Defective iron supply for erythropoiesis and adequate endogenous erythropoietin production in the anemia associated with systemic-onset juvenile chronic arthritis

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Defective Iron Supply for Erythropoiesis and Adequate Endogenous Erythropoietin Production in the Anemia Associated With Systemic-Onset Juvenile Chronic Arthritis

By Mario Cazzola, Luisa Ponchio, Fabrizio de Benedetti, Angelo Ravelli, Vittorio Rosti, Yves Beguin, Rosangela Invernizzi, Giovanni Barosi, and Alberto Martini

Systemic-onset juvenile chronic arthritis (SoJCA) is associated with high levels of circulating interleukin-6 (IL-6) and is frequently complicated by severe microcytic anemia whose pathogenesis is unclear. Therefore, we studied 20 consecutive SoJCA patients with hemoglobin (Hb) levels <12 g/dL, evaluating erythroid progenitor proliferation, endogenous erythropoietin production, body iron status, and iron supply for erythropoiesis. Hb concentrations ranged from 6.5 to 11.9 g/dL. Hb level was directly related to mean corpuscular volume (r = .82, P < .001) and inversely related to circulating transferrin receptor (r = -.81, P < .001), suggesting that the severity of anemia was directly proportional to the degree of iron-deficient erythropoiesis. Serum ferritin ranged from 18 to 1,660 µg/L and was unrelated to Hb level. Bone marrow iron stores were markedly reduced in the three children investigated, and they also showed increased serum transferrin receptor and normal-to-high serum ferritin. All 20 patients had elevated IL-6 levels and normal intracellular growth of erythroid progenitors. Endogenous erythropoietin (epo) production was inappropriate for the degree of anemia as judged by both the observed to predicted log (serum epo) ratio (0.95 ± 0.12) and a comparison of the serum epo-Hb regression found in these subjects with that of thalassemia patients. Multiple regression analysis showed that serum transferrin receptor was the parameter most closely related to hemoglobin concentration: variation in circulating transferrin receptor explained 61% of the variation in Hb level (P < .001). In 10 severely anemic patients, amelioration of anemia following intravenous iron administration resulted in normalization of serum transferrin receptor. Defective iron supply to the erythron rather than blunted epo production is the major cause of the microcytic anemia associated with SoJCA. A true body-iron deficiency caused by decreased iron absorption likely complicates long-standing inflammation in the most anemic children, and this can be recognized by high serum transferrin receptor levels. Although oral iron is of no benefit, intravenous iron saccharate is a safe and effective means for improving iron availability for erythropoiesis and correcting this anemia. Thus, while chronically high endogenous IL-6 levels do not appear to blunt epo production, they are probably responsible for the observed abnormalities in iron metabolism. Anemia of chronic disease encompasses a variety of anemic conditions whose peculiar features may specifically correlate with the type of cytokine(s) predominantly released.

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MATERIALS AND METHODS

Patients and study design. All consecutive patients observed at the Department of Pediatrics of the IRCCS Policlinico S. Matteo Hospital between April 1991 and June 1994 who fulfilled the criteria for a diagnosis of SoJCA were considered for the study. Informed consent was obtained from the parents. Follow-up studies were continued until November 1994. This study was originally designed to identify the mechanism(s) of anemia in individual patients with SoJCA and to provide them with the most appropriate treatment. Therapeutic options included: (1) oral iron; (2) IV iron; (3) administration of recombinant human epo (rHuEpo). Oral iron was planned as a first-line treatment; nonresponders were then to be moved to IV iron. If a response was still not achieved and there was evidence of blunted endogenous epo (SoJCA) frequently develop severe microcytic anemia. The extreme microcytosis of these young patients indicates that markedly impaired iron supply to the erythroid marrow may be a major pathogenetic mechanism. Some of the investigators had previously found a strong correlation between serum IL-6 levels and joint involvement and thrombocytosis in SoJCA, suggesting that IL-6 may play a significant role in the pathogenesis of this disease. It is presently unclear whether IL-6 is also involved in the pathogenesis of anemia.

