Nonfatal Graft-Versus-Host Disease Occurring After Transfusion With Leukocytes and Platelets Obtained From Normal Donors

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There have been two previous reports of fatal graft-versus-host (GVH) disease following transfusion of leukocytes from normal donors for the supportive care of leukemic patients. We have reported a nonfatal case of GVH disease in a child with acute monoblastic leukemia who received leukocyte and platelet transfusions from normal donors. The dose of lymphocytes contained in both leukocyte and platelet concentrates obtained with a semicontinuous-flow cell separator is sufficient to cause GVH disease. Irradiation of all blood products administered to severely immunocompromised and myelosuppressed patients should be considered.

GRAFT-VERSUS-HOST (GVH) DISEASE occurs commonly in recipients of bone marrow transplants. It has also been reported as a complication of transfusion therapy both in children with agammaglobulinemia and in neonates who have received in utero transfusions for erythroblastosis fetalis. GVH disease has also frequently been reported as a complication of leukocyte transfusion therapy when donor leukocytes from patients with chronic myelogenous leukemia (CML) have been used. Two fatal cases of GVH disease have been reported in patients with leukemia who were receiving leukocytes obtained from normal donors.

Recently we observed a similar but nonfatal case of severe GVH disease in a child undergoing therapy for acute monoblastic leukemia who was receiving leukocytes and platelet transfusions from normal donors. The purposes of this report are to further substantiate the phenomenon of GVH disease following transfusions from normal donors and to discuss recommendations for preventive therapy.

CASE REPORT

A 6-yr-old male child was referred to Children's Hospital Medical Center following resection of a hemorrhagic terminal ileum and cecum secondary to an intussusception. Prior to transfer, the patient was pancytopenic, and he received transfusions of packed red blood cells on two occasions. Past medical history and family history were unremarkable. The child was febrile on admission and had diffuse abdominal pain and a nonsuppurative cellulitis involving the RLQ abdominal incision site and left antecubital fossa. His initial blood count showed the following: hemoglobin 11.5 g/dl; white blood cell count 1400 cu mm, with differential counts of 7% polys, 3% bands, 14% monocytes, 67% lymphocytes, and 9% atypical lymphocytes; platelet count 70,000/cu mm; 0.3% reticulocyte count. Initial bone
marrow aspirates and biopsies revealed moderate hypoplasia, and the patient was initially thought to have aplastic anemia. Serum IgG and IgM were normal, but IgA was markedly decreased.

The patient was treated with antibiotics, and on the third and fourth hospital days he also received leukocyte transfusions (Fig. 1). Normal random-donor leukocytes were obtained by the use of a continuous-flow cell separator (American Instrument Co., Silver Spring, Md.). Units were not irradiated; they contained on the average $0.19 \times 10^8$ leukocytes, of which $5 \times 10^7$ were lymphocytes. The patient rapidly became asymptomatic, but on the ninth hospital day the fever again developed and the patient was treated with antibiotics alone because his white blood cell count had risen spontaneously. Over the next 2 wk bone marrow aspirates demonstrated progressive replacement with monoblasts, and the diagnosis of AML was made. Cytoreductive treatment was begun on the 18th hospital day using vincristine, prednisolone, cytosine arabinoside, and adriamycin. At that time the blood count showed the following: hematocrit 23%; white blood cell count 3000/cu mm, with 25% polys, 6% bands, 29% lymphocytes, 29% monocytes, and 11% blast forms; platelet count 90,000/cu mm.

On the 25th day the patient received 4 units of random-donor nonirradiated platelets (male donors) because of severe thrombocytopenia. On the 26th day, leukocyte transfusions were added to antibiotic therapy because of documented Escherichia coli sepsis. Combined granulocyte and platelet harvest was obtained from random donors with a semicontinuous-flow cell separator (Haemonetics Corp., Natick, Mass.). The cell collections contained large numbers of mononuclear cells and were irradiated to 5400 rads. The mean numbers of granulocytes, lymphocytes, and platelets transfused daily were $0.8 \times 10^9$, $3.9 \times 10^9$, and $5.3 \times 10^9$, respectively. In addition, gastrointestinal sterilization with nonabsorbable antibiotics was begun. The patient's clinical status improved as subsequent cultures remained negative.

On the 35th hospital day the patient developed a maculopapular erythematous eruption on his scrotum, lower abdomen, and chest. This rash became more generalized over the next few days and was believed clinically to represent a drug reaction. However, diarrhea had become prominent by that time; liver function abnormalities worsened, and the rash became more intense. In addition, the patient continued to display severe pancytopenia beyond the usual time course expected from the cytoreductive therapy he had received.

