Antibody-Mediated Marrow Failure After Allogeneic Bone Marrow Transplantation

By Alan J. Barge, Gretchen Johnson, Robert Witherspoon, and Beverly Torok-Storb

Marrow graft failure observed in association with histocompatibility differences between donor and recipient is often attributed to rejection mediated by host-derived cytolytic T lymphocytes. The data presented in this report indicate that persistent host antibodies specific for donor antigen may also mediate graft failure, either by antibody-dependent cell-mediated cytotoxicity (ADCC), or complement-mediated cytotoxicity. In the case of HLA Class I disparity, where all donor cells express the target antigen, the presence of α-donor antibody was associated with complete graft failure and death. In the case of ABO blood group antigen disparity, the presence of α-donor antibody resulted in erythroid hypoplasia. The latter cases proved informative insofar as they established that host antibodies could persist for more than 18 months after chemotherapy and impair marrow function.

Patients and Methods

Patients. Clinical summaries of four patients are outlined briefly below. Patients 1 to 3 received bone marrow from ABO-incompatible, HLA-identical donors, and suffered isolated erythroid failure. Patient 4 received bone marrow from an HLA-haploidentical family member, and failed to establish a successful graft.

Patient no. 1: UPN 1627. A 22-year-old untransfused man with severe aplastic anemia, blood group O, Rh-positive, received an allogeneic bone marrow transplant from his HLA-identical, MLC-nonreactive female sibling, blood group B, Rh-positive, on March 3, 1982. His pretransplant anti-B titer was 1:8 and he received erythrocyte-depleted marrow, after conditioning with cyclophosphamide, 200 mg/kg. By day 28 posttransplant, his anti-B titer was 1:4 and a bone marrow aspirate showed normal myeloid and megakaryocyte engraftment, but absence of all but very immature erythroid elements. By day 54 he remained red cell transfusion-dependent, his anti-B titer having risen to 1:128. There was no peripheral blood reticulocytosis, and a repeat bone marrow aspirate showed no evidence of mature erythroid elements. Samples of serum and bone marrow were obtained for study at this point. He was treated with transfusion only until day 310, when his anti-B titer had fallen to 1:2 and he required no further transfusions.

Patient no. 2: UPN 1613. A 23-year-old woman, with Philadelphia-positive chronic myeloid leukemia in chronic phase, of blood group O, Rh-positive, received an allogeneic bone marrow transplant from her HLA-identical, MLC-unreactive female sibling, blood group A, Rh-positive. Her pretransplant anti-A titer was 1:2000 (IgG), and she underwent plasma exchange twice, lowering the anti-A titer to an undetectable level. Her conditioning regime comprised cyclophosphamide 120 mg/kg, and total body irradiation, 6 fractions at 2.0 Gy over 6 days, with lung shielding. Red-cell–depleted bone marrow was infused on February 19, 1982, and her initial posttransplant course was uncomplicated. However, from day 90 her hematocrit began to fall, without reticulocytosis. At this point, her anti-A titer was again elevated at 1:1000. Bone marrow examination revealed normal myeloid and megakaryocytic maturation, but virtual absence of recognizable erythroid elements. Samples of serum and marrow were obtained for study at this stage. Her anti-A titer rose to 1:8192 (IgG) over the course of the subsequent four months, and she remained red cell transfusion-dependent. The anti-A titer eventually fell to 1:2 after 9 months, and she at last developed a reticulocytosis.

Patient no. 3: UPN 4043. A 30-year-old woman with acute nonlymphocytic leukemia in first remission, blood group O, Rh-positive, received an allogeneic bone marrow transplant from her HLA-identical female sibling, blood group A, Rh-positive. Her pretransplant anti-A titer was 1:32 (IgG) and she underwent plasma exchange twice, the titer falling to 1:2. She received red-cell–depleted marrow on November 19, 1987, without immediate problem, after conditioning with cyclophosphamide 120 mg/kg, and...
total body irradiation, six fractions at 2.0 Gy over 6 days, with lung shielding. Bone marrow examination at day 21 revealed brisk myeloid and megakaryocytic engraftment, but no evidence of erythroid maturation. Her anti-A titer had risen to 1:128 by day 80, without reticulocytosis, and she remained red cell transfusion-dependent. Repeat bone marrow examination showed virtual absence of maturing erythroid elements, and samples of it and serum were studied at this stage. She remains well, with a persistent anti-A titer of 1:2 and dependent on red cell transfusions.