In the present work, we studied the mechanisms of anemia in 20 consecutive anemic children with SoJCA by evaluating endogenous epo production, erythroid marrow proliferation, body-iron status, and adequacy of iron supply to the erythroid marrow. In a companion report, we have already shown that intravenous (IV) iron administration can be effective in correcting severe long-lasting anemia associated with SoJCA.
production, rHuEpo administration was considered as the final therapeutic option.

To be included in the study patients had to display hemoglobin (Hb) levels <12 g/dL in two consecutive samples drawn more than 3 months apart, be available for regular follow-up during the study, have normal vitamin B12 and folate serum levels, and show no evidence of α- or β-thalassemia trait. No investigation for thalassemia was performed in children with at least one normal mean corpuscular volume (MCV) value in previous testing. Quantitation of Hb A2 in the patient and blood cell counts in the parents were routinely performed in cases showing exclusively low MCV values.

Twenty SoJCA patients met the above criteria: 13 males and 7 females with a median age of 7.7 years (range, 1.5 to 17.5). These 20 subjects included the 8 children previously reported in the companion report."14 All patients were treated with nonsteroidal anti-inflammatory drugs; 9 were given low-dose weekly methotrexate and 11 received low-dose prednisone. None of the participants showed evidence of blood loss as evaluated by three consecutive Hemocult tests of stools. Disease activity was assessed by measuring Westergren erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), and by counting the number of active joints.

After a preliminary work-up only the 17 patients showing Hb levels <10 g/dL were considered suitable for treatment. These subjects were first treated with vials of oral iron (ferrous sulphate) at a dose of 3 mg/kg/d for 8 weeks. Two children discontinued iron after a few days so that only 15 were evaluable. Forty-one of these 15 showed an increase in Hb level <1 g/dL at the end of this period and were considered unresponsive to oral iron; the remaining child showed an Hb increase of 1.2 g/dL.

The next therapeutic option, ie, IV iron therapy (iron oxide saccharate, Ferrum Hausman; Laboratorien Hausman, St Gallen, Switzerland) was offered to the 14 children unresponsive to oral iron and discussed with their parents in each case. Informed consent was obtained for 11 children. One of these patients was subsequently lost at follow-up so that 10 participants were available for the efficacy studies of IV iron administration, including the 8 previously reported children."14 From a mean post-oral iron value of 8.0 ± 0.8 g/dL, Hb levels increased to 11.5 ± 0.9 g/dL after IV iron administration (P < .001). This amelioration of anemia and the results of studies on endogenous epo production reported below made the prospective use of rHuEpo unnecessary.

**Hematological profile and iron status.** Blood counts were determined with a Coulter Counter Model S (Couler, Hialeah, FL). Reticulocyte counts were performed by microscopic observation after staining with brilliant cresyl blue and corrected to account for anemia."21 A corrected reticulocyte count more than three times basal level in an anemic patient is taken to indicate adequate red blood cell production (ie, peripheral hemolysis), whereas an index of less than three times basal is assumed to represent impaired red blood cell production."21

Body-iron status was evaluated by measuring serum iron, total iron-binding capacity, and serum ferritin. This latter was determined with a radioimmunoassay method (Ramco Lab, Houston, TX). Prussian blue staining of marrow iron was performed as previously described using the packages STATISTICA/Mac (Statsoft, Tulsa, OK), Exstattrix (Select Micro Systems Inc, Yorktown Heights, NY) and DeltaGraph Pro 3 (DeltaPoint Inc, Monterey, CA), all run on a Macintosh Quadra 650 (Apple Computer Inc, Cupertino, CA) per-
A reticulocyte count ranged from 0.7% to 2.6% and was unrelated to Hb level, indicating impaired red blood cell production.

Between Hb and MCV with logic parameters of the 20 SoJCA patients are compared with those of normal controls in Table 1.

Iron and transferrin saturation were markedly reduced in all children, whereas serum ferritin levels were normal or elevated, extending from 18 to 1,660 pg/L (Table 1). As shown in Fig 1, there was an inverse exponential relationship between Hb and MCV ($r = -0.82$, $P < .0001$). The corrected reticulocyte count ranged from 0.7% to 2.6% and was unrelated to Hb level, indicating impaired red blood cell production.

