Pathogenesis and treatment of aplastic anemia

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SUMMARY

Aplastic anemia is a group of hematologic disorders characterized by peripheral blood pancytopenia and a hypocellular bone marrow. Hematopoietic stem cells are decreased and the residual cells are often functionally defective. A preponderance of evidence suggests that acquired aplastic anemia is immunologically mediated: activated T-lymphocytes produce increased quantities of γ-interferon and tumor necrosis factor which result in suppression of hematopoiesis.

Choice of initial treatment for a patient with aplastic anemia depends upon a complex interaction between patient age, disease severity and the availability of a bone marrow donor. Patients with mild disease may be treated with observation, androgens, colony stimulating factors or immunosuppression. For patients with severe disease, either immunosuppression or bone marrow transplantation are indicated. As our knowledge of the pathogenesis of aplastic anemia improves, more effective, less toxic treatments should become available.

Aplastic anemia is not a single disease. Rather, it is a group of acquired and inherited disorders characterized by deficient hematopoietic stem cells, a hypocellular bone marrow, and peripheral blood cytopenias. Prognosis depends upon the severity of suppression of hematopoiesis. However, recent advances in treatment have greatly improved the outcome of affected individuals. This review focuses on the pathogenesis and treatment of acquired aplastic anemia.

PATHOGENESIS

The incidence of aplastic anemia is approximately 2-3 cases per million population in Europe and the United States. For unknown reasons the disease is 2 to 3 times more frequent in many Asian countries.

These estimates do not include patients in whom predictable bone marrow suppression develops after exposure to irradiation, chemotherapy or other drugs. Similarly, patients who develop marrow failure as an immediate precursor to acute leukemia or myelodysplastic syndromes are not considered to have aplastic anemia.

Normal blood formation requires replication and differentiation of hematopoietic stem cells in a microenvironment that includes stromal cells, lymphocytes and growth factors. Abnormalities in any of these systems could lead to bone marrow failure. There is general consensus that hematopoietic stem cell numbers are markedly decreased in patients with aplastic anemia (1). Residual stem cells appear to be qualitatively deficient as well. These deficits persist even in patients whose peripheral blood cell counts return to normal.

A defective bone marrow microenvironment might explain failure to recover from aplastic anemia. Defects in stromal cell growth and production of cytokines have been noted by some investigators (2). However, these defects are often mild and are seen only in a minority of affected individuals. In addition, the success of bone marrow transplants for aplastic anemia requires intact stromal cells since these cells remain of host origin.

Finally, hematopoietic stem cells from patients with aplastic anemia grow poorly when plated on stromal cells from normal individuals whereas stromal cells from patients with aplastic anemia effectively support growth of hematopoietic stem cells from normal individuals (3).

Aplastic anemia might also be due to inadequate production of hematopoietic growth factors. However, serum levels of erythropoietin, granulocyte colony-stimulating factor, thrombopoietin and Flt-3 ligand are usually greatly increased. Levels of stem cell factor are only slightly decreased. There is no evidence for abnormal growth factor receptors in patients with acquired aplastic anemia.

A variety of clinical and experimental data suggest that aplastic anemia is an immune-mediated disease. These include: a. failure of marrow from an identical twin to correct aplastic anemia unless preceded by immune suppression (4), b. autologous marrow recovery after rejection of an allogeneic marrow transplant, c. improvement of hematopoiesis after immunosuppressive treatments (antilymphocyte globulin, cyclosporine, cyclophosphamide), d. suppression of normal hematopoiesis by lymphocytes from patients with aplastic anemia and e. activated cytotoxic lymphocytes in blood and bone marrow of patients with aplastic anemia which decreased in numbers after immunosuppressive treatment (5). Recent studies suggest that activated lymphocytes suppress hematopoiesis by production of gamma interferon or tumor necrosis factor (6). These effects may be mediated by induction and activation of FAS with subsequent apoptosis (7).

The pathogenesis of most forms of inherited aplastic anemia is not known. However, the poor response of most of these patients to immunosuppressive therapy suggests that non-immunologic mechanism may be operative.

TREATMENT

Evaluation and comparison of treatments for aplastic anemia require a standard definition of disease severity. Most trials have adopted the criteria initially proposed by the International Aplastic Anemia Study Group (Table 1). Unfortunately, there has not been agreement on criteria for evaluating responses to therapy. At a recent consensus conference in Santa Margherita Ligure, the criteria in Table 2 were developed. Investigators are encouraged to use these guidelines to report their results.

