The Role of IL-15 in Inflammatory Activity of Patients With Rheumatoid Arthritis (RA)

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Interleukin 15 (IL-15), a new player in the cytokine network, is produced mainly by monocytes and can be detected in the rheumatoid synovium. The aim of this study was to investigate the role of IL-15 in patients with rheumatoid arthritis (RA). IL-15 cytokine was measured in sera and synovial fluid (SF) of 40 patients with rheumatoid arthritis (RA) and 20 patients with osteoarthritis and only in sera of 20 healthy control using an enzyme-linked immunosorbent method. Seven American College of Rheumatology (ACR) core set measures as well as IL-15 levels were sequentially monitored at the commencement. IL-15 had been detected in sera of 16 (40%) of 40 RA patients, in two (10%) of 20 osteoarthritis (OA) patients and two (10%) of 20 healthy controls. The mean level of circulating IL-15 was significantly higher in RA patients ($P < 0.001$). In SF, the mean IL-15 levels were significantly higher in RA patients (162.8±35.4) than in OA patients (32.5±5.5) ($P < 0.001$). In paired samples ($n=40$) of sera and SF from RA patients and OA patients, IL-15 levels were higher in the SF than in sera ($P < 0.001$) in RA but not in OA. Rheumatoid arthritis patients with detectable IL-15 ($n=25$) had higher tender joint scores ($P < 0.01$), swollen joint scores ($P < 0.001$), rheumatoid factor ($P < 0.001$), and long disease duration ($P < 0.05$) than those without ($n=15$). In conclusion, IL-15 was elevated significantly in the sera and SF of patients with active rheumatoid arthritis (RA) but not on OA patients and correlated well with several parameters of RA disease activity. These observations suggest that IL-15 production may be associated with an exacerbation of RA, and then IL-15 levels could be useful to assess disease activity.

Keywords IL-15, disease activity, rheumatoid arthritis

INTRODUCTION

RA is an autoimmune disease characterized by the proliferation of synovium and the infiltration of chronic inflammatory cells. Cytokines from synovium and inflammatory cells are thought to be important in the initiation and perpetuation of RA. In particular, macrophage derived cytokines, including tumour necrosis factor alpha (TNF-α) and IL-6, have been detected at high levels and many of these monokines function as potent proinflammatory molecules in the joints, and reflect the disease activity of RA (1,2).

In the course of the disease, the normal relatively avascular synovium becomes heavily infiltrated by a wide variety of cells, including B cells, macrophages, fibroblasts, neutrophil granulocytes, dendritic cells, and many other cells (3). The synovial lining increases to a thickness of up to 30 cell layers, presumably through influx of macrophages, and by expansion of synovial fibroblasts. The latter produce high amounts of proinflammatory cytokines, mainly TNF, IL-1, and IL-6. Furthermore, many other cytokines such as IL-17, IL-18, and IL-15 chemokines, and angiogenic molecules are present in the inflamed synovial membrane and drive the disease. Subsequently, these proinflammatory cytokines activate signal transduction pathways and transcription factors, which, in turn, control the transcription of cytokines (4).

Interleukin-15 (IL-15) is a cytokine produced by macrophages and fibroblast-like synoviocytes. It has a chemoattractant activity, which facilitates recruitment and activation of T-cells (5, 6). IL-15 shares most of the biological activities of IL-2 on several types of lymphocytes, including the proliferation and activation of T, natural killer (NK) and B cells (7).

Synovial T cells produced significant amounts of TNF upon incubation with IL-15, but not with IL-2 (8). IL-15–stimulated T cells also elicit TNF production of synovial fluid macrophages in a cell–cell contact–dependent fashion. In turn, TNF and IL-1 are able to induce IL-15 production in synovial fibroblasts and therefore might perpetuate local T cell proliferation (9). As it has been shown for many other cytokines, IL-15 potently synergizes with other cytokines to enhance T cell growth as well as proliferation.
and macrophage activation. IL-12 and IL-18 in combination with IL-15 promote macrophage activation at low concentrations. Hence, IL-15 is likely to play a role in RA.(10)

The cytokine environment in rheumatoid synovium was initially considered to be difficult for T-cell activation and expansion due to the deficiency of IL-2, the factor essential for T-cell growth (11). However, others have shown that IL-15, a novel identified T-cell growth factor was present at significant levels in RA joints. The presence of IL-15 in immunologically active levels in RA synovial fluid provides another mechanism for activation of T-cells, once present within the synovial milieu, which could occur in the presence of relatively low concentrations of IL-2 (5). Therefore, IL-15 should be added to the matrix of cytokines locally produced by fibroblast-i.e. synoviocytes (12).