Iron and transferrin saturation were markedly reduced in all children, whereas serum ferritin levels were normal or elevated, extending from 18 to 1,660 pg/L (Table 1). Although there was no relationship between Hb and serum ferritin, this latter parameter was directly related to ESR ($r = .50$, $P < .05$). In addition, patients with ESR values $>80$ mm/h had higher ferritin levels than those of patients with ESR values $\leq 80$ mm/h (Table 2).

Previous studies have recommended adjusted serum ferritin cutoffs of 60 to 70 µg/L for defining iron depletion in patients with concomitant inflammation. We used two cutoffs, 60 µg/L and the median value of 133 µg/L, to divide patients into subgroups and to identify any significant differences (Table 2). Patients with ferritin levels $<60$ µg/L had lower MCV ($P < .05$) but their Hb values did not differ significantly from those of patients with serum ferritin $\geq 60$ µg/L. Using a cutoff of 70 µg/L yielded identical results, whereas a cutoff of 133 µg/L (median value) was useless.

Circulating serum TfR levels ranged from 5.0 to 41.5 mg/L. As shown in Fig 1, there was an inverse exponential relationship between this parameter and Hb level ($r = -0.81$, $P < .0001$). Using the median value for serum TfR of 10.4 mg/L, SoJCA patients were split into two groups with significantly different Hb and MCV values (Table 2). Using the upper normal limit of 7.1 mg/dL would have resulted in two extremely unequal subgroups (4 vs 16 subjects).

Fig 1. Relationship between Hb level and MCV (A) and serum TfR (B).

### Table 1. Hematologic and Iron Status Parameters in 20 Patients With SoJCA and in a Normal Control Population

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patients (n = 20)</th>
<th>Reference Values (n = 30)</th>
<th>ANOVA (F test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb, g/dL</td>
<td>8.9 ± 1.4 (6.5-11.9)</td>
<td>13.2 ± 1.3 (11.4-15.5)</td>
<td>$P &lt; .001$</td>
</tr>
<tr>
<td>MCV, FL</td>
<td>66 ± 7 (55-77)</td>
<td>82 ± 3 (77-89)</td>
<td>$P &lt; .001$</td>
</tr>
<tr>
<td>Serum iron, µg/dL</td>
<td>18 ± 12 (5-47)</td>
<td>83 ± 22 (57-135)</td>
<td>$P &lt; .001$</td>
</tr>
<tr>
<td>Transferrin saturation, %</td>
<td>5 ± 4 (2-17)</td>
<td>25 ± 6 (16-38)</td>
<td>$P &lt; .001$</td>
</tr>
<tr>
<td>Serum ferritin, µg/dL</td>
<td>278 ± 416 (18-1660)</td>
<td>41 ± 15 (18-78)</td>
<td>$P = .004$</td>
</tr>
<tr>
<td>Serum transferrin receptor, mg/L</td>
<td>14.4 ± 9.7 (5.0-41.5)</td>
<td>5.4 ± 1.7 (2.8-9.9)</td>
<td>$P &lt; .001$</td>
</tr>
<tr>
<td>Serum erythropoietin, mU/mL</td>
<td>65 ± 60 (18-221)</td>
<td>17 ± 6 (8-36)</td>
<td>$P &lt; .001$</td>
</tr>
<tr>
<td>Erythropoietin O/P ratio</td>
<td>0.95 ± 0.12 (0.76-1.18)</td>
<td>0.96 ± 0.10 (0.76-1.16)</td>
<td>$P = .80$</td>
</tr>
<tr>
<td>Circulating BFU-E, no./µL*</td>
<td>225 ± 90 (85-385)</td>
<td>237 ± 72 (88-321)</td>
<td>$P = .64$</td>
</tr>
</tbody>
</table>

Reference values were obtained in 30 normal subjects matched for sex and age. Data are expressed as mean ± 1 SD with range in parentheses. * BFU-E were studied in 20/30 reference subjects.
MARROW IRON SUPPLY AND HIGH IL-6 LEVELS

The normal range, indicating that endogenous epo production was adequate for the degree of anemia. There was no relationship between the O/P ratio and circulating TfR, ESR, CRP, or IL-6 level.