Based on our understanding of its pathogenesis, optimal therapy of aplastic anemia should include ablation of ongoing autoimmunity and replacement of deficient or abnormal hematopoietic stem cells. Many treatments fail to meet one or both of these objectives.

Androgens

Androgenic steroids stimulate hematopoiesis (especially erythropoiesis). However, despite initial optimistic reports, a controlled trial showed no benefit for either oral or intramuscular androgen in patients with severe aplastic anemia (8). Responses may be better in patients with milder disease but there is no evidence that androgen treatment alters the rate of progression to more severe disease or decreases mortality.

Table I: Criteria for severity of aplastic anemia.

Severe aplastic anemia

Peripheral blood (any 2 or 3)

a.	PMN	< 500/ml
b.	Platelets	< 20,000/ml
c.	Reticulocytes	< 1% (singly corrected for hematocrit)*

Bone Marrow: hypocellular

Very severe aplastic anemia: severe aplastic anemia with PMN < 200/ml

Mild aplastic anemia: not meeting the criteria for severe disease

** Some investigators substitute an absolute reticulocyte count of 20,000/ml*

Table II: Proposed response criteria for response to treatment of aplastic anemia

General:
- responses must be confirmed by 2 or more CBC at least 4 weeks apart
- PMN must be unsupported by colony stimulating factor administration

Severe aplastic anemia

None:
- still severe

Partial:
- transfusion independent
- no longer meeting criteria for severe disease

Complete:
- hemoglobin normal for age
- PMN > 1,500/ml
- platelets >150,000/ml

Mild aplastic anemia

None:
- worse or not meeting criteria below

Partial:
- transfusion independence OR
- doubling or normalization of at least one cell line OR
- increase above baseline:
  - hemoglobin 3 gm/dl (if initially < 6)
  - PMN 500 /ml (if initially < 500)
  - platelets 20,000/ml (if initially <20,000)

Complete: same as criteria for severe disease

Colony Stimulating Factors

Trials of G-CSF, GM-CSF, IL-3 and SCF (stem cell factor) in patients with aplastic anemia have produced increases in neutrophils. However, trilineage responses were uncommon and long term responses frequently depended upon continued administration of the growth factor. IL-1 and IL-6 were ineffective. A combination of SCF plus G-CSF has shown initial promise.

Although trilineage responses are unusual with growth factors alone, they might be helpful when used in combination with immunosuppressive therapy. The latter treatment usually requires 2 to 4 months before an initial response is seen. Use of a colony stimulating factor during this interval might decrease infectious morbidity. The EBMT reported an 82% trilineage response rate and 92% survival of patients with severe aplastic anemia who were treated with antilymphocyte globulin, cyclosporine, methylprednisolone and G-CSF (9). However, in a recently completed controlled trial, the addition of G-CSF did not improve the response rate or decrease early morbidity of treatment with antilymphocyte globulin, cyclosporine and methylprednisolone (10).

Stimulation of defective hematopoietic stem cells by growth factors might accelerate development of myelodysplasia (MDS) or acute leukemia. In a recent study, 8 of 50 patients with severe aplastic anemia developed MDS or AML within 3 years of treatment with G-CSF plus cyclosporine (11). However, G-CSF treatment was unusually intensive and prolonged, no cases of MDS or AML developed after treatment with G-CSF alone, and bone marrow chromosome analyses were not performed prior to initiation of therapy. Other trials, using shorter courses and lower doses of G-CSF, have not shown an increased risk of developing MDS or AML.

Immunosuppression

Response of aplastic anemia to antilymphocyte globulin was first demonstrated in a rabbit model. Similar results were seen in a randomized clinical study (12). Complete plus partial response rates of 40 to 60 % were reported in this and subsequent trials. Addition of cyclosporine improved the response rate to 70 % (13). Patients with mild aplastic anemia may have a higher response rate than patients with severe disease (14). Neither haploidentical marrow nor androgen are necessary for optimal responses to antilymphocyte globulin. Addition of G-CSF has had marginal benefits on morbidity without a concomitant improvement in response rate. Currently, antilymphocyte globulin plus cyclosporine plus methylprednisolone is considered to be the immunosuppressive regimen of choice for treatment of aplastic anemia.

