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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 in vitro growth of erythroid progenitors. Endogenous erythropoietin (epo) production was appropriate 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-lasting 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.

ANEMIA ASSOCIATED with rheumatoid arthritis is considered the prototype of anemia of inflammation; its pathogenesis is multifactorial and cytokines appear to play a crucial role.[1] Tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1), whose levels are increased in active rheumatoid arthritis,[1] inhibit erythroid progenitor proliferation[2-5] and may blunt erythropoietin (epo) response to anemia.[6] These cytokines are also responsible for the alterations in iron metabolism that result in reduced iron supply to the erythroid marrow.[1] An impaired epo response to anemia has been shown in adult patients with active rheumatoid arthritis,[7-9] and recombinant human epo has been found to be effective in ameliorating anemia in patients with active disease.[9-12]

Children with systemic-onset juvenile chronic arthritis (SoJCA) frequently develop severe microcytic anemia.[13] The extreme microcytosis of these young patients indicates that markedly impaired iron supply to the erythroid marrow may be a major pathogenetic mechanism.[14] 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.[15,16] 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.[17] In a companion report,[18] we have already shown that intravenous (IV) iron administration can be effective in correcting severe long-lasting anemia associated with SoJCA.

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[19,20] 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

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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 A_2 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.[18] All patients were treated with nonsteroidal antiinflammatory 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 Hemoccult 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. Fourteen 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.[18] 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 (Coulter, Hialeah, FL). Reticulocyte counts were performed by microscopic observation after staining with brillant 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 detailed[22] to evaluate both iron deposition in individual erythroblasts and reticuloendothelial iron stores. The positivity of erythroblasts was scored on each bone marrow (BM) specimen using the following criteria: 0, no stainable granules; 1+, 1 or 2 stainable granules; 2+, 3–5 stainable granules; 3+, >5 stainable granules. The positivity of reticuloendothelial cells was scored using the following criteria: 0, no reactivity; 1+, a few slightly positive cells or a few extracellular granules; 2+, some positive cells; 3+, numerous positive cells; 4+, massive positivity.

Serum epo assay. Circulating epo levels were measured by a commercially available radioimmunoassay (Incstar Corp, Stillwater, MN) that uses recombinant human epo for tracer and standards.[23] To define epo levels as appropriate or inappropriate for a given degree of anemia, an exponential regression of serum epo versus hematocrit (Hct) was determined in reference subjects (102 normal individuals or patients with iron deficiency anemia, hemolytic anemia, or hypoplastic anemia), and the 95% confidence limits were defined.[23] For Hct values ≤40%, the regression equation was: log(epo) = 3.42 − (0.056 × Hct). For Hct values >40%, the regression equation was: log(epo) = 1.31 − (0.003 × Hct). Based on these equations, the observed/predicted log(epo) ratio (O/P ratio) was derived for each sample. The mean O/P ratio in reference subjects was 1.01 ± 0.11 (95% confidence interval: 0.80–1.22).

Circulating erythroid progenitor assay. Growth of erythroid progenitors from peripheral blood (PB) mononuclear cells was assayed as previously described in detail.[24] Light-density mononuclear cells were separated from PB by centrifugation on a Ficoll-Hypaque gradient (Pharmacia Biotech, Uppsala, Sweden) (density 1.077 g/mL) at 400*g* for 40 minutes at 20°C. Interface cells were washed and suspended in Iscove's modified Dulbecco's medium (IMDM; Seromed, Berlin, Germany). Briefly, 5×10^5 mononuclear cells were plated in 35-mm Petri dishes in 1-mL aliquots of IMDM containing 30% fetal bovine serum (HyClone, Logan, UT), 5×10^{-5} mol/L 2-mercaptoethanol, 2 IU of epo, and 0.9% (wt/vol) methylcellulose. Assays were performed in duplicate. After incubation for 14 days at 37°C in a fully humidified atmosphere supplemented with 5% CO_2, the number of colonies was scored using an inverted microscope. Burst-forming units-erythroid (BFU-E) were defined as bursts of 3 or more hemoglobinized subcolonies consisting of at least 200 erythroid cells.