To further evaluate the adequacy of endogenous epo production, we compared the serum epo-Hb regression observed in patients with SoJCA to that obtained in a group of 20 thalassemia intermedia patients. As shown in Fig 2, the regression curves did not differ significantly with respect to either slope or location, indicating that the two populations examined (SoJCA v thalassemia intermedia) were indistinguishable regarding the regulation of epo production.

In 6 children who were treated with IV iron saccharate and responded with amelioration or complete correction of anemia, serum epo was measured sequentially following iron administration. As illustrated in Fig 3, improvement in Hb concentration was associated with a proportional decrease in serum epo; all points fell within the previously defined 95% confidence interval, indicating physiological regulation of endogenous epo production.

Mean circulating BFU-E numbered 225 ± 90 per mL of blood, which was not significantly different from the mean normal value of 237 ± 72 (Table 1). There was no relationship between BFU-E counts and Hb level.

Multiple regression analysis for identification of factors influencing Hb level. Multiple linear regression analysis was performed to study the relationship between Hb concentration and the following parameters: reticulocyte count, white blood cell (WBC) count, platelet (PLT) count, serum iron, serum ferritin, serum TfR, ESR, IL-6, epo O/P ratio, circulating BFU-E. Serum TfR was found to be the one most closely related to Hb concentration: 61% of the variation in Hb level was explained by variations in serum TfR concentration. Multiple correlation coefficient increased the multiple correlation coefficient (multiple r) to 0.88; the combination of serum TfR, serum iron, and PLT count explained 74% of the variation in Hb concentration.

Influence of IV iron therapy on Hb level and circulating TfR level. Ten patients received IV iron saccharate and all responded with amelioration of anemia. As shown in Fig 4, this resulted in a progressive decrease in the circulating TfR level. Analysis of variance showed that changes in Hb level and serum TfR were significantly different from zero (P < .001).

DISCUSSION

Juvenile chronic arthritis is a clinically heterogeneous condition currently divided into different clinical forms based

### Table 2. Comparison Between Patients Showing Values for ESR, Serum Ferritin, or Circulating Transferrin Receptor (TfR) < or ≥ Defined Cutoffs

<table>
<thead>
<tr>
<th>Patients</th>
<th>Hb, g/dL</th>
<th>MCV, fl</th>
<th>Transferrin Receptor, mg/L</th>
<th>Serum Ferritin, μg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients (n = 20)</td>
<td>8.9 ± 1.4</td>
<td>66 ± 7</td>
<td>14.4 ± 9.7</td>
<td>278 ± 416</td>
</tr>
<tr>
<td>ESR ≤ 80 mm/h (n = 10)</td>
<td>9.0 ± 1.5</td>
<td>65 ± 6</td>
<td>15.0 ± 11.4</td>
<td>107 ± 98</td>
</tr>
<tr>
<td>ESR &gt; 80 mm/h (n = 10)</td>
<td>8.9 ± 1.4</td>
<td>67 ± 7</td>
<td>13.7 ± 8.2</td>
<td>449 ± 540</td>
</tr>
<tr>
<td>Ferritin ≤ 60 μg/L (n = 5)</td>
<td>8.1 ± 1.2</td>
<td>61 ± 3</td>
<td>20.8 ± 14.1</td>
<td>—</td>
</tr>
<tr>
<td>Ferritin &gt; 60 μg/L (n = 15)</td>
<td>9.2 ± 1.4</td>
<td>68 ± 7</td>
<td>12.2 ± 7.1</td>
<td>—</td>
</tr>
<tr>
<td>Ferritin = 133 μg/L (n = 10)</td>
<td>8.6 ± 1.2</td>
<td>65 ± 6</td>
<td>15.2 ± 11.2</td>
<td>—</td>
</tr>
<tr>
<td>Ferritin &gt; 133 μg/L (n = 10)</td>
<td>9.3 ± 1.4</td>
<td>67 ± 7</td>
<td>13.6 ± 8.4</td>
<td>—</td>
</tr>
<tr>
<td>TfR ≤ 10.4 mg/L (n = 10)</td>
<td>8.1 ± 1.2</td>
<td>61 ± 4</td>
<td>—</td>
<td>311 ± 492</td>
</tr>
<tr>
<td>TfR &gt; 10.4 mg/L (n = 10)</td>
<td>9.7 ± 1.0</td>
<td>71 ± 6</td>
<td>—</td>
<td>244 ± 349</td>
</tr>
</tbody>
</table>

