

Association of the Risk of Osteoarthritis With High Innate Production of Interleukin-1 β and Low Innate Production of Interleukin-10 Ex Vivo, Upon Lipopolysaccharide Stimulation

Naghmeh Riyazi,¹ Eline Slagboom,¹ Anton J. M. de Craen,¹ Ingrid Meulenbelt,¹ Jeanine J. Houwing-Duistermaat,¹ Herman M. Kroon,¹ Dirkjan van Schaardenburg,² Frits R. Rosendaal,¹ Ferdinand C. Breedveld,¹ Tom W. J. Huizinga,¹ and Margreet Kloppenburg¹

Objective. In a sibpair study of osteoarthritis (OA) patients, we investigated whether, upon stimulation with lipopolysaccharide (LPS), variations in the innate ex vivo production of interleukin-1 β (IL-1 β), IL-1 receptor antagonist (IL-1Ra), IL-10, and tumor necrosis factor α (TNF α) in whole-blood assays contribute to the risk of OA.

Methods. Data from 305 patients with OA at multiple sites (hand, knee, hip, and spine), whose median age was 60 years (range 43–79 years), were compared with those from 137 controls. OA was defined in accordance with the American College of Rheumatology criteria. Whole-blood samples were stimulated with LPS (10 ng/ml). In the supernatants, cytokines were measured by enzyme-linked immunosorbent assay. Odds ratios (ORs) were used as measures of the relative risk of OA in relation to quartiles of IL-1 β , IL-1Ra, TNF α , and IL-10 production. The ORs were adjusted for sex and age, and 95% confidence intervals (95% CIs)

were computed using robust standard errors to take into account the intrafamily effect.

Results. Subjects in the highest quartile of IL-1 β and IL-1Ra had an increased risk of OA (OR 3.3, 95% CI 1.4–7.9 and OR 8.0, 95% CI 3.7–17.4, respectively), while subjects in the lowest quartile of IL-10 had a 3-fold increased risk of OA (OR 3.1, 95% CI 1.5–6.5). High innate ex vivo production of TNF α was not associated with an increased risk of OA.

Conclusion. Subjects with a high innate ex vivo production of IL-1 β and IL-1Ra and low innate ex vivo production of IL-10 have an increased risk of OA. These results suggest that a proportion of the genetic susceptibility to OA may be encoded for by variations in innate cytokine activity.

Osteoarthritis (OA) is the most frequently occurring joint disease and is an important cause of pain and disability in the general population. The hereditary basis of this disease has become apparent from studies of familial aggregation as well as studies of twins. These studies have shown that a considerable fraction of variation in disease susceptibility can be explained by genetic factors (1). Nonetheless, despite numerous genome-wide scans and association studies that have identified many of the linkage regions, it remains unclear which genes are involved (2).

In recent years, there has been increasing interest in the role of cytokines as mediators of joint damage and inflammation in the pathogenesis of OA. The most direct observation linking OA and inflammation comes from studies in which an association was found between progression of structural joint changes and the presence of synovitis and inflammation in patients with OA (3).

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¹Naghmeh Riyazi, MD, Eline Slagboom, PhD, Anton J. M. de Craen, PhD, Ingrid Meulenbelt, PhD, Jeanine J. Houwing-Duistermaat, PhD, Herman M. Kroon, MD, PhD, Frits R. Rosendaal, MD, PhD, Ferdinand C. Breedveld, MD, PhD, Tom W. J. Huizinga, MD, PhD, Margreet Kloppenburg, MD, PhD: Leiden University Medical Center, Leiden, The Netherlands; ²Dirkjan van Schaardenburg, MD, PhD: Jan van Breemen Institute, Amsterdam, The Netherlands.

Address correspondence and reprint requests to Margreet Kloppenburg, MD, PhD, Leiden University Medical Center, Department of Rheumatology, C4-R, PO Box 9600, 2300 RC Leiden, The Netherlands. E-mail: G.Kloppenburg@lumc.nl.

