TREATMENT OF SEVERE APLASTIC ANEMIA WITH CYCLOSPORIN-A AND HIGH-DOSE METHYLPREDNISOLONE

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SUMMARY

15 patients with severe aplastic anemia were treated with an immunosuppresive regimen consisting of Cyclosporin-A(CsA) and highdose methylprednisolone (HMP). Initially CsA was given orally at a dosage of 10 mg/kg/day and monitoring the serum level of the drug, blood levels of 200-500 ng/ml were maintained thereafter. CsA treatment was continued at least 3 months at which time the treatment was terminated in unresponsive patients depending on the peripheral cell counts. HMP was administered simultaneously with CsA at 30 mg/kg/day IV for 3 days, 20 mg/kg/day IV for 3 days and 10 mg/kg/day IV for 4 days. Within 3 months improvement of hematopoiesis was seen in 6 patients (40 %). First signs of response were seen in 21-69 (34) days. This was followed by complete remission in 3 patients, partial remission in 2 patients and minimal improvement in 1 patient. Remissions were proved to be dependent on the continued administration of CsA. Sequential changes of T-cell subsets using APAAP method revealed an increasing ratio of T-helper cells to T-suppressor cells. This observation strongly suggests the role of T-cells in the pathogenesis of some cases of aplastic anemia.

INTRODUCTION

Aplastic anemias refer to a diverse group of potentially severe marrow disorders characterized by peripheral pancytopenia and a marrow that is largely devoid of hematopoietic cells but that retains the basic marrow architecture and stroma with replacement of hematopoietic cells by large amounts of fat. Aplastic anemia has been classified as severe when the following three criteria are present:

(*) anemia with corrected reticulocyte count <1% ,
(*) neutrophils <500 mm3,
(*) platelets <20.000 mm3.

In addition, marrow hypocellularity must be present (estimated as <25 % of marrow space) (1).

The pathogenesis of aplastic anemia remains elusive and different mechanisms are believed to be involved. Two dominant hypotheses have emerged: (I) a primary stem cell failure; that is, an intrinsic defect of the hematopoietic stem cell that renders it unable to differentiate; (II) an immunologically mediated attack either on the stem cell itself or on other components of the complex network of cells and factors that are necessary for normal hematopoiesis ,(1-3).

In the treatment of severe aplastic anemia (SAA) immune suppressive agents have been used widely. What immune suppressive agents do in these patients is unclear. They might eliminate a population of immune cells and interrupt an immune reaction against the donor stem cell, or they might eliminate residual defective stem cells from preferred architectural "niches" that are essential for normal stem cell differentiation, allowing access of the donor stem cells to these particular microenvironmental sites (2,3).

As the only available therapy in the era before bone marrow transplantation (BMT) and antilymphocyte and antithymocyte globulins (ALG and ATG, respectively), androgens have been widely used for the treatment of SAA. It is not clearly established that androgens induce remissions in severe aplastic anemia more frequently

than they occur spontaneously. It is now known that androgens improve hematopoiesis in a minority of patients only and apparently fail to do so in SAA (3,4). On the other hand ALG or ATG, complex heteroantisera raised in animals against thymic lymphocytes, can significantly ameliorate the course of aplastic anemia in many patients. These antisera are generally assumed to act by immunosuppression, but they also bind to many hematopoietic cells and may have other targets that are not presently defined. Unfortunately, different antisera vary considerably, in efficacy and there is no current method to assay the active components by means of other than in vivo therapeutic trials in humans (4).

Compared to other agents, cyclosporin-A (CsA) is characterized by an unique and potent immunosupressive activity (5). CsA acts primarily on T lymphocytes, possibly sparing supressor cell. It interferes with the elaboration of several lymphokines such as interleukin-2 (IL-2) and gamma interferon (IF), probably at the level of mRNA transcription (1-3). In this study cylosporin-A was used for the treatment of severe aplastic anemia in 15 patients, in combination with high dose methylprednisolone (HMP) and the results of the study, compared with that of other studies, are discussed in detail.

MATERIALS AND METHODS

15 patients (12 male and 3 female), all had severe aplastic anemia were included in this study. Main inclusion criteria were as follows:

1. Bicytopenia or pancytopenia with granulacytes $<1.0x10^9$ / L, reticulocytes $<60x10^9$ / L, platelets $<50x10^9$;

2. Hypocellular bone marrow in biopsy specimens.

Of the 15 patients, 4 had not been treated previously, 11 had required androgens and/or low dose corticosteroids 1-16 months before entry and 6 patients had also received ALG prior to this study with no repsonse.

All the patients were given CsA, 10 mg/kg b.i.d. orally, for the first week and monitoring the serum level of the drug, blood levels of 200-500 ng/ml (whole blood RIA) were maintained thereafter. CsA was continued for at least 3 months at which time the treatment was terminated in unresponsive patients. Along with CsA therapy, all patients received high dose methylprednisolone (HMP) administered simultaneously with CsA, 30 mg/kg/day IV for 3 days, 20 mg/kg/day IV for 3 days and 10 mg/kg/day IV for 4 days.

Response Criteria : According to the remissions achieved at the end of the study the patients were reclassified in 4 different groups :

Complete Response (CR) :
- Hemoglobin > 12 gr/dL,
- Granulocytes > $1,5 \times 10^9$ /L,
- Platelets > 100×10^9 /L.

Partial Remission (PR):
- Complete resolution of red cells and platelet transfusion requirements.
- Sustained increment of granulocyte, platelet or reticulocyte counts (at least 0.5×10^9/L granulocytes, 30×10^9 /L platelets or 30 x 109/L reticulocytes above baseline).

Minimal Improvement (MI) :
- Decrease in transfusion requirements or of the frequency of infections.