80 mm/h is the median ESR value of the study population; 60 μg/L is the discriminant cutoff proposed by Hansen and Hansen for the prediction of iron-responsive anemia in patients with rheumatoid arthritis, whereas 133 μg/L is the median ferritin concentration of the present patient population; 10.4 mg/L is the median TfR concentration. Comparison was performed by using one-way analysis of variance; values for serum ferritin were analyzed after log transformation. *P* values are shown just below the pertinent means; NS = P > .05.

### Table 3. BM Stainable Iron and Laboratory Parameters of Body-Iron Status in Three SoJCA Children

<table>
<thead>
<tr>
<th>Patient Age (yr)/Sex</th>
<th>Erythroblastic Score</th>
<th>Noneerythroblastic Score</th>
<th>Serum Ferritin, μg/L</th>
<th>Serum Transferrin Receptor, mg/L</th>
<th>ESR, mm/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>SoJCA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15/M</td>
<td>0</td>
<td>+</td>
<td>156</td>
<td>12.7</td>
<td>62</td>
</tr>
<tr>
<td>1.5/M</td>
<td>0</td>
<td>+</td>
<td>84</td>
<td>12.9</td>
<td>54</td>
</tr>
<tr>
<td>1.7/M</td>
<td>0</td>
<td>+</td>
<td>50</td>
<td>14.9</td>
<td>70</td>
</tr>
<tr>
<td>Normal values</td>
<td>18-54</td>
<td>++/++++</td>
<td>18-76</td>
<td>2.9-7.1</td>
<td>&lt;20</td>
</tr>
</tbody>
</table>
Fig 2. Relationship of serum epo to Hb concentration in 20 SoJCA (0) patients and in 20 β-thalassemia intermedia (●) patients. The exponential regression curves are shown (the upper one refers to SoJCA, the lower one to thalassemia intermedia). Regression equations were the following: In epo = 7.68 - 0.43 Hb (r = -0.73, P < .001) for SoJCA, and In epo = 7.61 - 0.40 Hb (r = -0.80, P < .001) for thalassemia intermedia patients. Univariate tests showed no significant difference between the two groups with respect to either Hb or serum epo. Multivariate tests (MANCOVA: Wilks' Lambda and Pillai-Bartlett Trace) showed no significant difference between the two regression curves with respect to either slope or location.

Fig 3. Relationship between Hb level and serum epo in seven SoJCA patients. These subjects were studied sequentially at different times as Hb level improved after IV iron therapy.

on symptoms at onset. The systemic form, or SoJCA, is characterized by chronic arthritis associated with high spiking fever, other systemic features and prominent laboratory evidence of inflammation. Because IL-6 plays an important role in the pathogenesis of SoJCA, this condition may also be viewed as a model for studying the effect of excessive endogenous IL-6 production on erythropoiesis and iron metabolism.

Severe microcytic anemia is found not only in children with SoJCA but also in adult patients with Still's disease, in whom IL-6 production is increased, and in mice transplanted with hematopoietic cells constitutively expressing human IL-6. Therefore, IL-6 is an attractive candidate as a mediator of severe microcytic anemia in chronic arthritis. Theoretically, IL-6 may inhibit erythroid progenitor proliferation, blunt epo production, or impair iron supply for erythropoiesis.

Available evidence argues against any inhibitory effect of IL-6 on erythroid progenitors. In fact, IL-6 does not impair in vitro erythroid colony formation whereas it can synergize with other hematopoietic growth factors to enhance hematopoiesis. Results of the present study indicate that the number of circulating BFU-E in SoJCA patients is in the normal range in spite of elevated IL-6 levels. Similarly, in a recent phase I-II study on cancer patients, the number of BFU-E was not affected by 7 days of recombinant human IL-6 treatment, excluding any suppressive effect on in vivo erythropoiesis.