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In OA, synovitis is believed to be a reactive process as a result of cartilage destruction and the release of cartilage-degradation products in the synovial fluid (4). Loss of cartilage matrix results from the secretion of enzymes by chondrocytes in response to catabolic cytokines produced within the joint (5). These cytokines are most likely released by cells in the synovial fluid and synovial tissue, as well as by chondrocytes. In addition to proinflammatory cytokines, antiinflammatory cytokines are also present in OA joint tissues. The balance among these cytokines may determine the severity of cartilage damage in OA (6).

Of the proinflammatory cytokines, interleukin-1 β (IL-1 β) and tumor necrosis factor α (TNF α) are the most prominent in OA. These cytokines have been shown in vitro to be expressed and to stimulate the production of other cytokines, such as IL-8, IL-6, and leukemia inhibitory factor, as well as their own production, leading to accelerated damage of articular tissue (5). Studies in animal models suggest that cartilage-destructive processes are mainly driven by IL-1 β , whereas TNF α is involved in the early stages of inflammation (7).

IL-1 receptor antagonist (IL-1Ra) is a competitive inhibitor of the IL-1 receptor, and its production rises in response to IL-1 β production. Although an excess level of IL-1Ra is found in OA articular tissue, it has been shown, in in vitro experiments, that a large amount of IL-1Ra is necessary to inhibit IL-1 β activity (5). Additional data supporting the role of IL-1 β in OA susceptibility is provided by genetic studies in OA that have demonstrated linkage on the 2q chromosome, which encompasses the ligand gene cluster at 2q12–q13, composed of *IL1B*, *IL1A*, and *IL1RN* (8–10).

A number of cytokines, such as IL-4, IL-10, and IL-13, have been shown in vitro to inhibit the activity of proinflammatory cytokines (11). Furthermore, these antiinflammatory cytokines have been found in increased levels in the synovial fluid of OA patients (5). Thus, the metabolic state of chondrocytes is under the influence of a complex network of cytokines. However, in OA, the exact roles of cytokines other than IL-1 β and TNF α in the activation and modulation of possible cascade reactions have not yet been clearly established (11).

Studies of twins have shown that the ex vivo production of cytokines induced by lipopolysaccharide (LPS) in whole-blood samples varies by 60–75% on the basis of heritability alone (12), and that subjects may be characterized as high or low producers of these cytokines (13). Polymorphisms in the promoter region of IL-1 β and IL-10 have been associated with variation in

the production of cytokines (14,15). Furthermore, variation in innate ex vivo cytokine production upon LPS stimulation has been associated with the susceptibility to and severity of diseases such as systemic lupus erythematosus (SLE) (16).

Given the role of cytokines in OA pathophysiology and the genetic variation of ex vivo cytokine production upon LPS stimulation in humans, the question arises whether genetic variation in cytokine production contributes to OA susceptibility. Therefore, in a cohort of patients with familial symptomatic OA at multiple sites, we investigated whether variations in the ex vivo production of IL-1 β , IL-1Ra, IL-10, and TNF α upon LPS stimulation, measured in whole-blood assays, contribute to the risk of OA.

PATIENTS AND METHODS

Patient population. The present study is part of the ongoing Genetics, Arthrosis and Progression (GARP) study. The GARP study is aimed at the identification of determinants of OA susceptibility and progression, and involves Caucasian sibpairs of Dutch ancestry. The population predominantly comprises individuals with symptomatic OA at multiple sites.

Recruitment and clinical evaluation. The index patients (probands; $n = 1,874$), ages between 40 and 70 years, had symptomatic OA in the hands, knees, or hips, which was diagnosed by rheumatologists, orthopedic surgeons, and general practitioners in Leiden, The Hague, Delft, Haarlem, and Amsterdam, The Netherlands. Patients were informed of the study by mail. Of the 1,874 patients, 833 interested probands were sent a questionnaire to obtain demographic data, medical history, symptoms and signs of OA, and family history of OA. Subsequently, eligible probands with a positive family history of OA in first-degree relatives ($n = 521$) were requested to introduce a sibling who had “joint complaints,” and this sibling was also asked to complete a mailed questionnaire. Of these probands, 353 had at least 1 sibling with “joint complaints.” One hundred thirty-nine of these siblings either did not meet the GARP criteria ($n = 47$) or were unwilling to participate ($n = 92$), resulting in 214 eligible sibpairs.