No Response (NR) :
- Persistent aplasia.

Pretreatment and posttreatment levels of T cell subsets were determined using alkaline phosphatase anti-alkaline phosphatase (APAAP) staining method.

The K671 DAKO APAAP KITTM was used for this purpose (Dako Corporation/DAKOPATTS). This system allows human T cells to be detected as well as their two major subsets helper/inducer cells and cytotoxic/supressor cells. Three primary monoclonal anti-bodies are provided in the kit. The antibody against helper/inducer T-cells (T4), reacts with a 55 kD M.W. protein designated CD4 in the human leucocyte antigen classification system. The antibody against supressor/cytotoxic T-cells (T8), reacts with a 33 kD M.W. protein designated CD8. The antibody against T-cell (T11), reacts with an antigen found on the majority of T-cells in peripheral blood and bone marrow as well as many neoplastic lymphoid T-cells in T-cells neoplasms. The antigen is a 50 kD M.W. protein which is associated with the receptor responsible for binding sheep red blood cells in the E-rosetting assay.

After the preparation of the reagents, staining was performed on routinely prepared air, dried cell smears and the slides were examined by conventional light microscopy using oil immersion objectives.

Staining by this system results in the formation of a bright red precipitate at the site of the target antigen. When scoring the reactions obtained with individual antibodies in cell smears, the number af positive and negative lymphoid cells were evaluated by counting 100 to 200 lymphocytes in each smear. Polymorphs and monocytes were not included to the count.

RESULTS

The patients were reevaluated using the remission criteria mentioned above, and it was found that overall response rate to therapy was 40 % (improvement of hematopoiesis was seen in 6 of the 15 patients). Of the 6 responders, 3 had complete remission (CR) (20 %), 2 had partial remission (PR) (13.3%) and 1 had minimal improvement (6.7 %). No response was achieved in 9 patients (60.0 %) (Table I). 4 of the 6 responders had been previously unresponsive to HMP or ALG alone.

The first signs of response was seen in 21-69 days (mean 34 days). Remission duration was found to be dependent on the continued administration of CsA. After the cessation of CsA in 3-11 weeks relapse occured in all patients. When treating these patients with CsA or CsA + ALG again, a second remission could be induced in one patient only, who remains in Cr. 2 of the 9 unresponsive patients underwent allogeneic bone marrow transplantation (BMT). One of them died of complications of BMT. The other is still alive in CR.

Pretreatment and posttreatment levels of T-cell subsets are shown in Table II. Although these values were within normal range an increasing ratio of T-helper cells to T-supressor cells was observed.

Toxicity: The types and incidence of adverse side effects of CsA in our patients are summarized in Table-III. These side effects seemed to be reversible and dose-dependent in every case. It is important to consider that all the patients received high dose methylprednisolone simultaneously with CsA. After the withdrawal of the drug all these findings returned to normal in various periods of time for each patient.

DISCUSSION

The efficacy of CsA in the treatment of aplastic anemia is the most exciting outcome of this study although CsA-induced remissions were reported previously by others (6-12) contrary reports also exist in the recent literature (13). A CsA-induced remission provides a strong clue in favor of the role of T lymphocytes at least for the maintenance of bone marrow aplasia in vivo. CsA interferes with the elaboration of several lymphokines such as interleukin-2 and interferon gamma (14,15) probably at the level of mRNA transcription (16). Available evidence for the action of this agent suggests that maximum efficiency would be anticipated at the time when antigen exposure first occurs at a very early stage of the immune response. At this stage, primary T-cell activation by antigens and mitogens is inhibited whereas clonal expansion of already activated T-cells can no longer be prevented (16). This may account for the apparent lack of respose in the majority of our patients. If CsA can induce remissions in patients with aplastic anemia, then inhibition of active hematopoietic suppressor activity by interference with the lymphokines such as interferon gamma are most likely explanations than stimulation of hematopoietic growth factor production, as has been postulated by some authors, for the mechanism of ATG induced remissions (17). The combination of CsA with antilymphocyte serum or high dose methylprednisolone, anticipated to be additive in the restoration of immune system restoration of the immune system balance. However of the 6 responders in our series, 4 patients had been previously unresponsive to high dose methylprednisolone alone. The results of treatment with both ATG and ALG are variable and may reflect differences in dosage and, duration of therapy, quality and type of protein used (18). Because of the small number of patients in our series it is not possible to draw any definitive conclusion about the long term effect of CsA in the treatment of severe aplastic anemia. The dose and schedule used for its administration and combined action of cytoreductive agents like ATG, ALG or HMP with CsA has yet to be determined.

TABLE - I Response rates to CsA treatment.

Unresponsive	Responsive			
NR	CR	PR	MI	Overall Response Rate
9 60.0 %	3 20.0 %	2 13.3 %	1 6.7%	6 40.0 %

TABLE -II Sequential Changes of T-Cell Subsets (APAAP).

	pretreatment	third month	normal
T_{11} (Total T cells)	75 + 12.9 %	81 + 14.2 %	(80 - 85 %)
T_4 (Helper T cells)	41 + 10.4 %	52 + 12.4 %	(40 - 65 %)
T_8 (Supressor T cells)	34 + 7.3 %	29 + 9.8 %	(20 - 40 %)
T_4/T_8 Ratio	1.20	1.79	(1.0 - 3.25)

TABLE -III Toxic effects of CsA treatment.

Mild Hypertension	:	26.6 %	(4/15)
Hepatotoxicity (SGPT > 100 U/L)	:	53.3 %	(8/15)
Nephrotoxicity (Creatinin > 2.0 mg)	:	26.6 %	(4/15)
Gingival Hyperplasia	:	33.3 %	(5/15)

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