An impaired epo response to anemia is believed to be typical of chronic disease. Faquin et al showed that several cytokines, including IL-1, TNF-α, and TGF-β, inhibited hypoxia-induced epo production from the hepatoma cell line Hep3B. Interestingly, however, the addition of IL-6 to hypoxic Hep3B cells resulted in dose-dependent stimulation of hypoxia-induced epo production. In phase I-II clinical trials on IL-6 administration to cancer patients, epo levels increased in a dose-dependent manner and decreased after cessation of IL-6. In the present study, no patient displayed evidence of defective endogenous epo production, suggesting that this latter mechanism is not involved in the pathogenesis of the anemia associated with SoJCA.

We believe that a large body of evidence points to the fact that the chronic anemia associated with excessive IL-6
production, including that of SoJCA, is mainly determined by a severe impairment of the iron supply to the developing erythroid cells. Administration of recombinant human IL-6 to humans\textsuperscript{35,36} first provokes a rapid and reversible dilution anemia due to an increase in plasma volume; it also results in a rapid decrease in serum iron\textsuperscript{34,35} and a tendency toward development of microcytosis.\textsuperscript{33} However, because of the short exposure to IL-6 in this setting, hypoferremia is unlikely to considerably contribute to anemia.

The question is different with SoJCA patients who are exposed to high levels of endogenous IL-6 for long periods of time. In the present study, serum TIR was the parameter most closely related to Hb concentration. The erythroid marrow is the main source of soluble TIRs, and receptor density on erythroblasts increases as erythropoiesis becomes iron deficient; this in turn results in increased release of truncated receptors and elevated serum levels.\textsuperscript{17} Therefore, the above relationship indicates that the degree of iron-deficient erythropoiesis is proportional to the severity of anemia. The utility of circulating TIR in diagnosing iron-deficient erythropoiesis in patients with active inflammatory processes has already been shown.\textsuperscript{97,38}

The defective supply of iron to developing erythroid cells, responsible for the anemia associated with SoJCA, may be the consequence of both severe reticuloendothelial iron block and true iron deficiency. Harvey et al\textsuperscript{13} suggested that the former mechanism is probably operating in most cases of active SoJCA. In our opinion, disease duration is also relevant to the pathogenesis of anemia. SoJCA patients at clinical onset\textsuperscript{13} as well as adult patients with Still's disease\textsuperscript{29} show the typical pattern of iron-deficient erythropoiesis caused by severe reticuloendothelial iron block. However, subjects with active rheumatoid arthritis have decreased iron absorption\textsuperscript{36} and our SoJCA patients with severe long-lasting anemia responded to IV but not to oral iron treatment, suggesting iron malabsorption. Impaired iron absorption can easily result in iron deficiency in a growing child with physiologically low body-iron stores. Data reported in Table 3 suggest that, although some iron may be trapped in the reticuloendothelial cells, a true iron deficiency is probably present in the most anemic children with long-lasting active SoJCA. Nonetheless, IV iron administration might not only correct iron deficiency but also overcome macrophage trapping to some extent, thereby also improving erythroid iron supply in patients whose major mechanism of anemia is a reticuloendothelial iron block.\textsuperscript{30}

Excessive production of IL-6 might be directly responsible for the observed abnormalities in iron metabolism. Studies in cellular and animal models indicate that this cytokine may enhance ferritin synthesis and increase hepatic uptake of serum iron.\textsuperscript{41,42} In turn, increased ferritin expression results in reticuloendothelial iron block and impairs iron absorption.

In conclusion, the chronic anemia associated with high IL-6 levels appears to be peculiar in that it is associated with adequate endogenous EPO production and is mainly caused by a defective iron supply for erythropoiesis. IV iron saccharate appears to be an effective treatment for SoJCA patients with severe microcytic anemia, especially for those showing elevated serum TIR levels. Individuals with persistent microcytic anemia unresponsive to oral iron should be considered for such treatment.

REFERENCES