The total number of mononuclear cells obtained per unit volume of PB after separation on a Ficoll-Hypaque gradient was calculated to serve as a basis for the determination of the number of bursts grown per unit volume of PB. The following formula was used:

$$\frac{\text{BFU-E}}{\text{mL}} = \frac{\text{BFU-E}}{\text{Dish}} \times \frac{\text{Mononuclear Cells/mL}}{\text{No. of Mononuclear Cells Plated}}$$

Measurement of serum transferrin receptor (TfR). The amount of circulating TfR was estimated by an enzyme-linked polyclonal antibody assay, using purified placental receptor-transferrin complexes as a reference standard and rabbit antibodies as described in detail elsewhere.[23] The mean serum TfR level in 165 normal control subjects was 5.0 ± 1.1 mg/L, with 95% confidence limits ranging from 2.9 to 7.1 mg/L.

Serum IL-6 assay. Serum IL-6 levels were measured using the hybridoma cell line B9 (kindly provided by Dr L. Aarden, Netherlands Red Cross, Amsterdam), as described in detail elsewhere.[15,25] Chinese hamster ovary cell–derived recombinant human IL-6 (Genzyme Corp, Boston, MA) was used to culture B9 cells and as a standard in the assay. IL-6 was expressed as picograms per milliliter.

Reference populations. To define a normal reference population, we selected 30 normal children well matched with respect to sex and age.

Evaluation of the adequacy of endogenous epo production relies primarily on comparison with reference patients. This was performed by using two different approaches. First, the observed/predicted log(epo) ratio (O/P ratio) was calculated in each patient as described above. Second, we compared the serum epo-Hb regression found in SoJCA patients with that observed in 20 patients with β-thalassemia intermedia.

Data analysis and presentation. Data were stored, analyzed, and reported with the packages STATISTICA/Mac (StatSoft, Tulsa, OK), Exstatix (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-

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

	Patients (n = 20)	Reference Values (n = 30)	ANOVA (F test)
Hb, g/dL	8.9 ± 1.4 (6.5-11.9)	13.2 ± 1.3 (11.4-15.5)	$P < .001$
MCV, FL	66 ± 7 (55-77)	82 ± 3 (77-89)	$P < .001$
Serum iron, μg/dL	18 ± 12 (5-47)	83 ± 22 (57-135)	$P < .001$
Transferrin saturation, %	6 ± 4 (2-17)	25 ± 6 (16-38)	$P < .001$
Serum ferritin, μg/dL	278 ± 416 (18-1660)	41 ± 15 (18-76)	$P = .004$
Serum transferrin receptor, mg/L	14.4 ± 9.7 (5.0-41.5)	5.4 ± 1.7 (2.6-9.9)	$P < .001$
Serum erythropoietin, mU/mL	65 ± 60 (18-221)	17 ± 6 (8-35)	$P < .001$
Erythropoietin O/P ratio	0.95 ± 0.12 (0.76-1.18)	0.96 ± 0.10 (0.76-1.16)	$P = .80$
Circulating BFU-E, no./mL*	225 ± 90 (95-365)	237 ± 72 (88-321)	$P = .64$

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.

sonal computer. Results were expressed as mean ± 1 SD unless otherwise stated. Simple and multiple linear regression, and nonlinear regression analysis were used to identify the parameters most closely related to Hb. The Student's *t*-test and/or the F test (one-way analysis of variance) were used to evaluate the probability of significant differences between groups. *P* values less than .05 were considered statistically significant.

RESULTS

Red blood cell counts and body iron status. The hematologic parameters of the 20 SoJCA patients are compared with those of normal controls in Table 1. Hb levels ranged from 6.5 to 11.9 g/dL and the anemia was microcytic in all the examined children, with MCV varying from 55 to 77 fL. As shown in Fig 1, there was a direct relationship between Hb and MCV ($r = .82$, $P < .0001$). 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 μg/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 ≤ 80 mm/h (Table 2).