The mechanism of action of antilymphocyte globulin is presumed to be mediated via immunosuppression. However, there is poor correlation between *in vitro* or *in vivo* tests of immunosuppressive capacity and the efficacy of antilymphocyte preparations for treatment of aplastic anemia. Of further interest is the fact that monoclonal antilymphocyte antibodies have had very low response rates when used to treat patients with aplastic anemia.

Cyclosporine alone is less effective than antilymphocyte globulin for treatment of aplastic anemia (15). Substitution of cyclosporine for antilymphocyte globulin resulted in lower response rates of patients with severe (16) and mild (17) aplastic anemia in recently completed randomized studies.

Following high dose cyclophosphamide preparative treatment, occasional patients recover autologous marrow function after rejecting an allogeneic bone marrow transplant. In a recent report, a regimen of high dose cyclophosphamide plus G-CSF was reported to improve marrow function in patients with newly diagnosed or previously refractory aplastic anemia (18). These data require confirmation and comparison with antilymphocyte globulin regimens before they are applied more widely.

Bone marrow transplantation

Preparative pretransplant immunosuppression and the infusion of hematopoietic stem cells from a healthy donor attack both critical problems in the pathogenesis of aplastic anemia. In previously "untransfused" patients, disease-free survival of 80-90 % can be obtained with matched sibling marrow transplants (19).

Prior transfusions result in an increased rate of graft rejection that can only be overcome by more intensive pretransplant preparative regimens (with attendant increased acute and long term toxicities). Recently a preparative regimen of cyclophosphamide plus antilymphocyte globulin has produced a 92 % 3 year survival rate without increased toxicities in patients with aplastic anemia; 87 % of these patients had been previously transfused (20).

Only 20-30 % of patients with aplastic anemia have a sibling who is a genotypic HLA-A,B,DR match. An additional 1 % have a relative who is a phenotypic match: outcomes of transplants using these donors are similar to those using a matched sibling (21). In contrast, bone marrow transplants from less well matched donors (mismatched relatives, matched unrelated donors, mismatched unrelated donors) have been plagued by a high rate of graft rejection, a high rate of graft versus host disease, and poor (20-30 %) survival (21,22). These problems may be decreased by using a combination of more intensive pretransplant preparation in combination with partial T-cell depletion of the marrow graft: survival of 50-60% was recently reported using this approach (23).

TREATMENT RECOMMENDATIONS

Newly diagnosed patients with aplastic anemia should have HLA typing performed to determine

whether they have a fully histocompatible related donor. Bone marrow chromosome studies should be done to detect occult malignancy or Fanconi anemia. Transfusions should be withheld unless there is a life-threatening problem. Medications that impair platelet function (eg, acetylsalicylic acid) should not be used.

Overall, immune suppression produces similar or slightly lower response rates when compared to matched related bone marrow transplants (24,25). However, patients treated with immune suppression have less complete hematopoietic recovery, relapses are more frequent, and progression to other marrow diseases (paroxysmal nocturnal hemoglobinuria, myelodysplasia, or acute leukemia) is more frequent (26-28). In addition, transfusion of patients treated with immune suppression may cause sensitization to transplantation antigens, thereby decreasing the chances for a successful marrow transplant in the future. Therefore, choosing the optimal treatment for a patient with aplastic anemia depends upon interactions of several factors: the patient's age, the neutrophil count and the availability of a phenotypically or genotypically matched related donor.

Patients with **mild aplastic anemia** may be observed. Alternatively they may be treated with an androgen, with specific colony stimulating factors, or with antilymphocyte globulin, cyclosporine, and methylprednisolone.

Patients with **severe aplastic anemia** who do not have a matched related donor should receive antilymphocyte globulin, cyclosporine and methylprednisolone. If a donor is available the choice of treatment is more complex. Patients less than 20 years old with any PMN count and those 20 to 40 years old with PMN < 200/ml should be considered for immediate bone marrow transplantation. Other patients should receive antilymphocyte globulin, cyclosporine and methylprednisolone. These choices reflect, in part, the increasing risks of bone marrow transplantation (especially graft versus host disease) in older individuals and the poorer response of children to immunosuppression.

Patients with severe aplastic anemia who are treated with immunosuppression should have a search for an alternative donor begun while awaiting response to treatment. About 90 % of responses will begin within 4 months. If there is no improvement by that time, consideration should be given to repeated immune suppression (successful in one third of patients) or a bone marrow transplant.

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