A skin biopsy was obtained on the 38th hospital day; it was diagnostic for acute GVH disease and confirmed the clinical impression of GVH involving intestine, liver, skin, and possibly bone marrow (Fig. 2). The bone marrow aspirate was hypocellular and contained a few histiocytes that were not phagocytosing white cells. HAA, cytomegalovirus titers, and heterophile were negative.
Antibiotic therapy was continued in the presence of pancytopenia, and only irradiated blood products were employed for patient support. Over the next 2.5 wk the patient became afebrile, and leukocyte transfusions were discontinued. Liver function abnormalities reverted to normal, and the patient’s general state improved somewhat. His rash continued to wax and wane over the next several weeks, and his diarrhea gradually resolved. A repeat skin biopsy on the 60th hospital day revealed changes suggestive of, but not diagnostic for, acute GVH disease.

The patient’s subsequent hospital course was complicated by malnutrition, chronic pancytopenia, and an episode of acute cholecystitis. However, he was discharged on the 110th hospital day. Bone marrow examination at that time revealed marked hypoplasia but no evidence of leukemia. Liver function studies were within normal limits, and the patient’s diarrhea and rash had completely resolved.

DISCUSSION

GVH disease is a clinical syndrome manifested by an erythematous skin rash, diarrhea, and abnormalities of liver function, with characteristic histopathologic changes in these target organs. Most commonly the diagnosis is based on clinical grounds and is confirmed by skin biopsy, which should reveal “vacuolar degeneration, dyskeratosis, eosinophilic bodies, epidermolysis, bullae, or total denudation of
epithelium. Although cytogenetic evidence of engraftment is desirable, it may be present only transiently or may not be demonstrable in the absence of sex difference or other chromosomal marker. We were unable to demonstrate cytogenetic evidence of engraftment in our patient, but he fulfilled all the clinical criteria for this syndrome.

Our patient was severely lymphocytopenic (200–600 lymphocytes/cu mm) and received multiple transfusions with blood products known to contain large numbers of immunocompetent lymphocytes. In experimental systems, fatal GVH disease can be induced by transfusion of as few as $10^7$ homologous lymph node cells per kilogram in the adult irradiated mouse. Our patient received on the average $19 \times 10^7$ lymphocytes/kg/day with his leukocyte transfusions, although only two of his transfusions were unirradiated (day 3 and 4 of hospitalization). His only other sources of lymphocytes were the random-donor platelet transfusions he received on day 25 of hospitalization (average of $2.8 \times 10^7$ lymphocytes/kg). The dosages of lymphocytes from our blood bank platelets contain a mean of $1.44 \times 10^8$ lymphocytes per unit (500 cc of whole blood).

Myeloid and lymphoid engraftment after leukocyte transfusion therapy from donors with CML is a well-documented phenomenon, and GVH disease has been reported from several sources. Both transient mild-to-moderate disease and fatal disease have been noted.

Lymphoid engraftment with post-thymic T cells and subsequent GVH disease are dependent on transfusion of immunocompetent lymphocytes capable of spontaneous division. In addition to myelosuppression, immunosuppression and lymphatic aplasia must presumably be present in patients who experience GVH disease. Aggressive cytoreductive chemotherapy protocols often result in just this set of circumstances. In an attempt to prevent GVH disease secondary to transfusion of immunocompetent post-thymic T cells, it is clear that either these cells must be separated from blood products prior to transfusion or their proliferative capacity must be altered so as to prevent engraftment. Currently available cell separation techniques are not sophisticated enough to fulfill the former requirement.

Irradiation of blood products has been proposed by several authors, but it remains controversial. Survival in F, hybrid irradiated mice following transfusions with maternal bone marrow is reasonably good. However, Smith and Vos were able to demonstrate that subsequent transfusion with maternal lymph node cells shortened survival of graft and host, presumably secondarily to GVH disease. Irradiation of maternal lymph node cells prior to transfusion abrogated this killing effect and permitted prolonged survival.

Irradiation of blood products at dosages sufficient to destroy T lymphocytes does little to alter granulocyte function or erythrocyte survival. Mitogen-stimulated lymphocytes retain only 1.6% of their $^3$H-thymidine uptake after 5000 rads and none after a 7500-rad dose. In addition, following exposure to 1500 rads, the blastogenic response to tetanus toxoid and allogeneic lymphocytes is reduced by 95%, and following 3500 rads it is completely ablated.

This is the third reported case of GVH disease following transfusion of leukocytes and platelets from normal donors for the supportive care of leukemic patients. At the present time we recommend irradiation of all blood products administered to patients who are severely immunocompromised and myelosup-
pressed. Further reporting and investigation of similar cases may help to identify patients who are at greatest risk for GVH disease and allow for more specific recommendations.

REFERENCES

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Nonfatal graft-versus-host disease occurring after transfusion with leukocytes and platelets obtained from normal donors

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