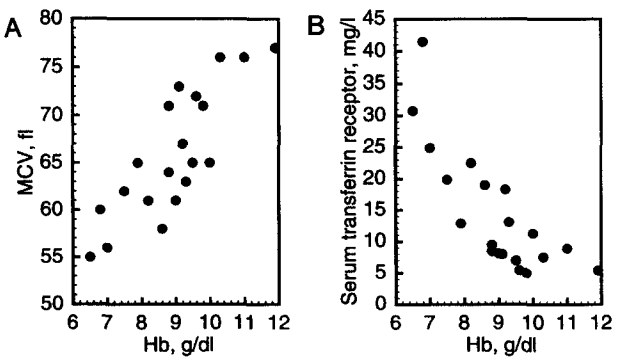

Fig 1. Relationship between Hb level and MCV (A) and serum TfR (B).

Previous studies have recommended adjusted serum ferritin cutoffs of 60 to 70 μg/L for defining iron depletion in patients with concomitant inflammation.[26,27] 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 ≥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 ($r = -.81$, $P < .0001$). 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 *v* 16 subjects).

Following parents' consent, BM examination was performed in three children whose Hb ranged from 6.7 to 7.8 g/dL in order to decide whether to proceed to IV iron. As shown in Table 3, none of the three children showed stainable iron in the erythroblasts and all presented markedly reduced reticuloendothelial iron stores; serum ferritin was normal to high, whereas serum TfR was clearly elevated. This pattern was consistent with a combination of iron deficiency and inflammation. A previous study on adult patients with Still's disease, ie, the adult counterpart of SoJCA,[28] also showed a reduction in erythroblastic iron but normal to increased nonerythroblastic iron with markedly elevated serum ferritin, as typically found in inflammation.

IL-6 levels. The severity of anemia was not correlated with disease activity, as judged by ESR or by the number of active joints (data not shown). All patients showed increased serum levels of IL-6; the median value was 485 pg/mL (range, 49 to 1,476 pg/mL). In our laboratory, 95% of normal subjects have undetectable IL-6 levels and the remaining 5% show values from 5 to 18 pg/mL.[25]

Although there was no relationship between Hb and IL-6 levels, linear regression analysis showed that IL-6 concentration was directly related to platelet count ($r = .64$, $P = .001$).

Endogenous epo production and erythroid proliferation. As shown in Table 1, all children had O/P ratio values within

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

Patients	Hb, g/dL	MCV, fL	Transferrin Receptor, mg/L	Serum Ferritin, μg/L
All patients (n = 20)	8.9 ± 1.4	66 ± 7	14.4 ± 9.7	278 ± 416
ESR ≤ 80 mm/h (n = 10)	9.0 ± 1.5	65 ± 6	15.0 ± 11.4	107 ± 98
ESR > 80 mm/h (n = 10)	8.9 ± 1.4	67 ± 7	13.7 ± 8.2	449 ± 540
	NS	NS	NS	$P < .01$
Ferritin ≤ 60 μg/L (n = 5)	8.1 ± 1.2	61 ± 3	20.8 ± 14.1	—
Ferritin > 60 μg/L (n = 15)	9.2 ± 1.4	68 ± 7	12.2 ± 7.1	—
	NS	$P < .05$	NS	
Ferritin ≤ 133 μg/L (n = 10)	8.6 ± 1.2	65 ± 6	15.2 ± 11.2	—
Ferritin > 133 μg/L (n = 10)	9.3 ± 1.4	67 ± 7	13.6 ± 8.4	—
	NS	NS	NS	
TfR ≤ 10.4 mg/L (n = 10)	8.1 ± 1.2	61 ± 4	—	311 ± 492
TfR > 10.4 mg/L (n = 10)	9.7 ± 1.0	71 ± 6	—	244 ± 349
	$P < .01$	$P < .001$		NS

80 mm/h is the median ESR value of the study population; 60 μg/L is the discriminant cutoff proposed by Hansen and Hansen[26] 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 = $P > .05$.