After we obtained their informed consent, eligible sibpairs were invited to the outpatient clinic to undergo physical and radiographic examinations, provide blood and urine samples for assessment of biomarkers and DNA, and complete standardized questionnaires. All sibpairs were examined by one medical doctor (NR). Questionnaires were verified and data were collected on physical functioning and quality of life. Of the 214 sibpairs, 191 were included in the GARP study from August 2000 to March 2003.

Exclusion criteria. Patients with secondary OA and familial syndromes with a Mendelian inheritance pattern were excluded. Conditions that were considered to be secondary OA were 1) major congenital or developmental diseases and bone dysplasias, 2) major local factors such as severe scoliosis and hypermobility, 3) certain metabolic diseases associated with joint disease such as hemochromatosis and Wilson’s disease, 4)

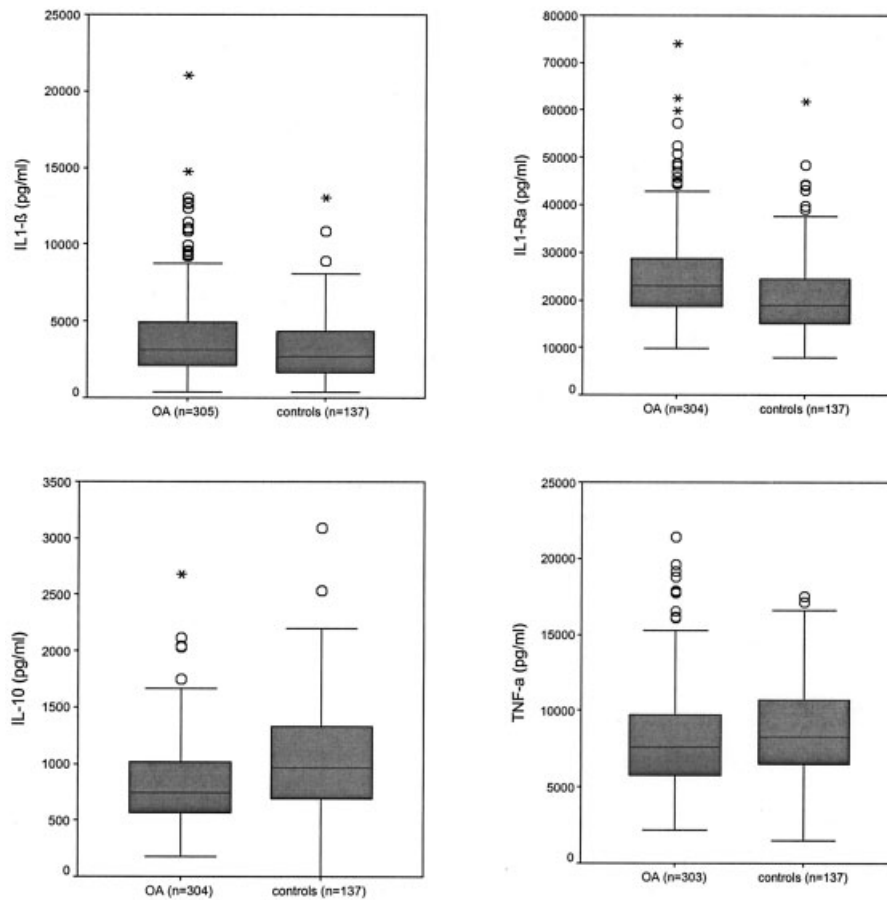


Figure 1. Ex vivo production of interleukin-1 β (IL-1 β), IL-1 receptor antagonist (IL-1Ra), IL-10, and tumor necrosis factor α (TNF- α) upon lipopolysaccharide stimulation in patients with osteoarthritis (OA) and in healthy controls. Data are presented as box plots, where the boxes represent the 25th to 75th percentiles, the lines within the boxes represent the means, and the lines outside the boxes represent the 5th and 95th percentiles. Asterisks indicate outliers.

