median) or (lower-upper quartile)] depending on distribution. Comparisons between the 2 groups of patients involved Student's t-test for age or BMI and Mann-Whitney test for all other measures. Correlations were tested with nonparametric Spearman correlation test. Twelve patients from the OA group were below the threshold of detection of IL-1ß. For these patients, the IL-1Ra/IL-1ß ratio was arbitrarily considered the maximal value of the ratio observed in the OA group. A 2-sided significance level was fixed at 5%. All tests involved use of SAS, version 9.13 (SAS Institute, Gary, IN, USA).

RESULTS

Characteristics of patients with OA and RA. The clinical characteristics of the patients are summarized in Table 1. Patients with OA (68% K&L grade III, 32% K&L grade IV) had a Lequesne index of 11.0 ± 3.8 and a mean pain value of 54 ± 19 on a VAS, whereas patients with RA had a mean DAS28 score of 4.7 ± 2.5. Patients with OA and RA differed significantly in age (66.1 ± 12.6 vs 54.1 ± 20 yrs, respectively; p = 0.02) and sex (women 31 of 42 patients with OA vs 6 of 11 patients with RA), but not BMI (26.7 ± 5.4 vs 24.3 ± 2.8 kg/m^2, respectively).

Measurement of IL-1ß, IL-1Ra, and ratio in SF. Detectable levels of IL-1ß in SF were found in 71% (n = 30/42) of patients with OA and all patients with RA. As expected, IL-1ß was in lower concentrations in the OA group than in the RA group [1.0 (1.0–1.0) vs 24.0 (5.0–77) pg/ml; p < 0.001] (Figure 1A) as was IL-1Ra, detected in all SF samples [614 (292.0–1951.0) vs 16977.0 (12771.0–19469) pg/ml; p < 0.001] (Figure 1B). The patients with OA showed a higher IL-1Ra/IL-1ß ratio than the RA group [1793 (584–6221) vs 773.5 (187.64–1570.5); p = 0.05] (Figure 1C).

Correlation between cytokine levels and clinical measures. In the whole OA group, IL-1Ra/IL-1ß ratio or cytokine levels were not correlated with disability as evaluated by pain on a VAS (rs = 0.01, p = 0.91) or Lequesne index (rs = 0.12, p = 0.42) (Table 2). No correlations were found when analysis was performed separately in patients with OA grade 3 or 4. In the RA group, IL-1Ra but not IL-1Ra/IL-1ß ratio or IL-

Table 1. Characteristics of patients with osteoarthritis (OA) or rheumatoid arthritis (RA).

Characteristics	OA, n = 42	RA, n = 11
Age, yrs	66.1 ± 12.6	54.1 ± 20*
Sex (F/M)	31/11	6/5*
Body mass index, kg/m^2	26.7 ± 5.4	24.3 ± 2.8
Pain, VAS 0–100 mm	54 ± 19	NA
Lequesne index	11 ± 3.8	NA
Kellgren-Lawrence grade (%)	III (68), IV (32)	NA
DAS28 score	NA	4.7 ± 2.5