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,[23] 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 ($r = -.78$, $P < .0001$). Addition of serum iron and PLT count significantly 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 heterogenous condition currently divided into different clinical forms based

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

	BM Iron (Perl's)				
Patient Age (yr)/Sex	Erythroblastic, Score	Nonerythroblastic, Score	Serum Ferritin, μg/L	Serum Transferrin, Receptor, mg/L	ESR, mm/h
SoJCA					
15/M	0	+	156	12.7	62
1.5/M	0	+	84	12.9	54
1.7/M	0	+	50	14.9	70
Normal values	18-54	++/+++	18-76	2.9-7.1	<20

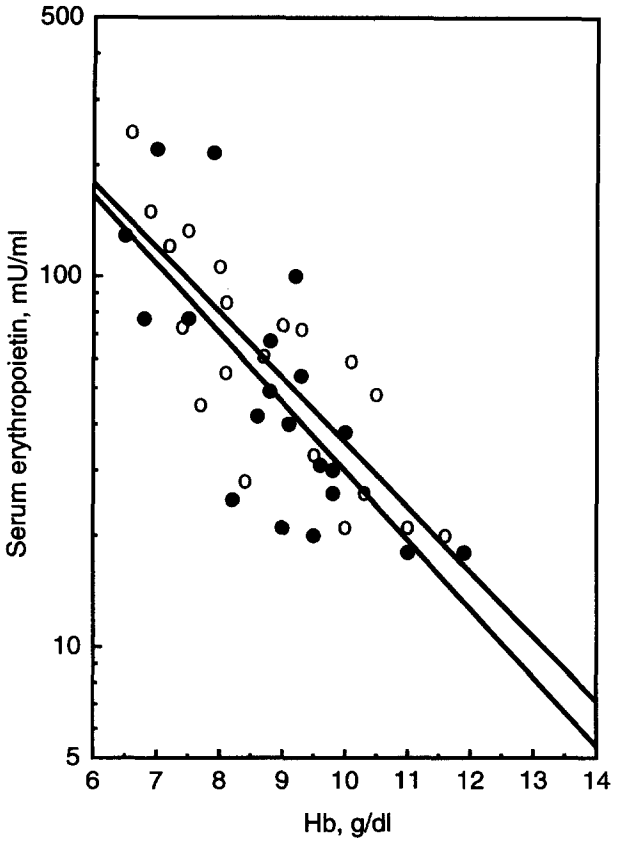

Fig 2. Relationship of serum epo to Hb concentration in 20 SoJCA (●) patients and in 20 β-thalassemia intermedia (○) patients. The exponential regression curves are shown (the upper one refers to SoJCA, the lower one to thalassemia intermedia). Regression equations were the following: ln epo = 7.68 − 0.43 Hb ($r = -.73$, $P < .001$) for SoJCA, and ln epo = 7.61 − 0.40 Hb ($r = -.80$, $P < .001$) 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.

on symptoms at onset.[19,20] 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,[15,16,25] 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,[29] and in mice transplanted with hematopoietic cells constitutively expressing human IL-6.[30] 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[31] whereas it can synergize with other hematopoietic growth factors to enhance hematopoiesis.[32,33] 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.[34]

An impaired epo response to anemia is believed to be typical of chronic disease.[1] Faquin et al[6] 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.[6] 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.[34,35] 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