**Patient no. 4: UPN 3264.** A 23-year-old man, with acute lymphoblastic leukemia in first persistent relapse, received an allogeneic bone marrow transplant from his haploidentical, MLC-reactive mother on April 24, 1986. Because of the demonstration of a cytotoxic antibody in his serum active against his donor cells, he underwent plasma pheresis twice prior to marrow infusion. Conditioning comprised cyclophosphamide 120 mg/kg, followed by total body irradiation, seven fractions at 2.0 Gy over 7 days, with lung shielding. At no stage following transplant did any evidence of engraftment ensue. Bone marrow aspirates and biopsies, on days 14, 21, and 28 showed no evidence of engraftment. Peripheral blood was obtained on day 28 for study.

In view of the failure of the first transplant, reconditioning with high-dose cyclophosphamide and antithymocyte globulin (ATG) was undertaken. He died 30 days after the second marrow infusion from interstitial pneumonia and fungal sepsis.

**Hematopoietic progenitor assays.** Bone marrow aspirates from the posterior iliac crest were obtained after informed consent as defined by the Internal Review Board of the Fred Hutchinson Cancer Research Center, Seattle, WA. Heparinized bone marrow aspirates were diluted, 1 to 5 in Hanks Balanced Salt Solution (HBSS) and the mononuclear cell fraction obtained by density gradient centrifugation over Ficoll-Hypaque (Lymphoprep Nycomed A.S., Oslo, Norway).

After washing in HBSS, the cells were plated for erythroid bursts (BFU-E) and granulocytic-monocytic colonies (CFU-GM) at 2 x 10^5/mL in 1.2% methyl cellulose, 12% screened fetal calf serum (FCS) (Flow Laboratories, Inc, McLean, VA), 2% BSA, and Iscove's medium (GIBCO Labs, Grand Island, NY), in the presence of 3.0 U/mL erythropoietin (Amgen Biochemicals, Thousand Oaks, CA), and colony-stimulating activities provided by phytohemagglutinin-stimulated lymphocyte-conditioned medium. Erythroid colonies (BFU-E) were plated in plasma clots with 0.5 units Epo/mL as described previously. BFU-E and CFU-GM were scored at day 14 by conventional inverted light microscopy. Plasma clots were harvested and stained at day 7 for enumeration of CFU-E.

**NK and CTL assay.** NK and CTL activity were evaluated by a 4-hour ^51Cr release assay. The K562 cell line was used as the target for NK activity, whereas donor and third-party EBV-transformed B-lymphoblastoid cell lines (B-cell) were used as targets to identify specific CTL activity. Fresh unstimulated patient and control PBMC were used as effectors. Spontaneous and maximum ^51Cr release were determined by incubating labeled targets in 200 µL culture medium or 200 µL 1% triton X-100. Each experimental condition was set up in triplicate and mean specific lysis calculated as follows: (experimental release – spontaneous release) x 100/maximum release – spontaneous release.

**Antibody-dependent cell-mediated cytotoxicity (ADCC).** Standard 4-hour ^51Cr release assays were used to determine whether patient serum could facilitate ADCC. Epstein-Barr virus (EBV) transformed B-lymphoblastoid cell lines established from the donor, recipient (patient no. 4), and a panel of HLA-defined normal individuals were used as targets. Briefly, ^51Cr-labeled B-line target cells were preincubated with patient serum for 30 minutes at 4°C, washed three times, then aliquoted at 10^4 cells/well in 96-well U-bottom plates. Normal human AB serum and antilymphocyte globulin (ALG 5 µg/mL) were used as negative and positive antibody controls, respectively. Patient or control PBMC were used as effectors.