inflammatory joint diseases such as rheumatoid arthritis (RA), 5) other bone diseases such as osteitis deformans and osteochondritis, and 6) intraarticular fractures. Patients with a shortened life expectancy were also excluded. Crystal deposition arthropathies (except in the case of severe polyarticular gout), diabetes mellitus, or thyroid conditions were not considered as exclusion criteria.

OA diagnosis. For the present study, all patients with symptomatic OA at multiple sites were included. Symptomatic OA at multiple sites was defined as the presence of OA symptoms in the hand joints or 2 or more of the following sites: hands, spine (cervical or lumbar), knee, or hip.

Symptomatic OA in the hand joints was defined according to the American College of Rheumatology (ACR) criteria (17), which requires the presence of pain or stiffness in the hand joints on most days of the prior month, in addition to 3 of the following 4 criteria: bony swelling of ≥ 2 of the 10 selected joints (second and third distal interphalangeal [DIP] joints bilaterally, second and third proximal interphalangeal [PIP] joints bilaterally, and first carpometacarpal [CMC1]

joint), bony swelling of ≥ 2 DIP joints, < 3 swollen metacarpophalangeal (MCP) joints, and deformity of at least 1 of the 10 selected joints. Symptomatic OA in the knee and hip was defined in accordance with the ACR recommendations for knee and hip OA (18,19). Knee OA was defined as pain or stiffness in the knee joints for most days of the prior month, and osteophytes at joint margins of the tibiofemoral joint (radiographic spurs). Hip OA was defined as pain or stiffness in the groin and hip region on most days of the prior month, in addition to femoral or acetabular osteophytes or joint space narrowing on radiographic examination. The presence of joint prostheses in the hips or the knees as a result of end-stage OA was included as OA in that particular joint. Spine OA (cervical and lumbar) was defined as pain or stiffness in the spine on most days of the prior month, in addition to a Kellgren/Lawrence (K/L) score of 2 in at least 1 disc or 1 apophyseal joint.

Radiographs. Conventional radiographs of the hands (dorsovolar view), knees (posteroanterior [PA] and lateral views of weight-bearing/semiflexed knees), hips (PA view),

lumbar (PA and lateral views), and cervical spine (anteroposterior, lateral, and transbuccal views) were obtained for all participants. This was performed in a standard manner with a fixed film-focus distance and a fixed joint position. All radiographs were obtained by a single experienced radiology technician. Conventional radiographs were scored by a single experienced musculoskeletal radiologist (HK) for osteophytes in the knees and hips and joint space narrowing in the hips.

In addition to the hands (DIPs, PIPs, and CMC1) and the discs and apophyseal joints of the cervical and lumbar spine, the hips and tibiofemoral joints of the knees were also scored according to the K/L scale, utilizing the original atlas (20). The K/L scale is a 5-point scoring system that rates the severity of OA in an ascending manner, based on the presence of osteophytes, joint space narrowing, sclerosis, and degenerative cysts. A K/L score of ≥ 2 defines disease severity in a particular joint.

Controls. For comparison, blood samples from 137 healthy control individuals were used. The control subjects belonged to 54 families who had participated as controls in a previous study on multiple sclerosis and SLE, and were spouses and first-degree relatives of the patients with SLE or multiple sclerosis (16).

