Enhanced Concentrations of Interleukin 16 Are Associated with Joint Destruction in Patients with Rheumatoid Arthritis

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ABSTRACT. ***Objective.*** To investigate the role of interleukin 16 (IL-16) in the development of rheumatoid arthritis (RA) and joint destruction.

Methods. We measured systemic IL-16 levels longitudinally in 39 patients with recent-onset RA, in 13 patients with initially undifferentiated arthritis who will develop RA over time, in 15 patients with undifferentiated arthritis, and in 12 healthy controls, and correlated the levels with joint damage and disease activity. Systemic IL-16 levels were measured by ELISA. Joint destruction was measured according to the Sharp method and the disease activity variables included C-reactive protein (CRP) and Disease Activity Score (DAS) measured at disease onset, and after one and 2 years of followup.

Results. A significantly increased IL-16 level in RA patients at disease onset [median (25th-75th percentile) 45.2 (37.7–82.4) pg/ml] was observed compared to both controls [30.4 (24.4–37.0) pg/ml, p = 0.0008], and to patients with undifferentiated arthritis [29.0 (21.5–52.4) pg/ml; p = 0.005]. The IL-16 levels of the patients who presented with undifferentiated arthritis but who developed RA over time were also increased [47.9 (34.5–108.2) pg/ml] compared to the controls (p = 0.004) and to the patients who over time remained diagnosed with undifferentiated arthritis (p = 0.01). We found that high IL-16 levels correlated positively with joint destruction during the 2-year followup (p = 0.02) and not with the disease activity variables.

Conclusion. Our results suggest that the cytokine IL-16 plays a role in the disease process underlying RA and joint destruction. (J Rheumatol 2004;31:35–9)

Key Indexing Terms:
INTERLEUKIN 16   RHEUMATOID ARTHRITIS   JOINT DAMAGE

Rheumatoid arthritis (RA) is a chronic autoimmune disease of unknown etiology. The rheumatoid synovium is characterized by an infiltration of T cells, macrophages, B cells, and proliferating fibroblasts[1], which eventually will lead to a chronic joint inflammation and joint destruction.

Interleukin 16 (IL-16), originally named lymphocyte chemoattractant factor, is a cytokine produced by, among others, T cells. IL-16 induces chemotaxis of CD4+ T cells, monocytes, and eosinophils, and also functions as a modulator of T cell activation[2]. In 2 cross-sectional studies[3,4], systemic IL-16 concentrations in patients with longstanding RA have been observed to be significantly elevated compared to controls without RA. Franz, *et al*[5] found highly increased levels of IL-16 in synovial fluid of patients with RA, which were higher than the systemic levels of IL-16 in these patients. They also observed that the synovial fibro-

blasts were a source of IL-16 using *in situ* hybridization experiments. Altogether these results indicate that synovial fibroblasts are a major source of IL-16 in RA and that the enhanced systemic levels most likely reflect the disease process in the joints.

One of the central questions in RA is why the disease becomes chronic. Studies on infiltrates of the synovial tissue have indicated that the composition of the infiltrate between early RA and other forms of arthritis differs[6]. There is an especially increased infiltrate of plasma cells, B cells, and macrophages in the synovial lining in patients with early RA. As IL-16 involvement is crucial in the chemotaxis of several cell types present in the inflamed joints of patients with RA, a unique feature of the synovial inflammation of patients who will develop RA would be the overproduction of IL-16. Such overproduction may lead to attraction of T cells to the joint. Moreover, the characteristics of T cells in the joint (hyporesponsiveness) may be explained in part by the IL-16 stimulation. It has been observed that IL-16 could inhibit T cell receptor (TCR) signaling, as indicated by its inhibitory effect on a mixed lymphocyte reaction[7]. To obtain more insight into the possible role of IL-16 in the pathogenesis of RA, we measured IL-16 levels in patients with recent-onset RA with mild and severe disease, patients with undifferentiated arthritis, and healthy controls. Further, the concentration of IL-16 was studied in patients with recent-

onset RA in a longitudinal setting to determine the relationship of IL-16 to joint damage and disease activity.