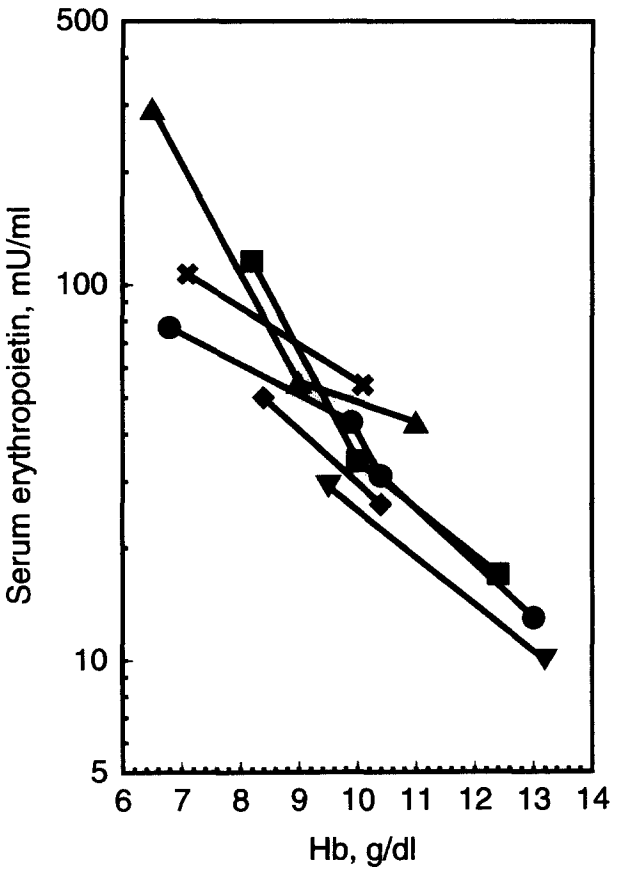

Fig 3. Relationship between Hb level and serum epo in seven SoJCA patients. These subjects were studied sequentially at different times as Hb level improved after IV iron therapy.

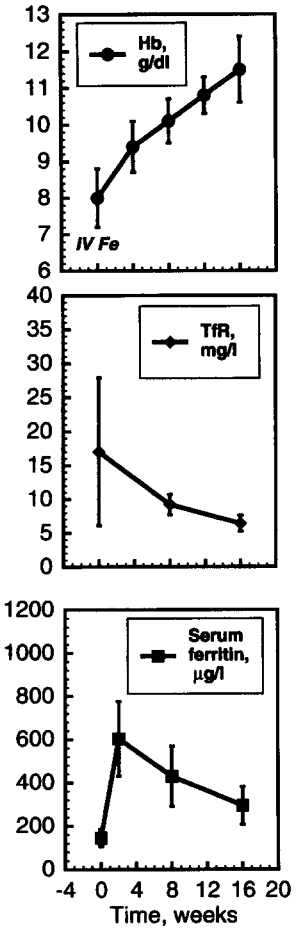

Fig 4. Time course of Hb, circulating TfR and serum ferritin after IV iron therapy in 10 SoJCA patients. Symbols and bars are mean values ± 1 SD (1 SEM for serum ferritin). Iron administration produced a sharp increase in serum ferritin followed by a subsequent slow decrease. This latter result was paralleled by increases in both Hb and serum TfR.

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[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[34,35] and a tendency toward development of microcytosis.[35] 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 TfR was the parameter most closely related to Hb concentration. The erythroid marrow is the main source of soluble TfRs, 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.[17] Therefore, the above relationship indicates that the degree of iron-deficient erythropoiesis is proportional to the severity of anemia. The utility of circulating TfR in diagnosing iron-deficient erythropoiesis in patients with active inflammatory processes has already been shown.[37,38]

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[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[13] as well as adult patients with Still's disease[28] show the typical pattern of iron-deficient erythropoiesis caused by severe reticuloendothelial iron block. However, subjects with active rheumatoid arthritis have decreased iron absorption[39] 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.[40]

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.[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 TfR levels. Individuals with persistent microcytic anemia unresponsive to oral iron should be considered for such treatment.

REFERENCES

1. Means RT, Krantz SB: Progress in understanding the pathogenesis of the anemia of chronic disease. Blood 80:1639, 1992

2. Johnson CS, Keckler DJ, Topper MI, Braunschweiger PG, Furmanski P: In vivo hematopoietic effects of recombinant interleukin-1α in mice: Stimulation of granulocytic, monocytic, megakaryocytic, and early erythroid progenitors, suppression of late-stage erythropoiesis, and reversal of erythroid suppression with erythropoietin. Blood 73:678, 1989

3. Johnson CS, Cook CA, Furmanski P: In vivo suppression of erythropoiesis by tumor necrosis factor-α (TNF-αa): Reversal with exogenous erythropoietin (EPO). Exp Hematol 18:109, 1990

4. Means RT, Dessypris EN, Krantz SB: Inhibition of human erythroid colony-forming units by tumor necrosis factor requires accessory cells. J Clin Invest 86:538, 1990

5. Means RT, Dessypris EN, Krantz SB: Inhibition of human erythroid colony-forming units by interleukin-1 is mediated by gamma interferon. J Cell Physiol 150:59, 1992

6. Faquin WC, Schneider TJ, Goldberg MA: Effect of inflammatory cytokines on hypoxia-induced erythropoietin production. Blood 79:1987, 1992