Whole-blood stimulation system. Whole-blood sample stimulation was performed as previously described (21). Briefly, blood samples were collected in pyrogen-free heparinized tubes (Endotube; Chromogenix, Mölndal, Sweden). Eight-milliliter whole-blood samples were diluted 1:1 with RPMI 1640 (Gibco Life Technologies, Paisley, UK) and stimulated with 10 ng/ml *Escherichia coli* LPS (Difco, Detroit, MI). To minimize the influence of circadian rhythms and measurement errors, blood samples were obtained between 8:00 AM and 11:00 AM, the time frame between blood collection and stimulation was <1.5 hours, and all stimuli were performed in the same endotoxin batch. The simultaneous measurement of all samples was possible, since spare samples from controls had been stored. One medium-diluted blood sample without LPS was used as a negative control. After 4-hour and 24-hour incubations, samples were centrifuged twice (at 600g) and the supernatants were stored at -70°C . TNF α was measured in 4-hour samples and IL-1 β , IL-1Ra, and IL-10 production was measured in 24-hour samples in one batch, by enzyme-linked immunosorbent assay according to the manufacturer's guidelines (Central Laboratory of the Blood Transfusion Service, Amsterdam, The Netherlands).

Statistical analysis. The difference in the innate ex vivo production (mean levels) of IL-1 β , IL-1Ra, TNF α , and IL-10 between the patients and the controls was calculated by linear mixed models with a random intercept to adjust for the familial effect within the sibpairs. Since the ex vivo production of cytokines was not normally distributed, a log transformation was performed. Estimates of fixed effects were reported with 95% confidence intervals (95% CIs), which excluded the value of 1 in case of statistical significance. The estimates represent the magnitude of the difference in the mean innate ex vivo production of IL-1 β , IL-1Ra, TNF α , and IL-10 between the patients and the controls.

Odds ratios (ORs), as measures of relative risk, were calculated using logistic regression to adjust for age and sex. To take into account the intrafamily effect, robust standard errors

were computed using the statistical program Stata, version 7.0 (Stata, College Station, TX). The ORs are presented with 95% CIs. Cytokines were divided into quartiles of production levels, based on the distribution in controls. Since pro- and anti-inflammatory cytokines act antagonistically, the modifying effect of one cytokine on the other was estimated by analyzing the effect of IL-1 β and IL-1Ra simultaneously and of TNF α and IL-10 simultaneously, using multivariate logistic regression models.

RESULTS

Of the 191 sibpairs included in the GARP study, 156 had symptomatic OA at multiple sites and were included in the present study. Seven patients were excluded from the analyses because no whole-blood samples had been obtained from these patients. Of the remaining 305 patients, levels of IL-1Ra and IL-10 were missing for 1 patient each and levels of TNF α were missing for 2 patients due to technical difficulties.

The characteristics of the 305 patients in the present study are shown in Table 1. The median age of the patients was 60 years (range 43–79 years) and the majority of the patients (82%) were women. Hand OA and spine OA were the most common types of OA in this patient population. Of the 248 patients with OA affecting multiple sites in the hands, 37 did not have symptomatic OA at other sites, although 35 of the 37 also had radiographic OA at other sites. Of the 246 patients with the diagnosis of spine OA, the apophyseal joints of 240 patients were affected by OA, irrespective of disc degeneration. The median age of the controls was 50 years (range 12–83 years) and 55% of the controls were women.

The mean innate ex vivo production of IL-1 β was 1.15 pg/ml higher in patients than in controls (95% CI 1.06–1.25). For IL-1Ra, the mean ex vivo production was 1.12 pg/ml higher in patients than in controls (95% CI 1.07–1.17). The mean ex vivo production of IL-10 was 1.04 pg/ml lower in patients (95% CI 0.90–14.9) and the mean ex vivo production of TNF α was 0.98 pg/ml lower in patients (95% CI 0.93–1.04) than in controls.