**RESULTS**

**Growth of BFU-E, CFU-GM, and CFU-E.** Table 1 summarizes the data from the three patients described above with primary erythroid failure. The data clearly demonstrate that, in the absence of autologous serum, normal numbers of erythroid bursts developed in marrow samples from all three patients, indicating that immature erythroid progenitors were clearly present in the marrow of each patient. CFU-E could not be grown from patient marrow, supporting data which suggest that these precursors express AB antigens and may therefore be targeted for destruction (data not shown).

The addition of patient serum alone to BFU-E assays variably reduced the number of bursts detected; however, the addition of both patient and normal AB serum as a source of complement caused a significant and reproducible reduction in the number of bursts. The numbers of CFU-GM grown in the same assay were not affected, in keeping with the erythroid-specific activity of the patients' sera. In addition, as summarized in Table 2, data from six experiments illustrate the specific anti-A activity of serum obtained from patient 3, which inhibited CFU-E growth from normal A" marrow, but not B" or O" marrow.

**NK, CTL, and ADCC-mediated cytotoxicity.** EBV-transformed B-lymphoblastoid lines (B1 through B3) were generated from donors of known HLA phenotype and used as targets for lytic assays in addition to K562 and CEM, a T-lymphoblastoid line.

Table 3 summarizes data which suggest that the graft failure observed in patient 4 could not be attributed to NK or CTL since these activities were not detected in patient PBMC. In contrast, results of the ADCC assay, using patient 4's PBMC and normal PBMC as effectors, indicate the presence of antibodies in patient serum that target donor cells for lysis. Of the six cell lines tested, only two, the donor B line and B2, appear to be susceptible to ADCC with patient serum. Given that the only HLA antigen disparity between the donor and patient is the presence of HLA-A28 on donor cells, it is reasonable to hypothesize that the antibodies mediating ADCC and possibly graft rejection include activities specific for A28. The fact that B2 also expresses A28 supports this hypothesis. Patient serum in the presence of effector cells was also found to inhibit growth of donor-derived erythroid bursts greater than 50% of control values (P < .05, data not shown).

<table>
<thead>
<tr>
<th>Serum Added</th>
<th>% Control BFU-E</th>
<th>% Control CFU-GM</th>
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<tbody>
<tr>
<td>Control</td>
<td>100 ± 1.2</td>
<td>105 ± 1.2</td>
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<tr>
<td>+</td>
<td>6 ± 1.5</td>
<td>78 ± 4.0</td>
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<td>–</td>
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ANTIBODY-MEDIATED MARROW FAILURE

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host antibody-producing cells, present pretransplant, can failure of one or more lineages. Firstly, it demonstrates that long periods after transplantation. In the case of patient 1, the antibody persisted for almost a year following transplant.

In an additional patient, not reported here, the antibody persisted for more than 18 months.

Secondly, it shows that these persisting antibodies are capable, in vitro, of bringing about killing of specific targets. In the case of recipients of ABO-incompatible transplants, the in vitro studies indicate that the antibody in patient serum alone can interfere with the maturation of erythroid progenitors; however, the addition of a source of complement significantly inhibits erythroid burst formation. In the case of ABO incompatibilities, the antibodies against blood group antigens can only affect erythroid precursors at a relatively late stage of development, since the blood group antigens are not expressed until after the BFU-E stage. For this reason, the marrows of patients 1 through 3 had detectable BFU-E, but not CFU-E, and demonstration of anti-erythroid activity required that the bursts be exposed to the antiserum throughout the 2-week culture period so that the maturing progeny that express the blood group antigens could be exposed to the antibodies. Since blood group antigens are not expressed on the nonerythroid elements of the engrafting marrow, growth of progenitors from other lineages, eg, CFU-GM, occurs normally. Therefore, patients with this complication simply require red cell transfusion support until the antibody levels have fallen.

Patient 4 demonstrates what may be the more catastrophic case of persisting host antidonor antibody directed against an antigen present on all elements of the transplanted marrow.

### DISCUSSION

These investigations provide a number of insights into the theoretical mechanisms by which engrafting bone marrow can be targeted by persistent host antibodies, resulting in failure of one or more lineages. Firstly, it demonstrates that host antibody-producing cells, present pretransplant, can survive cytoreductive chemoradiotherapy, and persist for long periods after transplantation. In the case of patient 1, the antibody persisted for almost a year following transplant.

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### REFERENCES


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