7. Baer AN, Dessypris EN, Goldwasser E, Krantz SB: Blunted erythropoietin response to anaemia in rheumatoid arthritis. Br J Haematol 66:479, 1987

8. Hochberg MC, Arnold CM, Hogan BB, Spivak JL: Serum immunoreactive erythropoietin in rheumatoid arthritis: Impaired response to anemia. Arthritis Rheum 31:1318, 1988

9. Takashima N, Kondo H, Kashiwazaki S: Suppressed serum erythropoietin response to anemia and the efficacy of recombinant erythropoietin in the anemia of rheumatoid arthritis. J Rheumatol 17:885, 1990

10. Pincus T, Olsen N, Russel IJ, Wolfe F, Harris ER, Schnitzer TJ, Boccagno JA, Krantz SB: Multicenter study of recombinant human erythropoietin in correction of anemia in rheumatoid arthritis. Am J Med 89:161, 1990

11. Salvarani C, Lasagni D, Casali B, Macchioni P, Boiardi L, Rossi F, Rivasi P, Portioli I: Recombinant human erythropoietin therapy in patients with rheumatoid arthritis with the anemia of chronic disease. J Rheumatol 18:1168, 1991

12. Bergström J: New aspects of erythropoietin treatment. J Intern Med 233:445, 1993

13. Harvey AR, Pippard MJ, Ansell BM: Microcytic anaemia in juvenile chronic arthritis. Scand J Rheumatol 16:53, 1987

14. Cazzola M, Pootrakul P, Bergamaschi G, Huebers HA, Eng M, Finch CA: The adequacy of iron supply for erythropoiesis: In vivo observations in humans. J Lab Clin Med 110:734, 1987

15. de Benedetti F, Massa M, Robbioni P, Ravelli A, Burgio GR, Martini A: Correlation of serum interleukin-6 levels with joint involvement and thrombocytosis in systemic juvenile rheumatoid arthritis. Arthritis Rheum 34:1158, 1991

16. de Benedetti F, Robbioni P, Massa M, Viola S, Albani S, Martini A: Serum interleukin-6 levels and joint involvement in polyarticular and pauciarticular juvenile chronic arthritis. Clin Exp Rheum 10:493, 1992

17. Cazzola M, Beguin Y: New tools for clinical evaluation of erythropoiesis and iron status in man. Br J Haematol 80:278, 1992

18. Martini A, Ravelli A, Di Fuccia G, Rosti V, Cazzola M, Barosi G: Intravenous iron therapy for severe anaemia in systemic-onset juvenile chronic arthritis. Lancet 344:1052, 1994

19. Brewer EJ Jr, Bass J, Baum J, Cassidy JT , Fink C, Jacobs J, Hanson V, Levinson JE, Schaller J, Stillman JS: Current proposed revision of JRA criteria. Arthritis Rheum 20:195, 1977 (suppl)

20. Wood PHN: Special meeting on: Nomenclature and classification of arthritis in children, in Munthe E (ed): The Care of Rheumatic Children. Basel, Switzerland, Eular, 1978, p 47

21. Hillman RS, Finch CA: Red Cell Manual (ed 5). Philadelphia, PA, Davis, 1985

22. Invernizzi R, Cazzola M, De Fazio P, Rosti V, Ruggeri G, Arosio P: Immunocytochemical detection of ferritin in human bone marrow and peripheral blood cells using monoclonal antibodies specific for the H and L subunits. Br J Haematol 76:427, 1990

23. Beguin Y, Clemons GK, Pootrakul P, Fillet G: Quantitative assessment of erythropoiesis and functional classification of anemia based on measurements of serum transferrin receptor and erythropoietin. Blood 81:1067, 1993

24. Carlo Stella C, Cazzola M, Ganser A, Bergamaschi G, Pedrazzoli P, Hoelzer D, Ascari E: Synergistic antiproliferative effect of recombinant interferon gamma with recombinant interferon alpha on chronic myelogenous leukemia hematopoietic progenitor cells (CFU-GEMM, CFU-Mk, BFU-E, CFU-GM). Blood 72:1293, 1988