The aim of this study was to assess IL-15 levels in the sera and the synovial fluid (SF) of RA patients and correlated it with the clinical and laboratory parameters of rheumatoid arthritis (RA) disease activity.

SUBJECTS, MATERIALS AND METHODS

This work was conducted in Microbiology & Immunology department and Rheumatology & Rehabilitation Department, Faculty of Medicine Zagazig University in the period from July 2005 to March 2006 on 60 patients who were divided as follow: Patient group (Group I): this group included 40 suffering from rheumatoid arthritis (13). The mean age of the RA patients (15 males and 25 females) was 48.3±5.2 years. The mean disease duration was 76.4±20.6 months. Group (II): included 20 patients with osteoarthritis. These subjects had clinical and radiological features of Osteoarthritis (OA) according to the criteria of American College of Rheumatology (ACR) (14). The mean age of the OA patients (8 male and 12 females) was 50.4±6.21 years. Control group (Group III): this group included 20 normal subjects who were chosen as a control for blood sampling only (no synovial fluid sampling) The mean age of the healthy controls (8 males and 12 females) was 51.1±6.62 years. Oral informed consent was obtained from each patient. All patients included in this study were subjected to the following: Complete history taking, Full clinical examination, Blood sampling, Synovial fluid sampling [except normal control group, no synovial fluid sampling].

Clinical and laboratory assessments:

In patients with RA, clinical assessments were performed at commencement by rheumatologist, trained in making standardized assessments. Seven clinical variables (American College of Rheumatology (ACR) core set measures) were evaluated, as defined by the ACR(15): tender joint count, swollen joint count, pain as recorded on a 100-mm visual-analogue scale, physician’s global assessment of disease activity, patient’s global assessment of disease activity, degree of disability, as measured by a Health Assessment Questionnaire. ALL patients were subjected also to the following:

1-erythrocyte sedimentation rate (ESR).
2- C-reactive protein (CRP) and Rheumatoid factor (RF) which were detected by using latex agglutination test (Avitex-- Latex test, supplied by Omega Diagnostic Limited).

Cytokine measurements:

Measurement of IL-15 levels in the sera and synovial fluid of RA and OA patients as well as in sera of healthy control by a solid phase sandwich ELISA technique, using the Biosource human IL-15 kit, USA.

Principle of the method:

The Hu IL-15 kit is a solid phase sandwich Enzyme Linked-ImmuNo-Sorbent Assay (ELISA). An antibody specific for Hu IL-15 has been coated onto the wells of the microtiter strips provided. Samples, including standards of known Hu IL-15 content, control specimens, and unknowns, are pipetted into these wells, followed by the addition of a biotinylated second antibody. During the first incubation, the Hu IL-15 antigen binds simultaneously to the immobilized (capture) antibody on one site, and to the solution phase biotinylated antibody on a second site. After removal of excess second antibody, Streptavidin-Peroxidase (enzyme) is added. This binds to the biotinylated antibody to complete the four-member sandwich. After a second incubation and washing to remove all the unbound enzyme, a substrate solution is added, which is acted upon by the bound enzyme to produce color. The intensity of this
colored product is directly proportional to the concentration of Hu IL-15 present in the original specimen. Optical density read at 450 nm using a microplate reader within 30 minutes. The sensitivity of the method (the minimum detectable dose) is <10 pg/ml. 

Statistical analysis:
Statistical analysis was done by using SPSS (Statistical Package for Social Science) statistical software for windows, version 10. The data were presented in the form of mean and standard deviation. Student (t) test was used for comparison of quantitative data. Correlation between two variables was performed using Spearman’s rank correlation coefficient. P < 0·05 was considered statistically significant.

RESULTS
This study was conducted on two groups of subjects, the patient group (60) which included (40) patients with rheumatoid arthritis (RA), 20 patients with osteoarthritis (OA), and the control group which included 20 healthy volunteers.

The results of the study were collected, statistically analyzed and tabulated and illustrated as follows:
The demographic data of the studied groups were shown in table (1). There was no significant difference between studied groups in age, gender and duration of disease.

