Increased Serum Interleukin 22 in Patients with Rheumatoid Arthritis and Correlation with Disease Activity

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ABSTRACT. Objective. To analyze the role of interleukin 22 (IL-22) in rheumatoid arthritis (RA).

Methods. IL-22 serum levels were measured in 83 patients with established RA under treatment with disease-modifying antirheumatic drugs and in 30 healthy controls matched for age and sex. Patients were assessed for clinical and laboratory variables. Correlations of IL-22 serum levels with disease activity measures [Clinical Disease Activity Index (CDAI) and Disease Activity Score for 28 joints (DAS28)], serological markers, bone erosions, and demographic factors were assessed. Peripheral blood mononuclear cells (PBMC) from 30 patients with RA and 14 controls were purified and stimulated in vitro with phorbol myristate acetate (PMA)/ionomycin. IL-22 production by PBMC and in serum was investigated by ELISA.

Results. IL-22 levels were increased in patients with RA compared with controls (mean 432.37 pg/ml and 67.45 pg/ml, respectively; p < 0.001). Levels of IL-22 correlated with DAS28 and CDAI measures. Rheumatoid factor (RF) positivity was correlated with higher levels of IL-22 in patients with RA (mean 575.08 pg/ml; p = 0.001). The presence of bone erosions was associated with high IL-22 levels (p = 0.0001). PBMC stimulated with PMA/ionomycin expressed higher levels of IL-22 in patients with RA than controls but this was not significant (mean 584.75 pg/ml and 295.57 pg/ml; p = 0.553).

Conclusion. IL-22 is elevated in the serum of patients with established RA. Elevated serum IL-22 allows discrimination between patients with different clinical and laboratory measures and indicates the potential of IL-22 as an additional tool for assessment of activity in RA, particularly in patients with RF antibodies and longterm disease. (First Release May 15 2012; J Rheumatol 2012;39:1320–5; doi:10.3899/jrheum.111027)

Key Indexing Terms:
INTERLEUKIN 22 RHEUMATOID ARTHRITIS DISEASE ACTIVITY
DISEASE ACTIVITY SCORE 28 CLINICAL DISEASE ACTIVITY INDEX

Rheumatoid arthritis (RA) is a systemic chronic inflammatory autoimmune disease characterized by synovial proliferation and progressive damage of multiple joints. RA is a disease that mainly targets the synovial membrane, cartilage, and bone. At times, systemic manifestations may occur. It is associated with increased mortality and affects 0.4% to 1% of the population. More than one mechanism could contribute to the disease. Cytokines are directly implicated in the pathogenesis of RA as part of a complex regulatory network related to specific immunological processes that promote autoimmunity, chronic inflammation, and tissue destruction. T cell activation and migration are involved in these mechanisms, and these cells adopt a proinflammatory phenotype. RA has been thought to be a Th1- associated disorder, but at present the Th17 phenotype has been identified and associated to RA. Th17 cells have been clearly implicated in animal models of RA, i.e., collagen-induced arthritis (CIA), the SKG mouse, and gene-targeted mice deficient in interleukin 1 (IL-1) receptor antagonist. The functional role of these T cell subsets in human arthritis has been recently clarified.

Th17 cell differentiation is regulated by the transcription factors signal transducer and activator of transcription 3 (STAT3), retinoic acid receptor-related orphan receptor-γt (RORγt), and aryl hydrocarbon receptor, and is driven by...
transforming growth factor-β (TGF-β), IL-1, and IL-6; IL-23 is required to expand and stabilize the cell population. In addition to IL-17A, Th17 cells produce IL-17F, IL-21, IL-22, and IL-26, as well as chemokines such as CC chemokine ligand 20 (CCL20; also known as MIP3α)9,10,11. Recently IL-17A production and IL-22 expression have been demonstrated to occur independently of TGF-β signaling12.