Table 1. Characteristics of the 305 patients with familial symptomatic osteoarthritis (OA) at multiple sites

Women, no. (%)	251 (82)
Age, median (range) years	60 (43–79)
Body mass index, median (range) kg/m ²	26 (19–46)
Subtypes of symptomatic OA, no. (%)	
Hand	248 (81)
Spine	246 (81)
Hip	76 (25)
Knee	107 (35)

Table 2. Risk of having OA in relation to quartiles of innate ex vivo production of IL-1 β , IL-1Ra, IL-10, and TNF α upon lipopolysaccharide stimulation in whole-blood assays*

Cytokine, quartiles (pg/ml)	No. in quartile		Adjusted OR (95% CI)†
	OA patients (n = 305)	Controls (n = 137)	
IL-1β			
397–1,589	45	34	1.0
1,590–2,724	80	35	1.7 (0.9–3.4)
2,725–4,392	92	34	3.2 (1.5–6.6)
4,393–21,019	88	34	3.3 (1.4–7.9)
IL-1Ra			
7,908–15,002	25	34	1.0
15,003–18,942	53	35	2.3 (1.0–5.4)
18,943–24,445	98	34	5.4 (2.1–14.2)
24,446–73,932	128	34	8.0 (3.7–17.4)
IL-10			
4–689	130	34	1.0
690–967	81	34	0.74 (0.4–1.5)
968–1,331	65	35	0.73 (0.3–1.5)
1,332–3,088	28	34	0.32 (0.2–0.7)
TNFα			
1,534–6,462	107	34	1.0
6,463–8,309	74	34	0.62 (0.3–1.2)
8,310–10,722	62	35	0.93 (0.4–2.0)
10,723–21,405	60	34	0.58 (0.3–1.2)

* Levels of interleukin-1 receptor antagonist (IL-1Ra), IL-10, and tumor necrosis factor α (TNF α) were missing for 1 patient, 1 patient, and 2 patients, respectively, due to technical difficulties. OA = osteoarthritis.

† Odds ratios (ORs) are adjusted for age and sex. The 95% confidence intervals (95% CIs) are based on robust standard errors to take into account the intrafamily effect.

Table 2 shows the risk of having OA according to quartiles of IL-1 β , IL-1Ra, TNF α , and IL-10 production. Subjects in the highest quartile of IL-1 β production had a 3-fold increased risk of having OA (OR 3.3, 95% CI 1.4–7.9), and subjects in the highest quartile of IL-1Ra production had an 8-fold increased risk of OA (OR 8.0, 95% CI 3.7–17.4), both in comparison with those in the lowest quartile. Furthermore, a trend toward an increased risk of OA was seen with increasing ex vivo production of IL-1 β and IL-1Ra.

Subjects in the highest quartile of IL-10 production had a decreased risk of OA (OR 0.32, 95% CI 0.2–0.7). When high IL-10 production served as the reference, based on data showing that a low innate production of IL-10 predisposes individuals to an inflammatory state, a trend toward an increased risk of OA was observed with decreasing ex vivo IL-10 production, in comparison with individuals in the highest quartile (second quartile OR 2.1, 95% CI 1.0–4.6, third quartile OR 2.4, 95% CI 1.1–5.4, fourth [lowest] quartile OR 3.1, 95% CI 1.5–6.5). High innate ex vivo production

of TNF α was not associated with an increased risk of OA.

In order to assess the independent effect of each cytokine, IL-1 β and IL-1Ra together and TNF α and IL-10 together were analyzed using multivariate models. These analyses did not materially affect the estimates found in the univariate model (data not shown).

DISCUSSION

Subjects with high innate ex vivo production of IL-1 β and IL-1Ra and low IL-10 production upon LPS stimulation had an increased risk of familial OA at multiple sites. High innate ex vivo production of TNF α did not increase the risk of OA.

