



Cyclosporin-A in severe refractory anemia of myelofibrosis with myeloid metaplasia: a preliminary report

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Abstract

Background and Objective. Severe anemia is the outstanding problem in approximately 50 percent of patients with myelofibrosis with myeloid metaplasia (MMM). The present trial was based on the considerations that abnormal immune responses are frequently associated with MMM and that cyclosporin A (Cy-A) has proven to be effective in improving anemia in autoimmune disorders. The aim of this study was to evaluate the effect of Cy-A on anemia of MMM.

Design and methods. We studied 10 patients with MMM and severe anemia who were not responsive to corticosteroids. Eight of them showed evidence of immune defects (direct or indirect Coombs' test, antinuclear or antimitochondrial antibodies, circulating immune complexes). Cy-A was delivered orally in two refracted doses of 5 mg per kilogram bw every day and the serum level of the drug was maintained between 100 and 200 ng/mL for at least 6 months. Clinical effects were measured by calculating a normalized transfusional need (NTN), and response was defined as about a 30% reduction in the initial transfusion requirement. Hematologic parameters, s-Epo, s-TfR, s-IL2R and lymphocyte flow cytometric analysis were also evaluated. The results were analyzed with the Student's t-test.

Results. Only 6 patients completed the entire 6 months of planned therapy. Three of these responded, with one no longer needing transfusions. A high CD4/CD8 ratio was predictive of response (mean value 4.7 ± 3.5 in responders versus 0.9 ± 0.4 in non-responders, $p=0.06$).

Interpretation and Conclusions. An immunomediated mechanism negatively affects erythropoiesis in MMM. Cy-A may be effective for patients with severe refractory anemia in this disease.

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Key words: cyclosporin A, anemia, myelofibrosis with myeloid metaplasia, immune disorders, CD4/CD8 ratio

Myelofibrosis with myeloid metaplasia (MMM) is a chronic myeloproliferative disorder with a heterogeneous clinical picture. Approximately 50% of patients have severe anemia at diagno-

sis or during the evolution of the disease.¹ First line treatment with corticosteroids and androgens is effective in one-third to half of all cases;² thus red blood cell (RBC) transfusions are frequently needed. The present study tested the efficacy of cyclosporin A (Cy-A) in treating the anemia of patients with MMM who were refractory to first line treatments. The rationale for this study stems from the considerations that Cy-A selectively inhibits immune responses mediated by T lymphocytes and has proven to be effective in improving anemia in disorders sustained by autoimmune mechanisms such as aplastic anemia,³ pure red cell aplasia,⁴ and immune hemolytic anemia.⁵ Disruption of the immune response with the development of Coombs'-positive autoimmune hemolytic anemia, nephrotic syndrome, antinuclear antibodies, rheumatoid factor, lupus-type anticoagulant and hypocomplementemia have all been documented in MMM.⁶⁻¹² T lymphocyte activation has also been reported and the poor prognostic significance of elevated soluble IL-2 receptor has been evidenced.¹³ These alterations suggest either clonal involvement of the lymphocyte population in the myeloproliferative disorder or secondary activation of the immune system due to abnormal monocyte-macrophage function. Another reason for this study was that patients responding to Cy-A have recently been described in other clonal myeloid disorders like myelodysplastic syndromes.^{14,15}

Materials and Methods

Subjects

Ten patients with MMM and severe anemia were evaluated according to a protocol approved by the hospital's ethical committee. MMM was diagnosed on the basis of bone marrow biopsy demonstrating moderate to severe bone marrow fibrosis, peripheral blood morphology showing immature erythroid and myeloid cells, tear drop erythrocytes, absence of absolute monocytosis, and splenomegaly. Myeloid metaplasia was documented by ferrokinetics in one patient, and by histological examination of the removed spleens in three others.

Patients were eligible to enter the study if they suffered from transfusion-dependent or pending-transfusion anemia, and showed no response to corticosteroid therapy (equivalent to a daily dose of 25 mg of

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prednisone) after 3 or more months of treatment. All patients had received other therapies, including androgens, 1-25-dihydroxyvitamin D3 or danazol. Three of them had been splenectomized from 1 month to three years earlier. None of the participants showed abnormal renal function (BUN and serum creatinine were normal) upon entry to the study.

Treatment

The patients were scheduled to receive a six-month course of Cy-A, 5 mg per kilogram per day orally. This dosage is recommended as a higher maintenance dose in most autoimmune diseases and was used in our study as an attack dosage. Cy-A was gradually tapered both to maintain plasma levels between 100 and 200 ng/dL and on the basis of renal function tests. The dose was reduced if the serum creatinine concentration increased 30% over baseline. The lower dosage was 3 mg per kilogram per day. Patients receiving corticosteroids at entry continued to receive them.