MATERIALS AND METHODS

Patients and controls. A special Early Arthritis Clinic (EAC) was started at the Department of Rheumatology of the Leiden University Medical Center, the only center for rheumatic patients in an area with 300,000 inhabitants. General practitioners in this area were motivated to directly refer patients when arthritis was suspected. All patients were seen within 2 weeks at the EAC. Diagnoses were made according to international classification criteria as described[8]. Patients diagnosed with recent-onset RA [n = 39, mean age 60 ± 17 yrs, 64% women, 84% rheumatoid factor (RF) positive, 72% shared epitope (SE) positive, median (25th-75th percentile) Disease Activity Score (DAS) 3.6 (3.0–4.3), and median C-reactive protein (CRP) 34 (15–60) mg/l] and patients with undifferentiated arthritis [n = 15, mean age 45 ± 14 yrs, 67% women, all RF negative, 57% SE positive, median DAS 2.0 (1.5–2.8), and median CRP level 12 (3–33) mg/l] from the EAC were included in this study. Another 13 patients [mean age 44 ± 17 yrs, 62% women, 15% RF positive, 54% SE positive, median DAS 2.6 (2.2–2.8), and median CRP level 9 (5–30) mg/l] who had undifferentiated arthritis at the EAC inclusion but developed RA over time were also included (at the 1-year followup, these patients fulfilled the classification criteria for RA[9]). All patients with RA were promptly treated with disease modifying antirheumatic drugs (DMARD) 2 weeks after inclusion in the EAC in addition to nonsteroidal antiinflammatory drugs (NSAID). The initial treatment of patients with RA consisted of chloroquine or salazopyrine. If disease control was insufficient according to the clinical judgment of the rheumatologist, or in case of intolerance, the rheumatologist was free to choose another DMARD. Five patients received prednisone at some point during the 2-year followup. The patients with undifferentiated arthritis were not treated with DMARD. Finally, 12 healthy controls (mean age 45 ± 13 yrs, 58% women) were also included in this study.

Clinical and laboratory examinations. Clinical variables of disease activity were assessed at study entry, at RA diagnosis, and thereafter at one year and 2 years. Radiographs of hands and feet were measured according to the modified Sharp-van der Heijde score[10]. These radiographs were scored in random order for the presence of erosions and joint space narrowing by an experienced rheumatologist blinded for the clinical data. The intraclass correlation coefficient for the assessor's scoring was 0.95. The disease activity of the patients was measured with the DAS3[11] and CRP[11]. The formula of the DAS3 was as follows: DAS3 = 0.54 × (√Ritchie score) + 0.065 × (number of swollen joints) + 0.33 × ln erythrocyte sedimentation rate + 0.224. We used a modified DAS3 in this study. All joints were assessed as in the Ritchie Articular Index except for the acromioclavicular, subtalar, and midtarsal joints. For the swollen joint index the metacarpophalangeal, proximal interphalangeal, and metatarsophalangeal joints were scored as one unit.

IL-16 ELISA. Blood samples were collected from which citrate plasma was prepared according to standard procedures, and stored frozen at –20°C until assayed. Blood samples from the RA patients were collected at disease onset before treatment and at one and 2-years' followup. Blood samples at disease onset were available from the patients with undifferentiated arthritis. The IL-16 levels in samples from the healthy controls were measured at a single time point. Plasma levels of IL-16 were measured using an ELISA according to the manufacturer's procedures (Roche Molecular Biochemicals, Germany). Briefly, patient or control plasma and known concentrations of human IL-16 (hIL-16) were added to streptavidin-coated wells simultaneously with a biotin-labeled capture antibody and a peroxidase-conjugated detection antibody. Following the washing step, tetramethylbenzidine was added as a substrate for the peroxidase bound in the complex and determined photometrically at 450 nm with an automated plate reader (Bio-Tek EL312e microplate ELISA reader; D.I Biotech Ltd., Seoul, Korea). The IL-16 levels were then determined by comparison with the linear part of the standard curve. The detection limit was 8 pg/ml. The

duplicate measurements differed less than 10%, and therefore we used the mean of the measurements.

Statistical analysis. Data were analyzed by Mann-Whitney U test. Overall changes in time of the IL-16 levels were analyzed with the nonparametric Friedman test. Correlations between several variables that were repeatedly measured over time were calculated using a multivariate mixed ANOVA model with patient number as random factor. All tests were 2-tailed and p ≤ 0.05 was considered significant.

RESULTS

Elevated IL-16 levels in RA patients. To investigate the role of the cytokine IL-16 in RA, systemic IL-16 levels were analyzed in the RA patients and compared to levels in patients with undifferentiated arthritis and healthy controls. At baseline, before treatment, the median systemic IL-16 level was significantly higher in the RA patients [median (25-75th percentile): 45.2 (37.7–82.4) pg/ml] compared to patients with undifferentiated arthritis [29.0 (21.5–52.4) pg/ml; p = 0.005] and healthy controls [30.4 (24.4–37.0) pg/ml; p = 0.0008; Figure 1].

