Posthepatitic Severe Aplastic Anemia—An Indication for Early Bone Marrow Transplantation

By Bruce M. Camitta, David G. Nathan, Edwin N. Forman, Robertson Parkman, Joel M. Rappeport, and Tessa D. Orellana

Despite the use of androgens, corticosteroids, antibiotics, and blood product support, survival in posthepatitic severe aplastic anemia remains about 10%. Histocompatible bone marrow transplantation in this illness has been shown to be feasible. Therefore, marrow transplantation is indicated early in the course of severe aplasia following hepatitis. Delay of this procedure exposes patients to increasing risks of sepsis, hemorrhage, and sensitization to transplantation antigens by multiple transfusions. Weeks, not months, should elapse between diagnosis and transplantation if an appropriate donor, intensive supportive care, and a trained transplantation team are available.

MODERATE, TRANSIENT depression of one or more peripheral blood elements is a common occurrence during viral hepatitis. However, peripheral pancytopenia with varying degrees of bone marrow hypoplasia is relatively rare. Since the association of aplastic anemia and hepatitis was first reported, approximately 100 cases have been published. The number of pa-
tients with aplasia secondary to subclinical, hence undetected, hepatitis re-
mains, of course, entirely speculative.

Before 1955, long-term survival of more than 20% of patients with aplastic
anemia of all etiologies was exceptional. With the advent of broad-spectrum
antibiotics, corticosteroids, androgens, and blood product support, many cen-
ters now report 50% recovery. However, if only severe cases [those with
granulocytes (PMN) less than 500/cu mm, or platelets below 20,000/cu mm,
or severely hypocellular bone marrow] are considered, survival remains 10–20% at
best. Posthepatitic marrow aplasia, with few exceptions, falls into this
category of severe disease.

Reconstitution of human marrow by transplantation of marrow from an
identical twin donor is now a well-recognized therapeutic approach to aplastic
anemia. Recently, Thomas and co-workers reported complete hematopoietic
reconstitution in two of four nontwin cases using histocompatible sibling
donors. Further data from the same group indicates a nearly 50% success rate
in a larger series of patients. Included in the engrafted long-term survivors is a
patient with posthepatitic marrow aplasia. In this paper, we report a
similar success. The available literature regarding severe aplastic anemia second-
dary to hepatitis is also reviewed. It is our contention that analysis of these
data clearly indicates the advisability of early bone marrow transplantation
in this disorder.

CASE REPORT

T.D., a 14-yr-old white male, had Australia antigen negative hepatitis diagnosed at another
institution on September 30, 1972. Mild thrombocytopenia and neutropenia were noted at this
time. Treatment consisted of rest and adequate nutrition. Ten days later, epistaxis and gingival
bleeding occurred. Peripheral blood counts showed pancytopenia.Bone marrow aspirate was
hypocellular, with more than 95% of the cells being lymphocytes, reticulum cells, and plasma
cells. A diagnosis of aplastic anemia was made. There was no history of contact with or
ingestion of known marrow suppressant agents at any time before development of marrow
aplasia.

The patient’s course is summarized in Fig. 1. Oxymetholone (200–300 mg/day), prednisone
(10–40 mg/day), and supportive therapy was instituted. Despite these measures, pancytopenia
progressed. Gastrointestinal and mucocutaneous hemorrhage increased, necessitating frequent
transfusions. Oral moniliasis and severe gingivitis required appropriate antimicrobial therapy.

By early November, marrow transplantation was considered because of the patient’s poor
progress. Histocompatibility and blood typing showed the boy’s 16-yr-old brother to be a com-
patible marrow donor. Thus, when multiple bone marrow biopsies on November 29 again showed
pronounced aplasia, transplantation was elected.

MATERIALS AND METHODS

Review of the Literature

One-hundred-two reports of pancytopenia supposedly occurring less than 9 mo after the onset
of hepatitis were reviewed. Twenty-two were discarded from our analysis because of
concomitant intake of potentially myelosuppressive drugs, presence of a second disease, or lack
of sufficient information. Data on the remaining eighty are summarized in Tables 1 and 2.

Informed Consent

After a potential marrow donor had been identified, the parents were informed about transplant
procedures and consented to a discussion by us with the 16-yr-old donor, who also gave his
Fig. 1. Hematologic values and liver functions of patient T.D. pretransplant. Arrows (↓) indicate red blood cell transfusions.

informed consent to the procedure. The methods by which such informed consent is achieved will be the subject of a subsequent report.