In sera, 16 out of 40 RA patients had detectable concentrations of IL-15 (40%), whereas two out of 20 (10%) of the OA patients and two (10%) of the 20 healthy controls had detectable IL-15 (P < 0·001). The mean±SD level of circulating IL-15 was significantly higher in RA patients than in both OA patients and healthy controls (84.8±14.3, 23.5±4.2 and 21.9±3.8 respectively and the P < 0·001) (Table 2).

In SF, the number of RA patients with detectable IL-15 in SF were 25 (62.5%) while only 2 patients with OA had detectable concentrations of IL-15 (10%) (P < 0·001). The mean±SD level of IL-15 were also significantly higher in RA patients than in OA patients (162.8±35.4 and 32.5±3.5 respectively and the P<0.001. (Table 2).

In the paired samples of sera and SF from RA and OA patients, IL-15 levels were significantly higher in the SF than in the sera in RA patients (P < 0·001). However, the levels of IL-15 were not significantly different between the sera and SF in patients with OA. (Table 2)

Table (3) shows the correlation between IL-15 and laboratory & clinical measures of disease activity of patients with active rheumatoid arthritis (RA). Patients with RA (n=40), in whom clinical assessment was performed at base line, were divided into two groups: patients with detectable concentrations of IL-15 (n=25) and those without detectable concentrations of IL-15 (n=15). The above mentioned categories of disease activity of RA were then compared for the two groups. There were no significant differences in pain score, patient’s global assessment, physician’s global assessment, patient’s self-assessed disability, and ESR between the two groups. However, patients with detectable IL-15 had a significant higher tender joint score (P<0·01), swollen joint score (P < 0·001), disease duration (P<0·05), rheumatoid factor (P < 0·001), and CRP (P<0·05), compared with those without.

Fig (1) shows highly significant positive correlation between IL-15 levels and tender joint score (r=0·512, P < 0·001) . also swollen joints score correlated positively with IL-15 levels (r=0·492, P < 0·001). Fig (2)

Table (1): Demographic characteristics in studied groups.

<table>
<thead>
<tr>
<th></th>
<th>RA</th>
<th>OA</th>
<th>Control</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>48.3±5.2</td>
<td>50.4±6.21</td>
<td>51.1±6.62</td>
<td>NS</td>
</tr>
<tr>
<td>Gender</td>
<td>Female/male</td>
<td>15/25</td>
<td>12/8</td>
<td>12/8</td>
</tr>
<tr>
<td>Disease duration</td>
<td>76.4±20.6</td>
<td>72.5±18.2</td>
<td>-</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS= Not significant.
Table (2): Levels of IL-15 in patients with RA and osteoarthritis (OA) and healthy control

<table>
<thead>
<tr>
<th>IL-15 pg/ml</th>
<th>RA (n=40)</th>
<th>OA (n=20)</th>
<th>Control (n=20)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sera</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>positivity (%)</td>
<td>16 (40%)</td>
<td>2 (10%)</td>
<td>2 (10%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>84.8±14.3*</td>
<td>23.5±4.2</td>
<td>21.9±3.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Synovial Fluid</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positivity (%)</td>
<td>25 (62.5%)</td>
<td>2 (10%)</td>
<td>–</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>162.8±35.4</td>
<td>32.5±5.5</td>
<td>–</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table (3): Comparison of clinical and laboratory parameters between patients with detectable IL-15 and those without

<table>
<thead>
<tr>
<th>Variables</th>
<th>Patients with detectable IL-15 (n=25)</th>
<th>Patients without detectable IL-15 (n=15)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease duration (months)</td>
<td>80.2±8.5</td>
<td>72.6±16.8</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Tender joint score*</td>
<td>17±6</td>
<td>11±3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Swollen joint score*</td>
<td>14±4</td>
<td>6±2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Visual analogue pain scale (mm)</td>
<td>52±17.2</td>
<td>50±14</td>
<td>NS</td>
</tr>
<tr>
<td>Physician’s global assessment</td>
<td>5.1±2.1</td>
<td>4.9±1.6</td>
<td>NS</td>
</tr>
<tr>
<td>Patient’s global assessment</td>
<td>5.0±2.5</td>
<td>5.0±2.3</td>
<td>NS</td>
</tr>
<tr>
<td>Degree of disability</td>
<td>1.7±0.6</td>
<td>1.6±0.5</td>
<td>NS</td>
</tr>
<tr>
<td>ESR (mm/h)</td>
<td>45.5±9.2</td>
<td>42.8±11</td>
<td>NS</td>
</tr>
<tr>
<td>C-reactive protein‡</td>
<td>24 (96%)</td>
<td>11 (73.3%)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Rheumatoid factor‡</td>
<td>25 (100%)</td>
<td>10 (66.7%)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Mean±SD                     ‡ Positive cases. NS= Not significant.