Elevated IL-16 in patients with undifferentiated arthritis who will develop RA. To further investigate the role of IL-16 in RA, we were also interested in the IL-16 levels in patients with initially undifferentiated arthritis who later developed
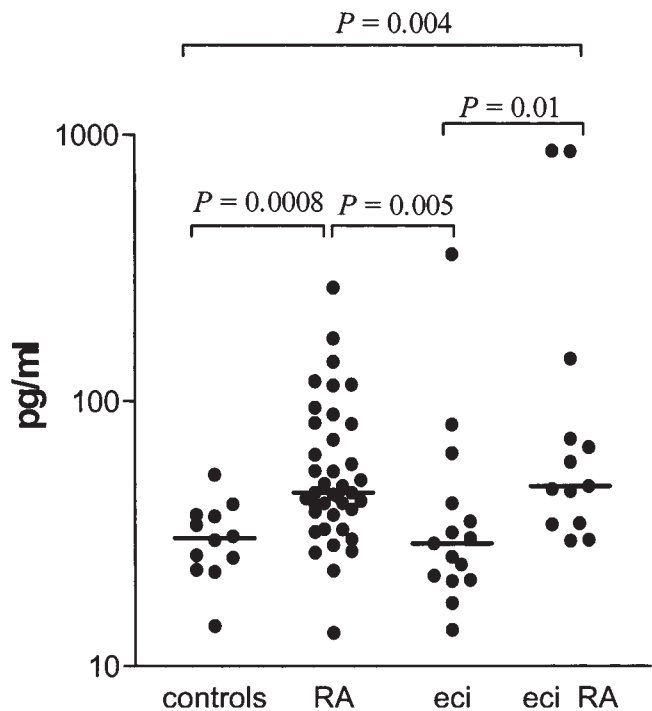
Figure 1. Median baseline plasma levels of IL-16 (pg/ml) at disease onset for patients with RA, patients with undifferentiated arthritis (eci), patients with undifferentiated arthritis that will develop into RA (eci RA), and controls. IL-16 is significantly increased in the RA patients compared with controls and patients with undifferentiated arthritis, while no significant difference was observed between the controls and patients with undifferentiated arthritis. Patients with undifferentiated arthritis who will develop RA had significantly higher IL-16 levels compared to the controls and other patients with undifferentiated arthritis.

RA. Interestingly, the patients who had undifferentiated arthritis before they developed RA also had significantly higher IL-16 levels before they developed RA [47.9 (34.5–108.2) pg/ml] compared to the healthy controls (p = 0.004) and to the patients who were still categorized with the diagnosis of undifferentiated arthritis at 1-year followup (p = 0.01), but not when compared to patients with RA (p = NS) (Figure 1).

Elevated IL-16 over time in RA patients. Next, the IL-16 levels over time were analyzed in these RA patients. Table 1 shows that also over time the IL-16 levels remained significantly higher compared to the controls. Subsequently, the RA patient group was divided into a progressive and a mild disease group based on the joint damage progression of these patients (more or less than 10 Sharp points during 2 years' followup). In the progressive patient group (n = 13), we observed a further significant increase in the IL-16 level at one year (p = 0.015; Wilcoxon signed ranks test) with overall significant changes over the 2 year followup (p = 0.028) [at disease onset: 48.8 (30.3–76.7) pg/ml; at one year: 72.0 (43.8–102.1) pg/ml; at 2 years: 59.0 (36.5–74.0) pg/ml]. However, in the mild disease group (n = 16), a stable, high IL-16 level was found during the 2-year followup [at disease onset: 47.8 (38.8–93.2) pg/ml; at one year: 58.8 (42.9–76.4) pg/ml; at 2 years: 56.7 (48.6–67.4) pg/ml].

Concentration of IL-16 is associated with joint damage. To further elucidate the role of IL-16 in relation to disease activity and joint damage, a multivariate mixed ANOVA test was performed with the disease activity variables CRP, DAS, and the joint damage score during the 2-year followup. A significant positive correlation between IL-16 levels and joint damage in RA patients was observed during the 2-year followup (R = 0.3, p = 0.023; Figure 2). Further, no significant association was observed over time between IL-16 and the DAS score or the CRP level (Figures 3 and 4).