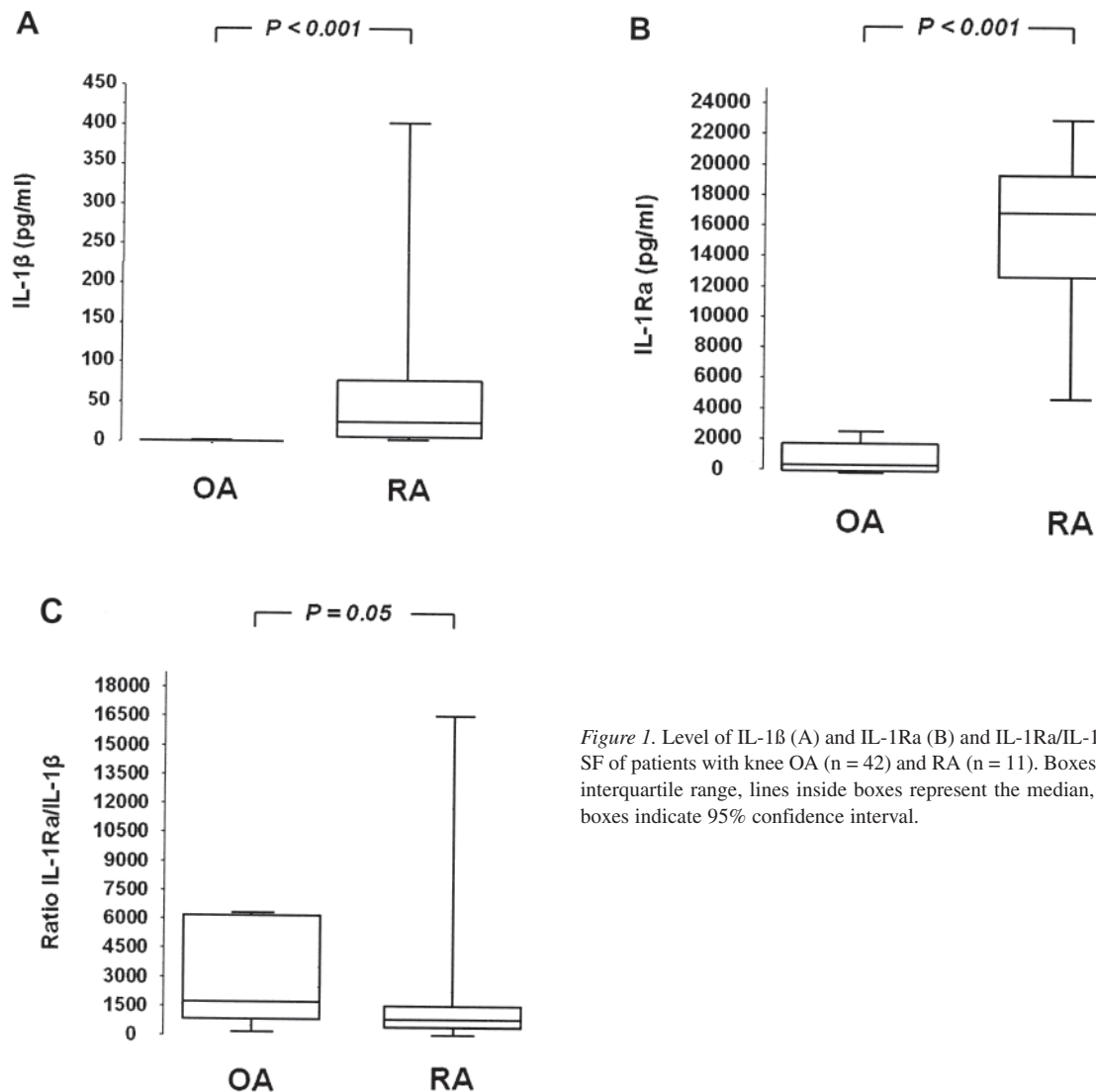Results are expressed as mean ± SD. * p < 0.05. VAS: visual analog scale. NA: not applicable.

Figure 1. Level of IL-1ß (A) and IL-1Ra (B) and IL-1Ra/IL-1ß ratio (C) in SF of patients with knee OA (n = 42) and RA (n = 11). Boxes represent the interquartile range, lines inside boxes represent the median, lines outside boxes indicate 95% confidence interval.

1ß level was correlated with disability as measured by the DAS28 (rs = 0.61, p = 0.05), and total WBC count was correlated with level of IL-1Ra (rs = 0.72, p = 0.01) and IL-1ß (rs = 0.73, p = 0.01) but less so with IL-1Ra/IL-1ß ratio (rs = 0.63, p = 0.04).

DISCUSSION

Our study provides new insight into the pathophysiology of painful knee OA with effusion. We observed a naturally high IL-1Ra/IL-1ß ratio in the OA knee joint and cytokine levels of IL-1ß or IL-1Ra in OA SF not correlated with pain or disability.

IL-1ß is involved in cartilage degradation during OA[17,18]. Hence, IL-1ß inhibition could be an attractive therapeutic target to slow the extracellular matrix degradation characterizing the OA process[19,20]. Initial studies of IL-1Ra therapy in knee OA used an intraarticular route to deliver the receptor antagonist into the knee joint to reach the cartilage lesions[11,12]. The hypothesis that IL-1ß inhibition could alleviate pain of knee OA is empirical and mainly relies on the observation that IL-1ß-stimulated chondrocytes produce a high level of the pain mediator prostaglandin E_2 (PGE_2)[18,20-22].

Inflammation is assumed to play a critical role in processing nociceptive input, both peripherally and centrally. In the presence of inflammation, the input from these nociceptors initially increases[23]. It is likely that a variety of inflammatory cytokines participate in this process, including tumor necrosis factor-α (TNF-α).

However, no accurate data from the literature demonstrate an association between joint levels of IL-1ß and IL-1Ra and OA symptoms in humans. To evaluate the strategy of IL-1ß inhibition for the treatment of painful knee OA, we examined the production of cytokines by surrounding cells and tissues in SF, and found no significant correlation between IL-1Ra/IL-1ß ratio or concentration of both

Table 2. Correlations between IL-1Ra/IL-1ß ratio, level of IL-1Ra and IL-1ß, WBC count, and disease activity in patients with osteoarthritis (OA) or rheumatoid arthritis (RA)*.