Histocompatibility Studies

Histocompatibility antigen (HLA) and two-way mixed lymphocyte (MLC) studies were performed in standard fashion.\textsuperscript{83,85} The recipient’s serum was checked for lymphocytotoxic anti-

Table 1. Characteristics of Posthepatitic Aplastic Anemia in 80 Cases Reported in the Literature

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean</th>
<th>Range</th>
</tr>
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<tbody>
<tr>
<td>Age (yr)</td>
<td>18</td>
<td>1-74</td>
</tr>
<tr>
<td>Sex</td>
<td>Male 49</td>
<td>Female 27</td>
</tr>
<tr>
<td>Maximum recorded</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bilirubin (total, mg/100 ml)</td>
<td>Mean 17</td>
<td>1-59</td>
</tr>
<tr>
<td>SGOT (U/ml)</td>
<td>Mean 915</td>
<td>50-4300</td>
</tr>
<tr>
<td>Interval from hepatitis to aplasia (wk)</td>
<td>Mean 8</td>
<td>0-36</td>
</tr>
<tr>
<td>Hematologic data lowest recorded</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PMN (per cu mm)</td>
<td>85% &lt; 1000</td>
<td>73% &lt; 500</td>
</tr>
<tr>
<td>Platelets (per cu mm)</td>
<td>90% &lt; 50,000</td>
<td>65% &lt; 20,000</td>
</tr>
<tr>
<td>Marrow cellularity</td>
<td>8% normal—increased</td>
<td>92% moderately—markedly decreased</td>
</tr>
</tbody>
</table>
Table 2. Outcome of Posthepatitic Aplastic Anemia in 80 Cases Reported in the Literature

<table>
<thead>
<tr>
<th>Died (70)</th>
<th>87%</th>
</tr>
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<tbody>
<tr>
<td>Interval from aplasia to death (wk)*</td>
<td>Mean 11</td>
</tr>
<tr>
<td>alive (10)</td>
<td></td>
</tr>
<tr>
<td>Complete remission no transplant</td>
<td>6</td>
</tr>
<tr>
<td>Transplanted</td>
<td>5</td>
</tr>
<tr>
<td>Partial improvement</td>
<td>2</td>
</tr>
<tr>
<td>Continued aplasia</td>
<td>2</td>
</tr>
</tbody>
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*Excluding three patients (see text).

Body. Complete red cell antigen and gamma globulin allotypes were determined by routinely accepted procedures.

Transplantation Techniques

The patient was placed in reverse isolation in a regular hospital room. The entire room including the floor, windows, and half-way up the walls was cleaned daily with TOR (Huntington Laboratories, Huntington, Ind.). Food and all other items entering the room were sterilized. Betadine baths were given daily, and a thin film of Betadine was left on the entire body. Topical ointments and a nonabsorbable bowel prep were given to further decrease bacterial colonization.

Marrow procurement techniques have been previously described. Transplantation procedures (summarized in Table 3) were based on work by Santos' and Thomas' groups in experimental animals and in patients with leukemia and aplastic anemia. After immunosuppression with cyclophosphamide, transfusion with washed frozen red cells or irradiated (4500 rads) white cells and platelets prevented transfer of immunocompetent foreign lymphocytes into the immunodeficient host. The transplanted marrow was not irradiated.

RESULTS

Review of the Literature

As indicated in Table 1, average age at the onset of hepatitis was 18. Seventy-five per cent of the patients were less than 20 yr old. Approximately two-thirds of the patients were male. There was no significant influence of age or sex on characteristics of the illness or its outcome.

Aplasia developed within 2 mo of the onset of hepatitis in 75% of the cases. Liver functions and histology at that time usually revealed subacute or resolving hepatic disease. In addition, maximum recorded values for total bilirubin and SGOT did not indicate an unusual severity of hepatic insult.

Table 3. Transplantation Procedures

<table>
<thead>
<tr>
<th>Day</th>
<th>Procedure</th>
</tr>
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<tr>
<td>Day -6</td>
<td>Transfusion of donor blood to stimulate immune patient cells potentially reactive against donor marrow.</td>
</tr>
<tr>
<td>Days -5 through -2</td>
<td>Cyclophosphamide 50 mg/kg/day i.v. over 30 min to eliminate reactive cells.</td>
</tr>
<tr>
<td>Day -1</td>
<td>Rest—cyclophosphamide cleared from body.</td>
</tr>
<tr>
<td>Day 0</td>
<td>Transplant</td>
</tr>
<tr>
<td>Days +1, 3, 6, 11 then weekly until day 90</td>
<td>Methotrexate 10 mg/sq m i.v. to ameliorate potential graft-versus-host disease.</td>
</tr>
</tbody>
</table>
Reported hematologic values are somewhat limited. Nevertheless, pancytopenia was severe in all but a few cases. Survivors tended to have higher peripheral blood counts and less damaged marrows, but there was much overlap, and the differences were not significant.