The primary endpoint of the study was improvement of anemia. Even though the general policy was to transfuse RBC when Hb levels dropped below 80 g/L, different transfusional regimens were active during the study. In order to compare various regimens among patients and at different periods of time, a normalized transfusional need (NTN) was calculated, defined as the number of RBC units theoretically needed to maintain the Hb value equal to 100 g/L. By assuming a linear relationship between the blood supplied and the Hb concentration actually maintained, the following proportion was used:

$$N. \text{ of RBC units given in one month} : \text{normalized mean Hb (g/L) maintained during the month} = \text{NTN} : 100$$

The normalized mean Hb value maintained in the monthly period was calculated by the area under the curve of all the Hb concentration values measured during that month. Patients whose NTN did not fall by 30% of the basal value for 2 consecutive measurements were considered not to have responded to the treatment. Biochemical studies, including tests of renal and hepatic function, were performed every month.

Assessment of erythropoiesis

Hematologic parameters, serum erythropoietin (s-Epo) and serum transferrin receptor (s-TfR) were tested at the time of enrolment and every month during treatment. Hemoglobin concentration, PCV, red blood cell count, erythrocyte indices and reticulocyte count were obtained with an automatic cell counter (ELT-800, Ortho Diagnostic System, Massachusetts, USA). S-Epo and s-TfR levels were determined using venous blood sampled without anticoagulant. Serum was separated by centrifugation within 12 hours of collection and stored at -20°C until thawed for assay. An enzyme-linked immunosorbent assay (CLINIGEN,

Amgen Diagnostic, Thousand Oaks, CA, USA) was employed. Intra- and inter-assay variation coefficients differed at the different sample concentrations but were always less than 10%.

Assessment of immune function

Tests for antinuclear antibodies (ANA, ENA, anti-dsDNA), antimitochondrial and anti-smooth muscle antibodies (AMA, ASMA), direct and indirect Coombs' and circulating immune complexes (CIC) were performed at the time of enrolment and on completion of treatment.

The serum interleukin-2 receptor (s-IL-2R) was assayed as a marker of T-lymphocyte activation using an enzyme immunoassay kit (T Cell Diagnostics, Inc. Woburn, MA, USA). Peripheral blood mononuclear cells were examined by flow cytometry analysis at the time of enrolment and every month during treatment. One hundred mL of whole blood were incubated with monoclonal antibodies (MoAbs) conjugated with FITC or PE and harvested with a Multi-Q-Prep technique (Coulter Ltd, Hialeah, FL, USA). Flow cytometry (two-color) was performed with an EPICS XL-MCL (Coulter, Ltd). T and B cells were identified by MoAbs against CD2, CD3, CD4, CD8, CD19 and CD20, and activated cells were detected by MoAbs HLA-DR and CD25, while NK cells were identified by CD56. All MoAbs were purchased from Coulter except CD56, which was obtained from Becton Dickinson (Mountain View, CA, USA).

Statistical analysis

Quantitative variables were compared with the two-tailed Student's t-test. A *p* value less than 0.05 was considered significant.

Results

Clinical and hematologic features

Mean age, duration of disease, duration of corticosteroid therapy before the study, the number of patients requiring blood transfusions and the number of those requiring concomitant medications before and during the trial are reported in Table 1. The hematologic parameters are also described in the same table. Besides anemia, 7 patients also presented leuko- or thrombocytopenia. Bone marrow biopsy documented a normocellular picture in one, hypocellular in 8, and selective erythroid aplasia in 1 patient.

Immunologic features

Laboratory signs of immunodysfunction were found in 8 patients: documented by a positive indirect Coombs' test in 3 patients who had been repeatedly transfused, by a direct Coombs' test in one, an ANA titer of 1:160 with a diffuse pattern in 2, an AMA titer of 1:40 in one and CIC in 2.

Flow cytometry analysis revealed that the percentage of CD4 cells was below the normal range in 1

Table 1. Baseline characteristics of the 10 study patients with MMM.