	OA, n = 42		RA, n = 11	
	r_s	p	r_s	p
IL-1Ra/IL-1ß ratio and				
Pain (VAS 0–100 mm)	0.01	0.91	NA	NA
Lequesne index score	0.12	0.42	NA	NA
DAS28 score	NA	NA	0.62	0.06
WBC count	0.24	0.11	0.63	0.04[†]
IL-1Ra level and				
Pain (VAS 0–100 mm)	–0.01	0.92	NA	NA
Lequesne index score	0.16	0.28	NA	NA
DAS28 score	NA	NA	0.61	0.05[†]
WBC count	0.17	0.25	0.72	0.01[†]
IL-1ß level and				
Pain (VAS 0–100 mm)	–0.19	0.29	NA	NA
Lequesne index score	0.008	0.96	NA	NA
DAS28 score	NA	NA	0.68	0.06
WBC count	0.01	0.91	0.73	0.01[†]

* By nonparametric 2-tailed Spearman rank-order correlation. NA: not applicable; SF: synovial fluid; WBC: white blood cell; IL-1ß: interleukin 1ß; IL-1Ra: interleukin 1 receptor antagonist. DAS: Disease Activity Score; VAS: visual analog scale. [†] Statistically significant.

cytokines and clinical outcomes in patients with knee OA. Previous studies also failed to demonstrate a correlation between levels of IL-1ß, TNF-α, nitric oxide, or PGE_2 in synovium or SF and symptoms in knee OA[24-26]. These data might suggest that pain in knee OA is primarily provoked not solely by inflammation, as evaluated by the levels of these proinflammatory mediators, but rather by different mechanisms involving other tissues such as subchondral bone[23,27]. In our patients with severe OA (K&L grade IV), it is possible that part of the pain originated from subchondral bone. Indeed, the origin of pain in OA is poorly understood and the discordance between radiographic OA and the occurrence of knee pain is well documented[28]. In cross-sectional studies of magnetic resonance imaging (MRI) in knee OA, bone marrow lesions, periarticular lesions, knee effusions, and, to a lesser extent, synovitis, are more often present in people with knee pain than in those without pain[23,29].

We found elevated IL-1ß and IL-1Ra concentrations, approximately 20 and 25 times greater, respectively, in SF of patients with RA than in patients with OA. IL-1Ra levels in SF of patients with OA (median 614 pg/ml) or RA (median 16,977 pg/ml) were much higher than the circulating levels found in previous studies of both diseases or in the general population. For example, the serum concentration of IL-1Ra was approximately 300 to 400 pg/ml in active RA[30,31] and about 100 to 150 pg/ml in patients with OA or in healthy subjects[32,33]. As well, IL-1Ra concentration in SF was correlated with total WBC count in patients with RA but not in patients with OA. Our data suggest that IL-1Ra is synthesized locally in part in OA and RA joints and that WBC could be an important source of IL-1Ra within the rheumatoid joint[15].

A large molar excess (10- to 100-fold) of IL-1Ra is required to efficiently inhibit the IL-1ß effect *in vitro*. In animal studies, a 100- to 1000-fold molar excess of IL-1Ra is necessary to block a systemic response to IL-1ß[2,13]. In our study, a high IL-1Ra/IL-1ß ratio (median 1793) occurred naturally in SF of patients with OA at an advanced stage of the disease. This result suggests that the degradation of cartilage matrix occurred despite a balance in favor of the receptor antagonist. Hence, endogenous inhibition of IL-1ß by IL-1Ra might not be sufficient to abrogate the cartilage degradation during late OA. However, we did not explore the ratio in SF from patients with early OA, and our data obviously do not preclude that therapeutic inhibition of IL-1ß could slow the structural progression of knee OA and may benefit patients with a low ratio.

We also found a higher IL-1Ra/IL-1ß ratio in SF of patients with OA than in those with RA. Low-dose corticosteroids in patients with RA might be enough to trigger this ratio difference. Indeed, dexamethasone can decrease IL-1Ra production by stimulated chondrocytes[4].

Our work has several limitations. Because of the small sample sizes, we may have missed some weak associations, and we thus cannot establish definite conclusions. In addition, the cross-sectional design of our study precluded addressing whether the assessed concentration of cytokines predicted change in pain in patients with OA.

Our findings reveal not only an absence of correlation between pain and IL-1ß level in SF but also the pre-existence of a high IL-1Ra/IL-1ß ratio in patients with OA. This suggests that intraarticular injection of IL-1Ra in knee OA might be self-limited in patients with a naturally high ratio in SF.

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