25. de Benedetti F, Massa M, Pignatti P, Albani S, Novick D, Martini A: Serum soluble interleukin 6 (IL-6) receptor and IL-6/soluble IL-6 receptor complex in systemic juvenile rheumatoid arthritis. J Clin Invest 93:2114, 1994

26. Hansen TM, Hansen NE: Serum ferritin as indicator of iron responsive anaemia in patients with rheumatoid arthritis. Ann Rheum Dis 45:596, 1986

27. Balaban EP, Sheehan RG, Demian SE, Cox JV, Frenkel EP: Evaluation of bone marrow iron stores in anemia associated with chronic disease: A comparative study of serum and red cell ferritin. Am J Hematol 42:177, 1993

28. Montecucco C, Caporali R, Invernizzi R: Iron status in Still's disease. Lancet 345:58, 1995 (letter)

29. Houssiau FA, Devogelaer JP, van Damme J, Nagant de Deuxchaisnes C, van Snick J: Interleukin 6 in synovial fluid and serum of patients with rheumatoid arthritis and other inflammatory arthritis. Arthritis Rheum 31:784, 1988

30. Brandt SJ, Bodine DM, Dunbar CE, Nienhuis AW: Dysregulated interleukin 6 expression produces a syndrome resembling Castleman's disease in mice. J Clin Invest 86:592, 1990

31. Vreungdenhil G, Lowenberg B, van Ejik HG, Swaak AJG: Anaemia of chronic disease in rheumatoid arthritis: raised serum interleukin 6 (IL-6) levels and the effects of IL-6 and anti-IL-6 on in vitro erythropoiesis. Rheumatol Int 10:127, 1990

32. Bernad A, Kopf M, Kulbacki R, Weich N, Koehler G, Gutierrezramos JC: Interleukin-6 is required in vivo for the regulation of stem cells and committed progenitors of the hematopoietic system. Immunity 1:725, 1994

33. Jacobsen SE, Ruscetti FW, Okkenhaug C, Lien E, Ortiz M, Veiby OP, Keller JR: Distinct and direct synergistic effects of IL-1 and IL-6 on proliferation and differentiation of primitive murine hematopoietic progenitor cells in vitro. Exp Hematol 22:1064, 1994

34. van Gameren MM, Willemse PHB, Mulder NH, Limburg PC, Groen HJM, Vellenga E, de Vries EGE: Effects of recombinant human interleukin-6 in cancer patients: a phase I-II study. Blood 84:1434, 1994

35. Nieken J, Mulder NH, Buter J, Vellenga E, Limburg PC, Piers DA, de Vries EGE: Recombinant human interleukin-6 induces a rapid and reversible anemia in cancer patients. Blood 86:900, 1995

36. Atkins MB, Kappler K, Mier JW, Isaacs RE, Berkman EM: Interleukin-6-associated anemia: Determination of the underlying mechanism. Blood 86:1288, 1995

37. Ferguson BJ, Skikne BS, Simpson KM, Baynes RD, Cook JD: Serum transferrin receptor distinguishes the anemia of chronic disease from iron deficiency anemia. J Lab Clin Med 19:385, 1992

38. Nielsen OJ, Andersen LS, Hansen NE, Hansen TM: Serum transferrin receptor levels in anemic patients with rheumatoid arthritis. Scand J Clin Lab Invest 54:75, 1994

39. Weber J, Werre JM, Julius HW, Marx JJM: Decreased iron absorption in patients with active rheumatoid arthritis, with and without iron deficiency. Ann Rheum Dis 47:404, 1988

40. Bentley DP, Williams P: Parenteral iron therapy in the anaemia of rheumatoid arthritis. Rheumatol Rehabil 21:88, 1982

41. Hirayama M, Kohgo Y, Kondo H, Shintani N, Fujikawa K, Sasaki K, Kato J, Niitsu Y: Regulation of iron metabolism in HepG2 cells: A possible role for cytokines in the hepatic deposition of iron. Hepatology 18:874, 1993

42. Kobune M, Kohgo Y, Katp J, Myazaki E, Niitsu Y: Interleukin-6 enchances hepatic transferrin uptake and ferritin expression in rats. Hematology 19:6, 1994