Eighty-seven percent of the patients died despite treatment with antibiotics, corticosteroids, blood products, and (in half of the cases) androgens (Table 2). Three expired after 12, 14, and 48 mo of aplasia. Excluding these, the average survival postaplasia was 11 wk. Two-thirds of the patients died in less than 2 mo. Since androgen therapy, if efficacious at all, usually requires at least 2 mo before benefit is observed, it is not surprising that androgen-treated patients did not have a more favorable outcome. One severely ill patient died of graft-versus-host disease (GvH) following an incompatible marrow transplant. Two other moribund patients died shortly after receiving donor marrow. Details in these cases are not given, and it is not even clear whether the marrow was given following adequate immune suppression. Three patients died after transplantation with a regimen similar to ours.

Nine patients survived without transplantation, five achieving complete remission. Of the seven nontransplanted responders, three improved in less than 1 mo, while six improved before 2 mo—prior to expected benefit from androgens. One patient, critically ill, survived following marrow transplantation.

Thus, posthepatitic aplasia appears to be a severe, rapidly progressing illness with few survivors despite intensive supportive therapy. It was in this setting that our patient was transplanted.

Transplantation

1.5 x 10^10 marrow cells (2.3 x 10^8 cells/kg) were administered shortly after aspiration from the donor's iliac crests. The patient's subsequent course is summarized in Fig. 2. Hematologic improvement began on day 10 with appearance of nucleated red cells and granulocytes in the peripheral blood. Bone marrow at this time was still very hypocellular, but small foci of production of all cell lines were present. A reticulocytosis began on day 12. No blood products were needed after day 17. On day 21, platelets began to rise. A bone marrow on day 24 showed solid engraftment. As hematologic values improved, isolation procedures were gradually relaxed. By discharge on day 52, blood values were normal.

Mild graft-versus-host disease (GvH) developed on day 6. Fever, eosinophilia, rash, and exacerbation of liver function abnormalities occurred, then gradually declined. The latter two abnormalities persisted.

At 5 mo and 9 mo after transplantation, skin and liver biopsies were performed. The skin showed nonspecific changes with hyperkeratosis, minimal lymphocyte and plasma cell infiltration and no perivascular infiltration. The liver biopsies showed progressive changes consistent with subacute or chronic active hepatitis with a widespread cellular infiltrate consisting mainly of lymphocytes, plasma cells, and small numbers of eosinophils and neutrophils. Lymphocytes and neutrophils were prominent around vessels and bile ducts in portal regions. Necrosis and regeneration of liver cells were seen. Fat droplets were evident in some cells. Viral cultures of liver biopsy material were negative. Australia antigen and antibody studies were repeatedly negative. Tests for
antinuclear antibodies and LE cell preparations were negative. Complement levels (C3) were normal. A high titer (1:250) of cytomegalovirus antibody was detected. The virus could not be detected in body fluids. Electron microscopy of the liver sections did not reveal lesions consistent with cytomegalovirus infection.

For 4 mo postengraftment, the patient lived at home, with visitors restricted to a tutor and his immediate family. These limitations were slowly lifted, as evidence of immunologic reconstitution (measured by immunoglobulin levels, response to immunizations, in vitro responses of lymphocytes to mitogens, etc.) accrued.

Three lines of evidence point to the patient's present marrow being donor in origin. First, there was rapid restoration of marrow function despite previous severe aplasia, deteriorating condition, and massive doses of cyclophosphamide. Second, GvH was present. Third, Cw red cell antigen, absent pre-transplant, is now present in all red cells. The donor and one parent are Cw positive. All other blood groups in donor and recipient also became identical.

Nine months after transplantation, when marrow cellularity was normal, Howell-Jolly bodies and siderocytes were observed in the peripheral blood. Ten months following transplantation an episode of *Listeria monocytogenes* septicemia was successfully treated. A technium sulfur colloid spleen scan revealed decreased splenic function. The chronic active hepatitis persisted. The patient's skin became sclerodermatous. The marrow graft remained intact.

**DISCUSSION**

Bone marrow aplasia may theoretically be secondary to defects in the marrow microenvironment or to qualitative or quantitative defects of the precursor stem cells. Although animal models of these so-called soil and seed problems exist, the defect in human aplastic anemia has been uncertain.
Combined experience in our institution and other centers has shown that prompt, persistent marrow engraftment can be achieved in 80% of patients with severe marrow aplasia posthepatitis or secondary to other causes. Equal or greater success is possible without immunosuppression in identical twins. Finally, presensitization to donor antigens or inadequate host immunosuppression probably account for most cases of graft rejection. Therefore, it seems reasonable to postulate a stem cell defect in most cases of aplastic anemia in man.