IL-22 is a member of the IL-10 cytokine family and is related to different T cell subsets. IL-22 is presumed to play a role in pathogen defense, wound healing, and tissue reorganization, therefore IL-22 modulates tissue responses during inflammation. IL-22 is involved in the induction of an acute-phase response in vivo and chemokines and matrix metalloproteinases in vitro13. IL-22 takes part in the adaptive response by the activation of CD4 T cells and in the innate response by the activation of lymphocytes such as natural killer cells and lymphoid tissue inducer-like cells14. IL-22 signals through a heterodimeric receptor complex consisting of the IL-10R2 and IL-22R115,16. The IL-22R1 subunit is strongly expressed in the skin, kidney, and tissues of the digestive and respiratory systems and synovial fibroblasts13. Binding of IL-22 to this receptor leads to activation of STAT3 signaling cascades and Akt and mitogen-activated protein kinase pathways14. The role of IL-22 as a Th17-effector cytokine has been demonstrated and it is most highly expressed by this cell subset17. Expression of this proinflammatory cytokine is induced by intradermal injection of IL-22 in mice13. The functional role of IL-22 is also associated with chronic inflammatory diseases such as psoriasis, inflammatory bowel disease (IBD), and RA13,14,18,19,20. Both psoriasis and IBD predispose to spondyloarthritis, including axial disease, therefore examination of this cytokine was relevant in ankylosing spondylitis21. IL-22 has also been implicated in the pathogenesis of reactive arthritis22.

In IBD, IL-22 expression was detectable in CD4-positive T cells and was related to the expression of other inflammatory cytokines18. Increased levels of IL-22 in patients with psoriasis23, Sjögren’s syndrome24, and RA19,20,25 have been described, suggesting that it has proinflammatory properties and is important in these autoimmune diseases13. In RA, IL-22 possibly induces the proliferation of synovial fibroblasts and production of chemokines19. In animal models of collagen-induced arthritis, mice deficient in IL-22 were less susceptible to panus formation and incidence of arthritis26. IL-22 levels have been associated to radiographic progression in RA20. Nevertheless, there are currently no studies demonstrating a positive correlation between levels of IL-22 and disease activity. We investigated serum levels of IL-22 in patients with RA to determine its relations to disease activity and severity.

MATERIALS AND METHODS

Study population. A total of 83 patients with RA (80 women, 3 men; mean age 53 ± 10.6 yrs) were recruited from the Department of Rheumatology at Hospital das Clínicas da Universidade Federal de Pernambuco (UFPE). Current medications were recorded (Table 1); 6 patients were taking biological agents (adalimumab and 1 etanercept). The diagnosis of RA was established by the presence of 4 or more American College of Rheumatology 1987 diagnostic criteria27. About 30 healthy volunteers matched for age and sex (mean age 44 ± 10.8 yrs) were included as controls, and all were free of any rheumatologic conditions. Peripheral blood samples were obtained from patients and controls.

Demographic, clinical, and laboratory data were collected from hospital records or by questionnaire and reviewed by experienced physicians. Table 1 presents demographic and clinical findings in patients with RA.

Laboratory features of patients with RA [erythrocyte sedimentation rate (ESR), rheumatoid factor (RF) positivity] were recorded. Individual disease activity was quantified using the Disease Activity Score for 28 joints (DAS28)28 and the Clinical Disease Activity Index (CDAI)29. Radiographs of hands were obtained from patients with RA and evaluated for the presence of erosions by an experienced rheumatologist blinded to the clinical data. All subjects gave written consent to participate. The study was approved by the ethics committee of the UFPE.

Peripheral blood mononuclear cells (PBMC). PBMC were obtained from heparinized blood from healthy, nonsmoking donors who had not taken any drugs for at least 15 days before sampling, and were isolated by standard density-gradient centrifugation over Ficoll-Hypaque (GE Healthcare). Cells were counted in a Neubauer chamber, and cell viability was determined by the trypan blue exclusion method. Cells were used only when viability was > 98%.

PBMC cultures. PBMC (1 x 10⁶ cells/well) from 30 patients with RA and 14 controls were cultured in 24-well plates in RPMI-1640 (Gibco) supplemented with 10% fetal bovine serum (Gibco), HEPES 10 mM (Gibco), and penicillin/streptomycin 200 U/ml (Gibco), and incubated at 37°C in a humidified 5% CO2 incubator. Cells were stimulated/not stimulated with phorbol myristate acetate (PMA) 100 ng/ml (Sigma) and ionomycin 1 µg/ml (Sigma). The supernatants were collected after 48 h and IL-22 levels were quantified.