IL-1 β contributes to the pathogenesis of OA by several pathways. In vitro, human articular chondrocytes, when stimulated by IL-1 β , dramatically increase the expression of matrix-degrading proteinases, such as matrix metalloproteinases (MMPs), and induce the production of nitric oxide, a catabolic mediator in cartilage (22). In addition, IL-1 β inhibits the biosynthesis of cartilage proteoglycans and collagens and leads to the synthesis of other inflammatory cytokines (11). The biologic activity of IL-1 β in OA tissue is further enhanced by the increased number of type I IL-1 receptors on OA chondrocytes and synovial fibroblasts, which may account for the increased susceptibility of OA cartilage to degradation by IL-1 β when compared with similar cartilage from subjects without arthritis (23). OA animal models provide further support for the role of IL-1 β in OA. In experimental models, intraarticular injections of IL-1Ra have been shown to block IL-1 β action and reduce OA progression (24). Furthermore, the in vivo transfection of the IL-1Ra gene into the OA knee joints of dogs or rabbits was shown to be successful in reducing the progression of OA lesions (25).

It has been suggested that IL-1Ra is released from cells along with IL-1 in response to the same stimulus and is thus present at high levels in OA patients (4,26). In the present study, we found that patients with a high innate ex vivo production of IL-1 β as well as IL-1Ra had an increased risk of OA. The simultaneous presence of an increased ability to produce IL-1Ra and IL-1 β indicates an increased responsiveness, possibly through a more sensitive IL-1 β and IL-1Ra gene activation route in this disease. Since the risk of OA in our study was associated with an independent innate production of both cytokines, genetic variants in both loci may contribute to this effect. A direct effect of the IL-1 gene cluster has been suggested by various studies reporting a

positive linkage to chromosome 2q12–q14, containing the IL-1 cluster, and OA (8–10). Leppävuori et al, for example, found linkage in this region with OA in the DIP joints (8). Two other groups of investigators found an association between the IL-1 gene cluster and the occurrence of end-stage symptomatic OA among patients who underwent joint replacement of the knee or hip (9,27), and most recently, Meulenbelt and associates found a predisposition for radiographic hip OA in carriers of the *IL1B* –211T allele and the *IL1RN* variable-number tandem repeat allele 2 (10).

In the present study, a high innate production of TNF α did not increase the risk of OA. Experiments in animal models of arthritis have shown that TNF α and IL-1 β have separate activities. These studies have shown that TNF α injections in rodents significantly reduce mononuclear cell infiltration in the joint, and that TNF α is substantially less important than IL-1 β in cartilage destruction (7). The distinct effects of TNF α and IL-1 have been shown in the streptococcal cell wall arthritis model. In this model it was shown that blocking IL-1 with antibodies produced little or no suppression of inflammation, but led to the normalization of the chondrocyte synthetic function. This was in contrast to TNF α blockade, which led to an efficient reduction in swelling but did not have an effect on chondrocyte synthetic function (28).

In our study, we also found a higher risk of OA in subjects with a low ex vivo production of IL-10. Anti-inflammatory cytokines such as IL-10 have been reported to be synthesized in increased amounts either spontaneously by synovial membrane and cartilage (4) or after stimulation of chondrocytes with IL-1 β or TNF α (11). Experiments in synovial tissue cultures have shown that IL-10 inhibits the production of TNF α and IL-1 β and decreases the levels of MMPs produced by macrophages and fibroblast-like synoviocytes (29). Although the anti-inflammatory effects of IL-10 have been demonstrated in RA, its role in OA is still unclear. Based on experimental data in arthritis models, it is, however, conceivable that a low innate production of IL-10 can contribute to the catabolic state found in OA. Furthermore, the low innate IL-10 production can be a direct effect of genetic variation at the IL-10 locus (12).

In OA, inflammation is believed to be important in the development and progression of disease (3,4). Although synovial inflammation in OA is often believed to be reactive to cartilage breakdown products in synovium (4,11), a recent study investigating synovial tissue samples from patients with early knee OA has shown a greater mononuclear cell infiltration and hyperplasia of

the synovial lining than in late OA, suggestive of deregulation of inflammatory pathways in early stages of this disease (30). This finding implicates the role of cytokines in early stages of disease development, even before extensive damage to the cartilage has taken place.