Mean age - yrs (range)	60.9 (42-72)
Sex - M/F	6/4
Mean duration of disease - months (range)	36.4 (3-147)
Concomitant corticosteroid therapy before and during the trial - no. of patients (%)	4 (40)
Mean duration of corticosteroid therapy before the study - days (range)	122 (20-540)
Transfusions - no. of patients (%)	8 (80)
Hemoglobin concentration at the beginning of the trial - mean (range) g/L	72 (48-95)
White blood cells count at the beginning of the trial - mean (range) $\times 10^9/L$	6.8 (1-24)
Platelet count at the beginning of the trial mean (range) $\times 10^9/L$	86.6 (9-236)
Spleen volume - no. of patients	
splenectomized	3
above the umbilical line	4
below the umbilical line	3
Bone marrow biopsy - no. of patients	
normocellular	1
hypocellular	8
erythroid aplasia	1

out of 8 patients and the percentage of CD8 cells was above normal in 5 out of 8, with low CD4 absolute counts in 3 patients and high CD8 counts in 2. Four patients had an increased percentage of CD3/HLA-DR-positive cells, indicating activation of cytotoxic T cells (Figure 1). The CD4/CD8 ratio was higher than normal in 2 out of 8 patients (3.2 and 8.8, respectively; normal values ranged from 1.3 to 2.9). Two of the 3 splenectomized patients showed the lowest values (respectively, 0.5 and 1). S-IL-2R was increased in 6 out of 8 patients (from 1103 to

6641 U/mL) with respect to the values detected in normal healthy subjects (range 237-943 U/mL).

Status of erythropoiesis

Reticulocyte count was normal or below normal in all but 3 patients, who presented with mild reticulocytosis (396 , 108 and $127 \times 10^9/L$, respectively). One of these also had decreased serum haptoglobin, mildly increased nonconjugated bilirubin (1.8 mg/dL) and a positive indirect Coombs' test. S-Epo ranged from 3.6 to 5031 mU/mL (reference range from 5 to 20 mU/mL). The ratio between observed and predicted values with respect to anemia¹⁶ was lower than 0.8 in one case, indicating that Epo production was inadequate for the degree of anemia. S-TfR ranged from 172 to 6818 ng/mL (normal reference values from 1470 to 3400 ng/mL). Only in one patient, who also presented a histological pattern of bone marrow hypoplasia, was the value lower than normal, documenting reduced erythropoiesis.

Response to cyclosporin treatment

Six of the 10 patients concluded the 6-month treatment schedule. One patient interrupted the drug treatment because of evolution toward blast transformation, 2 others did not comply with treatment and one developed progressive renal failure. Three of the 6 evaluable patients responded to treatment. One of these obtained complete independence from RBC transfusions. Before treatment he needed 6 units of RBC every month to maintain a mean pre-transfusional Hb level of 62 g/L. At the end of treatment his mean Hb concentration remained at 92 g/L without transfusions. Two other patients reduced their NTN from 2.9 and 1.8 units of blood/month to 1.2 and 1.1 , respectively. The improvement was slow in all patients: the reduction in transfusional need became apparent within 4 months after treatment was begun,

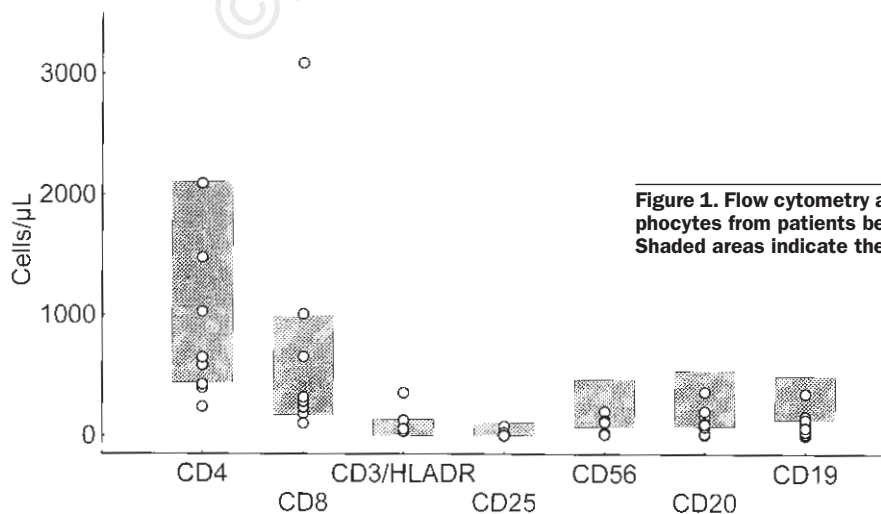


Figure 1. Flow cytometry analysis of peripheral blood lymphocytes from patients before treatment with Cy-A. Shaded areas indicate the normal range.

and the optimally responding patient was transfusion-independent at the sixth month.

During treatment, the reticulocyte count decreased in one patient who responded to therapy (from 77 to $49 \times 10^9/L$) and in another who did not respond (from 108 to $16 \times 10^9/L$). In both these patients s-TfR also declined: from 2242 to 1407 ng/mL in the former, and from 1679 to 834 ng/mL in the latter; however, a drop in sTfR (from $3,932$ to $2,388$ ng/mL) was also noted in a case that had no change in the reticulocyte count. No changes in the haptoglobin level were documented. S-Epo decreased in all patients during therapy: from a mean value of $1,491$ mU/mL, range $105-5,032$, to a mean value of 557 mU/mL, range $95-1,609$ ($p < 0.01$).