DISCUSSION

We observed high concentrations of IL-16 in patients with recent-onset RA compared to controls and patients with

Table 1. Clinical and laboratory variables of RA patients (n = 29) during a 2-year followup study. Data are shown as median (25th-75th percentiles).

	Baseline	One Year	Two Years
IL-16, pg/ml	49 (35–82)	62 (43–81)	58 (42–71)
DAS score	3.6 (2.7–4.4)	2.3 (1.5–3.2)	2.1 (1.4–2.9)
CRP, mg/l	32 (12–55)	9 (4–20)	8 (5–21)
Sharp score	1 (0–6)	5 (1–23)	6 (1–33)

DAS: disease activity score; CRP: C-reactive protein; Sharp score: radiological joint destruction measured according to the modified Sharp-van der Heijde method[10]. The median IL-16 level of healthy controls is 30 (24–37) pg/ml (p = 0.0008 compared to baseline IL-16 level of RA patients, p < 0.0001 compared to IL-16 level at one year, and p = 0.0005 compared to IL-16 level at 2 years).
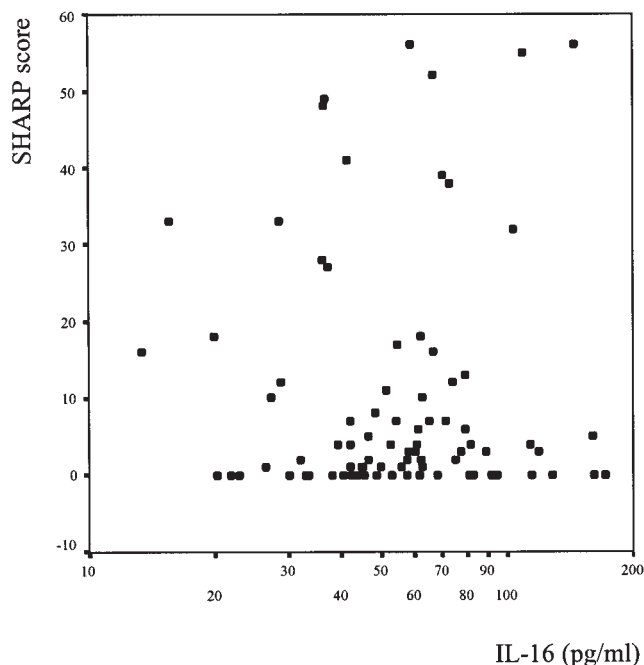

Figure 2. Correlation between IL-16 levels (pg/ml) and Sharp score from RA patients followed over 2 years using a multivariate mixed ANOVA model (n = 29; R = 0.3; p = 0.02).
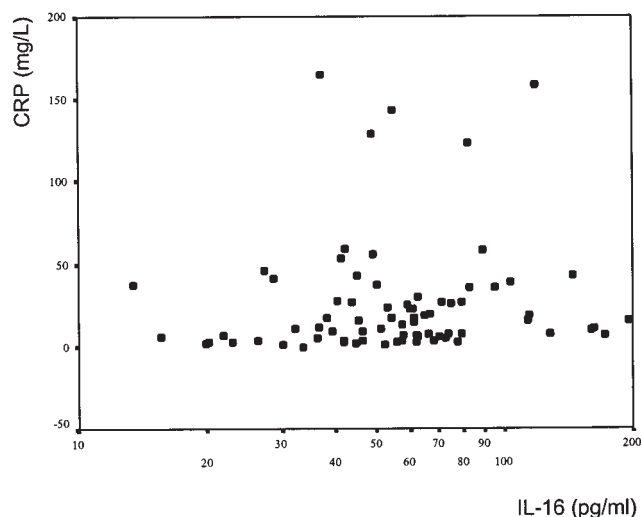

Figure 3. Correlation between IL-16 levels (pg/ml) and CRP levels (mg/l) in RA patients followed over 2 years using a multivariate mixed ANOVA model (n = 29; R = –0.15; p = 0.3).