In the present study, ex vivo production of cytokines in whole-blood assays was used to classify subjects as high or low producers. In whole blood, higher values of cytokine production and smaller individual variation were found in comparison with production by peripheral blood mononuclear cells (PBMCs) (31). The difference in the level of ex vivo cytokine production in whole-blood assays as compared with PBMC assays is suggested to be related to the isolation processes. The methods used to separate PBMCs modify lymphocyte: monocyte ratios, resulting in a substantial reduction in monocyte concentrations. The modified lymphocyte: monocyte ratios in isolated PBMCs may thus affect qualitative and quantitative production of cytokines. In whole-blood stimulation, not only are natural cell-to-cell interactions preserved, but also circulating stimulatory and inhibitory mediators are present at their normal concentration (32). A previous study by Fraenkel and associates (33) investigated the association of OA with PBMC production of IL-1 β , IL-1Ra, and TNF α upon LPS stimulation. The investigators failed to detect an increased production of these cytokines in 703 subjects with radiographic OA in the knees or the hands. The discrepancy between these findings and the results in the present study may thus be a reflection of the difference in the assays that were used and the different phenotype definitions that would possibly lead to selection of patient groups enriched for different genetic effects.

In order to minimize measurement errors, samples from patients and controls in this analysis were measured on the same day and with the same batch of reagents, although it has been shown that the misclassification of subjects as high or low cytokine producers merely on the basis of laboratory error is unlikely (21). All analyses in the present study were performed with correction for age and sex, since the patients in the present study were considerably older than the controls and consisted of more women. The LPS-induced production of cytokines has been shown to be sex- and age-specific (34).

The response rate in the GARP study was low, at 44%. This may be attributed partly to the recruitment procedure. In order to recruit probands who met both the criterion of multiple sites affected and the criterion of having a family history of OA, a very broad approach to patient recruitment was undertaken. OA patients

from participating centers who had knee, hip, or hand involvement were approached by mail to inform them of the study, including its familial aspect. It is conceivable that probands without a familial history of OA did not respond. This hypothesis is further supported by a higher response rate of 70% of the siblings.

It is unlikely that the selection procedure biased our results. In the present study, the innate *ex vivo* production of cytokines was investigated in patients with an endogenous susceptibility to OA, marked by a clear family history of this disease and involvement of multiple anatomic sites. Although patients were recruited from different clinical settings and the multiple-site involvement included the combination of different joint sites with OA, we believe that this phenotype is a homogeneous one. A separate analysis of the patient population (results not shown) showed that the *ex vivo* production of the cytokines under study in patients with hand involvement was not materially different from that among patients with knee or hip involvement. Furthermore, we did not observe an association between OA severity, as defined by the total K/L score and the number of joint sites affected, and the *ex vivo* cytokine production (results not shown).

The prevalence of OA among the controls in the present study was unknown. However, one can speculate what proportion might have had OA based on the prevalence of familial symptomatic OA at multiple sites. Although the exact prevalence of this phenotype has not been established in the population, a prevalence of <14% seems realistic. This estimation is based on data from the Rotterdam population-based study (1), in which generalized radiographic OA has been reported to occur in 14% of the population ages 55–65 years (Slagboom E: personal communication). We expect that the proportion of persons with OA included in the GARP study is lower due to the extra requirements for inclusion in the GARP study, which were the presence of symptoms and the familial nature of OA.

The present study results suggest that an innate variation in the production of IL-1 β , IL-1Ra, and IL-10 independently contributes to OA susceptibility in familial cases. Furthermore, these results complement data from *in vitro* studies and animal models that implicate IL-1 β as the prominent cytokine involved in the cartilage-destructive process, and stimulate research into treatment possibilities with structure modifiers such as IL-1 inhibitors (e.g., diacerein) (35).

Based on the results of the present study, further investigation of the role of IL-10, functional IL-10 polymorphisms, as well as IL-1 β and IL-1Ra in OA is

warranted. Whether these results can be generalized to other OA populations needs to be assessed in future studies, since the results in the present study are based on a patient population with a specific phenotype, comprising symptomatic OA, radiographically confirmed OA at multiple sites, and a clear familial predisposition for this disease.

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