A decrease in the CD4/CD8 ratio was observed in 3 of the 6 evaluable patients. This was due to a decline in the CD4 subset of lymphocytes. At the end of the sixth months of therapy, an indirect Coombs' test was found to be negative in 2 of the 3 previously positive patients. ANA and AMA tests were negative in all patients. No changes in leukocyte or platelet counts were detected. IL2R decreased in one patient who did not respond (from $6,641$ to $4,070$ U/mL).

Three patients continued to receive the drug for periods ranging from 6 to 12 months after the conclusion of the study and they maintained the hematologic response. Thereafter there was a progressive increase in their transfusional need and therapy was subsequently stopped.

Predictors of response

Patients who responded to Cy-A therapy had the most severe transfusional need. NTN ranged from 1.8 to 2.9 (mean 2.4 ± 0.5) in responders, while it varied from 0 to 2 (mean 0.9 ± 1 , $p = 0.04$) in nonresponders. All the responders had an intact spleen and a positive indirect Coombs' test. The characteristic that best divided the patients who subsequently responded to Cy-A was the CD4/CD8 ratio, which showed a mean value of 4.7 ± 3.5 (range 2.3 to 8.8) in responders and 0.9 ± 0.4 (range 0.47 to 1.4) in nonresponders ($p = 0.06$). There was no association between response to Cy-A and the previous or concomitant use of corticosteroids.

Plasma cyclosporin-A concentration

Among the 6 patients who concluded the treatment, the mean plasma Cy-A concentration was 124.5 ng/mL (range 59 to 185 ng/mL). There was no association between plasma Cy-A concentration and the rapidity of response.

Adverse effects

Four of the 10 patients treated with Cy-A suffered nephrotoxicity. Four experienced paresthesias. One patient developed gingival hyperplasia that improved after Cy-A was discontinued.

Discussion

The immunosuppressive properties of cyclosporin A were the reason for its use in patients with MMM and severe anemia in this trial. A high frequency of immune defects has been described in MMM patients⁶⁻¹² and most of the resulting abnormalities are able to produce anemia. As a matter of fact, 70% of our patients had alloantibodies against transfused red cells, autoantibodies against red cells, antinuclear or antimitochondrial antibodies. This frequency of immunodysfunction was similar to that reported in the literature, which ranges from 10 to 80%.^{6-12,17}

This preliminary report documents that Cy-A is not an easy therapy to administer in patients with MMM. Four of 10 participants did not complete the scheduled 6 months of treatment: one due to evolution towards the blast phase of the disease, 2 due to non-compliance and one because of toxic effects on renal function.

Three out of the 6 evaluable patients responded to therapy, and one of these experienced complete disappearance of a severe transfusional need. A similar complete remission has been recently reported in a single case by Pietrasanta *et al.*¹⁹ Response was evaluated on the basis of an index that assessed the theoretical transfusional need required to maintain a Hb level of 100 g/L, which was useful for comparing patients with different transfusional schedules. These results seem to indicate that Cy-A is able to modify mechanisms that negatively affect erythropoiesis. Even though no significant lymphocytosis within the bone marrow sections had been reported in our patients, high levels of s-IL2R were reported in 6 out of 8 patients studied. These serum concentrations did not change during Cy-A therapy and the basal levels were not different in responders and nonresponders. So, no conclusion can be drawn on the meaning of this immune response index in patients with MMM, on the mechanism underlying Cy-A action.

A study of red cell production parameters supports the hypothesis of a peripheral effect of an immunosuppressive agent. In fact, reticulocyte count and s-TfR decreased in 2 and 3 patients, respectively, during treatment, indicating a reduction of hemolysis rather than a direct effect on erythroid progenitors.

Very severe anemia and a high CD4/CD8 ratio were the features that best predicted response to therapy. An elevated CD4/CD8 ratio was an unexpected finding in our patients since a reduction of this parameter is reported in patients with high transfusional needs, and splenectomy is known to increase the CD8 count without a proven effect on the CD4/CD8 ratio.²⁰ This suggests that a more detailed characterization of bone marrow and peripheral blood lymphocyte population would be of interest in these patients.

Further studies involving more patients are needed to substantiate our findings and confirm the possibility of response prediction.

Contributions and Acknowledgments

GB was responsible for the conception and the design of the study. He followed the clinical assessment of patients with EC and RG, who also performed the immuno-assay (EIA) measurements, the data handling and the statistical analysis. EC wrote the paper. GI, as chief-director of the Laboratory of Clinical Immunology, collaborated in evaluation of immunologic data.

Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

Manuscript processing

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