undifferentiated arthritis. More interestingly, our study showed that patients with initially undifferentiated arthritis who later developed RA have high levels of IL-16 even before they fulfill the American College of Rheumatology classification criteria. Furthermore, we found that the systemic IL-16 levels in RA patients remained high over
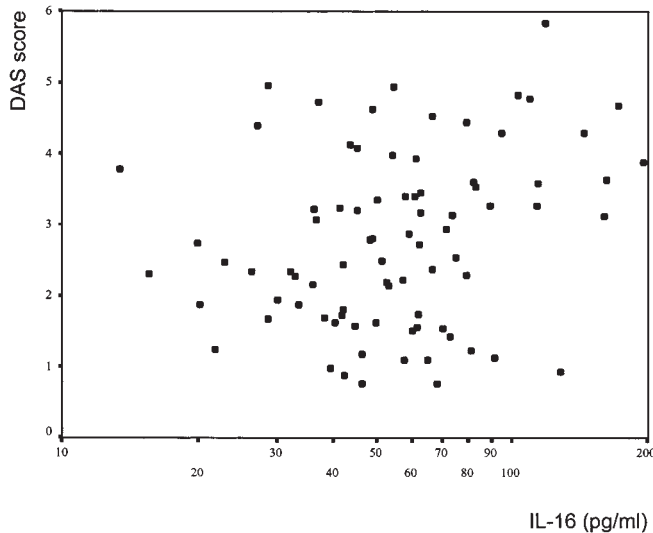
Figure 4. Correlation between IL-16 levels (pg/ml) and DAS in RA patients followed over 2 years using a multivariate mixed ANOVA model (n = 29; R = 0.2; p = 0.07).

time and that these IL-16 levels are associated with joint damage.

Cross-sectional studies[3-5,12] report high levels of IL-16 in RA patients. In line with these studies we also observed high levels of IL-16 in RA patients at disease onset compared to patients with undifferentiated arthritis as well as controls. More importantly, our study further showed in a longitudinal setting that IL-16 levels remained high in these RA patients. In addition, we showed that patients with undifferentiated arthritis who will develop RA already had significantly higher IL-16 levels at baseline, while the patients who remained with undifferentiated arthritis had IL-16 levels comparable to the controls. Further, it has been reported that in other autoimmune diseases, high levels of IL-16 are not genetically determined[13]. Altogether, these results indicate RA disease associated involvement of IL-16, rather than arthritis *per se*.

In this study, small differences exist in sex and age between the different patient categories. Although no influence of age or sex on IL-16 levels was observed, it is theoretically possible that these factors affect immune homeostasis and therefore IL-16 levels. To exclude this theoretical possibility, a formal study is needed.

RA is an autoimmune disease characterized by chronic synovitis in which T cells are thought to play a crucial role. Contradictory studies have recently been published reporting IL-16 as a proinflammatory cytokine[5] and also as an antiinflammatory cytokine in RA[14]. T cells, monocytes, and fibroblasts in the RA synovium were found to produce IL-16. After binding to its receptor molecule, CD4, IL-16 induces the stimulation of CD4+ T cells and causing them to migrate into the inflamed tissues and stimulating their proliferation[4]. On the other hand, the binding of IL-16 to

CD4 can also lead to induction of T cell anergy[7]. Moreover, the characteristics of T cells in the joint (hyporesponsiveness) may be explained in part by the IL-16 stimulation. To elucidate this dogma in RA, we measured IL-16 in RA patients in a longitudinal setting and investigated correlations with joint damage as measured by the Sharp score and the disease activity variables CRP and DAS. During the 2-year followup, we did not observe a significant correlation of the DAS or CRP with the IL-16 levels in RA. This is in accord with other studies in RA[3,5,12] that also reported no association of IL-16 with the disease activity variables, with the exception of one study[4]. An explanation for the association of IL-16 with joint damage in RA rather than disease activity could be that joint inflammation and cartilage destruction are not necessarily interdependent processes, as suggested by Van den Berg[15]. The confirmed finding that IL-16 is not associated with disease activity in RA is, however, in contrast to the situation in other autoimmune diseases, such as systemic lupus erythematosus (SLE), where elevated IL-16 levels were correlated with high disease activity[13].

We observed a significant positive correlation of IL-16 with joint damage during the 2-year followup. More importantly, we observed a further significant elevation of the IL-16 levels at one year in the subgroup of patients with progressive joint damage, while the IL-16 levels remained stable in the mild disease group. These findings therefore suggest an involvement of IL-16 in joint damage.

We also observed lower plasma IL-16 levels compared to serum IL-16 levels reported in other studies[3,4]. Previously we have shown in an SLE study[13] that IL-16 plasma levels were lower than those in matched serum levels, but significantly correlated.

In conclusion, our study shows that IL-16 levels are elevated in patients with RA and are involved in joint destruction. Further, IL-16 levels predict the development of RA in patients with undifferentiated arthritis.

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