Table 1. Demographic, clinical, and laboratory presentation of the patients with rheumatoid arthritis.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. patients</th>
<th>Age, yrs, mean (range)</th>
<th>Female/male</th>
<th>Disease duration, mean (range)</th>
<th>Rheumatoid factor (%)</th>
<th>Radiological erosions (%)</th>
<th>Treatment (%)</th>
<th>Nonsteroidal antiinflammatory drugs</th>
<th>Steroids</th>
<th>Methotrexate</th>
<th>Leflunomide</th>
<th>Antimalarial agents</th>
<th>Biologic therapy</th>
<th>Disease activity (%)</th>
<th>Disease Activity Score 28 joints</th>
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<tbody>
<tr>
<td></td>
<td>83</td>
<td>53 (28–77)</td>
<td>80/3</td>
<td>10.5 years (0.1–35.2)</td>
<td>Positive 56 (67)</td>
<td>Absent 27 (33)</td>
<td>Present 67</td>
<td>60%</td>
<td>63 (75.9)</td>
<td>51 (61.4)</td>
<td>33 (39.8)</td>
<td>14 (16.9)</td>
<td>6 (7.2)</td>
<td>7 (8.4)</td>
<td>Clinical remission</td>
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<td>Negative 16 (19.3)</td>
<td>Treatment (%)</td>
<td>Present 67</td>
<td>60%</td>
<td>63 (75.9)</td>
<td>51 (61.4)</td>
<td>33 (39.8)</td>
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<td>Mild disease</td>
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<td>Treatment (%)</td>
<td>Nonsteroidal</td>
<td>Present 67</td>
<td>60%</td>
<td>63 (75.9)</td>
<td>51 (61.4)</td>
<td>33 (39.8)</td>
<td>14 (16.9)</td>
<td>6 (7.2)</td>
<td>39 (47)</td>
<td>Moderate disease</td>
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<td>antiinflammatory</td>
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<td>14 (16.9)</td>
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<td>drugs</td>
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<td>60%</td>
<td>63 (75.9)</td>
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<td>14 (16.9)</td>
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<td>14 (16.9)</td>
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<td>28 (33.7)</td>
<td>Severe disease</td>
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CDAI: Clinical Disease Activity Index.
Measurement of serum IL-22 levels. Cytokines in the supernatants of cultures and in sera were assayed with an ELISA kit according to the manufacturer’s recommendation (R&D Systems). The lower limit of detection for the ELISA IL-22 kit was 15 pg/ml.

Statistical analysis. Associations of serum IL-22 levels with clinical and laboratory measures of patients with RA were analyzed by univariate comparisons using nonparametric tests (Mann-Whitney tests). p < 0.01 was considered a significant association and p < 0.05 a suggestive association. Results are shown considering the mean value. All quantitative data were plotted with GraphPad Prism 3.02 software. Variables with p < 0.2 at univariate analysis were retained for multivariate logistic regression analysis.

Correlations between serum IL-22 levels and DAS28 and CDAI were evaluated using Pearson correlation analysis. This analysis was carried out with Origin 8.0724 software (OriginLab, Northampton, MA, USA).

RESULTS

Serum IL-22 levels in RA patients and controls. In total, 83 patients with RA and 30 healthy controls were included in our analysis. Serum IL-22 levels were significantly increased in RA patients compared with controls (mean 432.37 pg/ml and 67.45 pg/ml, respectively; p < 0.001; Figure 1). Serum levels of IL-17 were higher in patients with RA but this finding lacked statistical significance. No correlation was detected between IL-17 levels and disease activity (data not shown).

Association of serum IL-22 levels and disease activity. Having found that IL-22 was elevated in patients with RA, we assessed whether serum levels of IL-22 correlated with disease activity.

There was significant correlation for serum IL-22 levels among the scores for DAS28 ($r^2 = 0.041$, $p = 0.037$; Figure 2A) and CDAI ($r^2 = 0.062$, $p = 0.013$; Figure 2B). Serum from patients with RA in clinical remission had significantly lower levels of IL-22 than serum from patients with mild, moderate, and severe disease.

Correlations of serum IL-22 with RF positivity. Patients with RA who were positive for RF had significantly increased levels of IL-22 compared to seronegative patients (mean 575.08 pg/ml and 136.37 pg/ml; $p = 0.001$; Figure 3).

Correlations of serum IL-22 levels with bone erosions. We assessed associations of IL-22 levels and presence of erosions. Serum IL-22 levels were higher in patients with bone erosions (mean 516.69 pg/ml) than in patients without erosions (mean 79.29 pg/ml; $p = 0.0001$; Figure 4).