Fig (1): Correlation of IL-15 with tender joint score.
DISCUSSION

Rheumatoid arthritis (RA) is a chronic inflammatory disease that associated with intense pain, irreversible joint destruction, systemic complications and a progressive functional decline. The disease is also associated with a decreased life expectancy of five to 15 years secondary to comorbid illnesses that may be exacerbated by the underlying pathophysiologic mechanisms. It is an immune mediated disease that develops in a genetically predisposed host. It is characterized by development of a hyperplastic synovial membrane (i.e. pannus) that has been infiltrated by B-cells and macrophages, which lead to the production and release of proinflammatory cytokines. In diseases such as RA, an imbalance develops between the proinflammatory cytokines (i.e. interleukin (IL)-1b, tumor necrosis factor-a (TNF-a, IL-6, IL-15)) and the anti inflammatory cytokines (i.e., soluble TNF receptors, IL-1 receptor antagonists)(16).

Involvement of IL-15 has been demonstrated in a number of autoimmune and infectious diseases, such as rheumatoid arthritis, sarcoidosis and leprosy, i.e. diseases where cellular immunity predominates. IL-15 is a potent T-cell attractant that is elevated in synovial fluid and synovial membranes in patients with RA. Some preliminary studies suggest this cytokine is involved in the perpetuation of RA synovitis(18).

In the present study IL-15 levels were significantly higher in sera of patients with RA than those of patients with OA or healthy controls, which confirms previous reports. Who found that RA patients had significantly higher serum levels of IL-15 (102.4 +/- 150 pg/ml; p = 0.0001) than systemic lupus erythematosus (SLE), patients (9.8 +/- 15.3 pg/ml), spondyloarthropathies (SSd) patients (7.9 +/- 14.6 pg/ml) and healthy donors' (5.2 +/- 11.6 pg/ml)(18).

By comparison between RA and OA Synovial fluid values, we found that RA synovial fluid level of IL-15 highly exceeded that estimated in SF of OA patients. This finding coincides with McInnes et al. (5, 8) and Thurkow et al. (19). Who were reported that IL-15 expressed at high concentrations in synovial fluid (SF) and ST from patients with RA. further suggesting that IL-15 is an important regulator of the inflammation in RA. They concluded that IL-15 probably contributes to the chronic inflammatory process in patients with RA.

Ziolkowska et al (20), also reported that synovial fluids of patients with rheumatoid arthritis, but not with osteoarthritis, contain high levels of IL-15 and IL-17.

In addition, in paired samples of the sera and SF, the study revealed that IL-15 levels were significantly higher in SF than sera in patients with RA, but there was no significant difference in patients with OA. This in
agreement with Fahmy et al. (21), how revealed that the synovial fluid of RA patients had a significant elevated level of IL-15 than that estimated in the serum.

In the present study there was a significant difference in disease duration between RA patients with detectable levels of IL-15 and those without. This in agreement with Gonzalez-Alvaro (18), who reported that RA patients with a disease evolution less than 2 years showed lower IL-15 levels (33.7 +/- 62.2 pg/ml) than those with long-term disease (152.4 +/- 64.6 pg/ml; p = 0.004) (18).

We demonstrated that patients with detectable levels of IL-15 in had higher tender joint scores, swollen joint scores, RF and CRP. Moreover, IL-15 levels correlated well with several parameters indicative of disease activity in RA, especially with the tender and swollen joint scores. There were significant positive correlations between; levels of IL-15, and both tender joint score and swollen joint score in RA patient. Our finding coincided with other investigator how reported that there was a relationship (p < 0.01) between the concentrations of TNF-alpha, IL-12, IL-15, and IL-18 cytokines in blood and synovial fluid with the quantity of the disease activity score in joints. Also they concluded that cytokines concentrations could be good indicators of the degree of the general activity of RA and could contribute to the interpretation of insufficiently well known views of the pathogenesis role and significance of cytokines in an active disease (22).

In conclusion, IL-15 was elevated significantly in the sera and SF of patients with active rheumatoid arthritis (RA) but not on OA patients and correlated well with several parameters of RA disease activity. These observations suggest that IL-15 production may be associated with an exacerbation of RA, and then circulating IL-15 levels could be useful to assess disease activity.

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