The exact pathogenesis of aplastic anemia posthepatitis is unknown. Autoimmune phenomena are unusual, but have not been systematically sought. Liver injury with failure to detoxify harmful metabolic products or failure to produce essential nutrients may occur, but an etiologic role for these processes is speculative. Stem cell damage secondary to viral infection of bone marrow remains the most likely possibility.

Direct damage to marrow by hepatitis virus should not be surprising. It is well known that hepatitis is a systemic illness. Moreover, other virus infections (both in man and animals) have been associated with pancytopenia. In this regard, murine hepatitis virus infection, in addition to causing peripheral blood changes, has also been shown to cause focal marrow aplasia. Although these changes resolved within several weeks, human disease might result from a larger inoculum or more “marrow toxic” virus. Interestingly, all patients tested have been Australian antigen negative. This may have etiologic significance or may simply be related to transience of antigenemia.

Anabolic hormone therapy has been reported to improve prognosis in certain cases of aplastic anemia. Others have been unable to confirm this finding. Indeed, comparably good results were seen in a clinic where only excellent supportive care was given. Perhaps these discordant findings are in part due to variability of etiologic agents in different series and to differences with time in one institution’s series. In addition to this, it has been our contention, and others, that aplastic anemia can be roughly divided into two categories. In the first group, with marked depression of PMN and platelets plus greater than 80%–85% lymphocytes in bone marrow sections, mortality approaches 90%. Most patients die of infectious or hemorrhagic complications within 3 mo, before spontaneous recovery or benefit from androgens might be expected. Mortality remains high and is little affected by androgens, even in those surviving this initial period of time. In the second group, less severe marrow damage results in higher peripheral blood counts. This affords protection against complications while allowing time for spontaneous or hormone-induced recovery.

Posthepatitic aplastic anemia is severe. Two-thirds of the patients die within 2 mo of developing aplasia. Thus, although at least some cases of aplastic anemia with other etiologies are androgen sensitive, in posthepatitic aplasia most patients succumb before early benefit (usually increased reticulocytes or decreased transfusion requirements, not elevations of needed platelets or PMN) from these agents would be observed. Clearly, more effective therapy is required.

Storb et al. have reported long-term survival of 45% of severe aplastics following histocompatible marrow transplantation. Preliminary data from this
institution in a smaller series indicates the probability of similar success. Included in results from these centers are two survivors in five cases of posthepatitic aplasia transplanted when critically ill. These data on a limited number of patients suggest that histocompatible transplantation, when feasible, may be the treatment of choice in this disorder. This procedure should be even more useful if carried out before serious complications of aplasia have occurred. Patients without histocompatible donors (two-thirds of the cases) would perform provide data for evaluation of the natural course of the disease or for comparison with other modes of therapy.

On the basis of the above considerations, we suggest the following approach to a newly diagnosed patient with posthepatitic severe aplasia:

Identical twins should be transplanted as soon as possible, especially if there is no evidence of active hepatic disease. Immunosuppression in these cases is not necessary. Other patients should have histocompatibility testing versus parents and siblings as soon as aplasia is diagnosed. While awaiting results androgens, corticosteroids (to improve capillary integrity), and supportive therapy should be given. Multiple blood product transfusion from random sources causes sensitization to histocompatibility antigens.4 In animals, even a single transfusion from the potential marrow donor markedly diminishes chances for subsequent marrow engraftment.102 Therefore, blood product support should be limited and only unrelated donors used. Washed frozen red cells may decrease sensitization. However, even this preparation may contain small amounts of transplantation antigens.103 Buffy-coat-poor red cells probably do not decrease sensitization.104

In the posthepatitic severe aplasia, if a matched donor is available, serious consideration should be given to immediate transplantation, especially if 1 or (at most) 2 mo have elapsed since diagnosis, or if complications have already occurred. Delay of this procedure while awaiting infrequent remissions exposes patients to increasing risks of sepsis, hemorrhage, and sensitization to transplantation antigens by multiple transfusions. Histocompatible donors may be used for long-term transfusion support only if an irrevocable decision against transplantation is made.105

Timing of transplant intervention in severe aplastic anemia of other etiologies is not yet as clear because of factors alluded to above. However, we speculate, on the basis of the group of severe aplastics of uniform etiology reported here, that similar considerations will eventually apply.

Addendum. Thirteen months after transplant, at the time of galley submission, the patient suddenly died of overwhelming septicemia. The bacteriologic and autopsy findings are pending. The fact that this patient developed chronic hepatitis and functional asplenia following acute hepatitis does not alter our opinion concerning the importance of early marrow transplantation in severe aplastic anemia following acute hepatitis.

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