Anaemia of chronic disease in rheumatoid arthritis

Raised serum interleukin-6 (IL-6) levels and effects of IL-6 and anti-IL-6 on in vitro erythropoiesis

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Summary. Serum and bone marrow from 21 patients with rheumatoid arthritis (RA) were studied in order to establish the pathogenetic role of interleukin-6 (IL-6) in anemia of chronic disease (ACD). Erythroid colony growth, using burst forming units of erythroblasts (BFUe) as a parameter, was impaired in ACD and not in nonanemic RA controls. Serum IL-6 was elevated in ACD and it correlated well with parameters of disease activity such as erythrocyte sedimentation rate and C-reactive protein. IL-6 addition to bone marrow cultures had inconsistent effects while anti-IL-6 addition resulted in impaired erythroid colony growth, suggesting stimulatory effects of IL-6 produced in the medium, which may be masked by simultaneous production of cytokines with suppressive effects. It was concluded that elevated serum IL-6 in ACD reflects disease activity. It probably plays no pathogenetic role in ACD. Its stimulatory effects on erythroid growth might counteract suppressive effects of other interleukins.

Key words: Rheumatoid arthritis – Anemia – Erythroid colony growth – Interleukin-6, IL-6, anti-IL-6

Introduction

Anemia is frequently seen in patients with active rheumatoid arthritis (RA) [1]. Many causes of anemia are associated with RA, such as deficiencies of iron [2], vitamin B12 [3], and folic acid [4]. The most frequent type of anemia seen in RA, however, is the anemia of chronic disease (ACD) [5]. Many studies have been carried out to examine pathogenesis of ACD in RA. Decreased iron release from the mononuclear phagocyte system (MPS) [6, 7], decreased iron absorption [8], and decreased erythropoietin responsiveness to the anemia [9] have been claimed to play a role in the pathogenesis of ACD. More recently, investigations have also been concerned with a possible role of interleukins as mediators. For instance, interleukin-1 (IL-1) and tumor necrosis factor (TNF) were able to suppress erythropoiesis in vitro [10, 11].

Interleukin-6 (IL-6) is a monokine with biological activities related to inflammatory responses [12, 13]. IL-6 levels were elevated in serum and synovial fluid of patients with active RA [14, 15]. About the effects of IL-6 on bone marrow only few data exist [16].

Here we report experiments dealing with a possible role of IL-6 in determining anemia in RA.

Patients and methods

Serum from 21 patients (6 male, 15 female) with classical or definite RA [17] and bone marrow from 21 RA patients and 5 normal donors were studied after obtaining patients' written informed consent. RA patients were divided into two groups: group I consisted of 9 nonanemic patients and group II of 12 patients with ACD. Patients who had iron, vitamin B12, or folic acid treatment recently, or patients with a present or past ulcer history, hematuria, hypernephrosis, positive occult fecal blood test, hemolysis, iron, vitamin B12, or folic acid deficiency, or decreased creatinine clearance were excluded. Patients using corticosteroids or cytostatic drugs were also excluded. Mean overall disease duration had been 7 years (range 4–17); 74% used long-acting antirheumatic drugs and 88% used nonsteroidal antiinflammatory drugs. Mean age was 64 years. These characteristics did not differ between groups I and II.

Laboratory procedures. Hemoglobin (Hb), hematocrit (Ht), reticulocytes, and ferritin and erythrocyte sedimentation rates (ESR) were measured using standard laboratory procedures. C-reactive protein (CRP) was assessed using immunodiffusion techniques and Rose test using sensitized sheep erythrocytes. A titer over 1/32 was considered positive [18].

IL-6 in serum was measured in the B9 assay [19]. Serum was heated for 30 min at 56 °C and a titration was added to 5000 B9 cells and compared with standard IL-6 preparation. After 3 days proliferation was measured by thymidine incorporation; 1 U/ml is a concentration that leads to one-half maximal proliferation. In 100 healthy individuals serum levels were less than 10 U/ml.