The CDAI, DAS28, RF, and erosion variables were significantly associated with IL-22 by univariate analysis ($p < 0.05$). Therefore, these variables were subjected to multivariate logistic regression analysis. In the first analysis we considered DAS28, RF, and erosions; DAS28 and RF measures were inde-
independently associated with IL-22 levels ($p = 0.046$ and $p = 0.013$, respectively). Then we analyzed CDAI, RF, and erosions; CDAI and RF were also independently associated with IL-22 levels ($p = 0.028$ and $p = 0.029$, respectively).

**IL-22 expression in PBMC following stimulation.** Next we analyzed the IL-22 patterns in vitro following stimulation with PMA and ionomycin. We observed increased levels of IL-22 in RA patients with RA compared to controls.

PBMC from 30 patients with RA and 14 controls were stimulated in vitro with PMA and ionomycin. There was low spontaneous secretion of IL-22 in both groups (mean 17.66 pg/ml in patients with RA, 63.85 pg/ml in controls). The levels of IL-22 in supernatants of PBMC from patients with RA after stimulation were higher than in control PBMC (584.75 pg/ml and 295.57 pg/ml, respectively), but this finding was not statistically significant ($p = 0.553$; Figure 5).

**DISCUSSION**

In our study, IL-22 levels were increased in patients with RA compared with those of healthy controls. In this series the levels of IL-22 correlated with disease activity (by DAS28 and CDAI). Of note, when samples from patients with RA were separated into RF-negative and RF-positive groups, there were significant differences in IL-22 serum levels. There was significant correlation between higher levels of IL-22 and positive RF. IL-22 levels were higher in patients with bone erosions. PBMC stimulated with PMA/ionomycin expressed higher levels of IL-22 in patients with RA than in controls, but this finding was not significant.

It has been suggested that IL-22 might play a role in RA pathophysiology\(^{14,20,21,25,26,30,31}\). Elevated levels of IL-22 have been demonstrated in RA synovial tissues and the lining and sublining layers of rheumatoid synovium expressed higher levels of IL-22R\(^{19}\). Endogenous IL-22 plays a proinflammatory role in collagen-induced arthritis in C57BL/6 mice, and this cytokine appears to be important for osteoclastogenesis and regulates antibody production\(^{26}\). Our findings are in accord with these observations, and are also in agreement with a recent study that described increased plasma levels of IL-22 in 30 patients with established RA with a mean disease duration of 10.7 years, although the patients had not received immunomodulatory drugs for 2 months\(^{30}\). In contrast, all our patients were under therapy. In our study the mean disease duration was 10.5 years and most patients had active disease, as noted. Another recent study showed levels of IL-22 were increased in almost 50% of patients with very early and active RA, and revealed no difference for disease activity measures between the groups of RA patients with high IL-22 and those with normal IL-22 levels; the patients assessed had active disease. Both groups of patients had never been treated with disease-modifying antirheumatic drugs and glucocorticoids. Elevated levels of IL-22 at baseline were significantly associated with bone erosions\(^{20}\). We found higher IL-22 levels in patients with bone erosions of hands. This finding supports the potential role of IL-22 as a predictive marker of bone destruction in RA. However, previous data demonstrated that levels of IL-22 were elevated in patients with established disease as well as in patients with very early disease\(^{25}\). Our data show that patients with longterm disease have increased serum levels of IL-22, supporting the idea that this proinflammatory cytokine may take part in the chronic inflammatory process in established RA, but to a lesser degree in very early disease.

Although IL-22 has been described to have a mild proinflammatory effect in arthritis\(^{32}\) this is not in line with our observation that patients differ in their clinical inflammatory measurements (DAS28 and CDAI) according to serum levels of IL-22, showing a linear trend (Figure 2). Our study is the first to associate the levels of IL-22 with severity of disease according to DAS28 and CDAI, demonstrating statistical significance in patients with established disease. However, this is not in agreement with a previous study that found no correlation between plasma levels of IL-22 and DAS28 in a similar group of 30 patients in China\(^{30}\). Our study analyzed a higher
The physiologic role of RF is to enhance immune complex clearance. Immune complexes are also present in the joint. Therefore, RF may contribute to the activity and chronicity of RA through complement-mediated pathways. Further, the RF-producing B cells in synovial tissue of patients with RA may act as antigen-presenting cells and present (foreign or self) antigens to T cells through uptake of immune complex-deposited IgG chains of IgM-RF can be directed to the Fcγ chains of IgG molecules. IgM-RF can be associated with bone erosions, this interleukin might be helpful in identifying patients with more destructive disease. Further studies are needed to clarify the role of IL-22 in RA.

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