Bone marrow was aspirated after posterior superior iliac crest puncture. Iron content was measured using Perls' Prussian blue
Table 1. Erythrocyte parameters, parameters of disease activity, IL-6, and burst forming units (BFUe) per 10⁵ cells in RA patients without anaemia and ACD

<table>
<thead>
<tr>
<th></th>
<th>Hb (mmol/l)</th>
<th>Ht (L/L)</th>
<th>Retics (0/00)</th>
<th>BFUe (col. per 10⁵ cells)</th>
<th>ESR (mm/h)</th>
<th>CRP (mg/l)</th>
<th>Rose reciprocal titer</th>
<th>IL-6</th>
<th>Ferritin (µg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonanemic RA (n=9)</td>
<td>7.9 (7.5-8.3)</td>
<td>0.39 (0.35-0.42)</td>
<td>17 (5-39)</td>
<td>260 (215-391)</td>
<td>34 (21-60)</td>
<td>8 (4-45)</td>
<td>32 (0-256)</td>
<td>39</td>
<td>(0-11) (12-86)</td>
</tr>
<tr>
<td>ACD RA (n=12)</td>
<td>6.6* (5.4-7.3)</td>
<td>0.31* (0.28-0.34)</td>
<td>17 (1-28)</td>
<td>191** (123-108)</td>
<td>74* (40-105)</td>
<td>62** (10-121)</td>
<td>32 (0-256)</td>
<td>29*** (0-82)</td>
<td>108** (39-410)</td>
</tr>
</tbody>
</table>

* P<0.001, ** P<0.01, ***P<0.10. Values are expressed as median with range

Table 2. Standard BFUe count (no addition; counts per 10⁵ cells incubated) and absolute (abs) and relative (%) change in BFUe count after addition of IL-6 (1000 U/ml) and anti-IL-6 (1000 U/ml) to the cultures in controls, nonanemic, and anemic RA patients

<table>
<thead>
<tr>
<th>Addition</th>
<th>Controls (n=5)</th>
<th>Nonanemic RA (n=3)</th>
<th>ACD RA (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>abs (%</td>
<td>abs (%)</td>
<td>abs (%)</td>
</tr>
<tr>
<td>None</td>
<td>356 (254-396)</td>
<td>100</td>
<td>246 (242-282)</td>
</tr>
<tr>
<td>IL-6</td>
<td>294 (168-318)</td>
<td>111 ± 2* (109-113)</td>
<td>155 (6-471)</td>
</tr>
<tr>
<td>Anti-IL-6</td>
<td>228* (180-244)</td>
<td>86 ± 10* (74-99)</td>
<td>135* (8-329)</td>
</tr>
</tbody>
</table>

* P<0.10, ** P<0.05, ***P<0.01; difference compared with standard BFUe count. Values expressed as median with range (percentages as mean and standard deviation)

Results

Parameters of erythropoiesis, disease activity and IL-6 in RA patients without and with anaemia

The BFUe count was significantly lower in group II compared with group I (Table 1). Reticulocytes did not differ between the two groups.

The ESR and CRP were significantly higher in group II. Hb correlated negatively with ESR (r=-0.71; P<0.001) and CRP (r=-0.68; P<0.005). The BFUe count correlated negatively with ESR (r=-0.65; P>0.005) and CRP (r=-0.66; P<0.01). No differences in Rose titer were found.

Serum IL-6 tended to be higher in group II. IL-6 correlated positively with ESR (r=0.46; P<0.025) and CRP (r=0.52; P<0.025) and negatively with Hb (r=-0.41; P<0.05) but not with BFUe.

Effects of addition of IL-6 and anti-IL-6 to bone marrow cultures (Table 2)

The BFUe count was higher in controls compared with ACD patients (P<0.05). The difference between controls (healthy donors) and nonanemics was not significant.