Fatal Graft-Versus-Host Disease Following Blood Transfusion in Hodgkin’s Disease Documented by HLA Typing

By Robert E. Dinsmore, David J. Straus, Marilyn S. Pollack, James M. Woodruff, Thomas J. Garrett, Charles W. Young, Bayard D. Clarkson, and Bo Dupont

Fatal graft-versus-host disease (GVHD) developed in a patient with Hodgkin’s disease treated with combined chemotherapy and radiotherapy following the transfusion of 2 U of packed red blood cells. Clinical features of the GVHD included the development of exfoliative dermatitis, progressive hepatic dysfunction, aplastic anemia, and finally progressive fatal pneumonia. GVHD was documented by skin biopsy and chimerism by HLA typing. The HLA phenotype of the patient’s skin fibroblasts (A3; Bw44 (w4)/A2, B15 (w4)) was appropriate for parental haplotypes and probably represented her true HLA phenotype. Lymphocytes from the patient (peripheral blood and lymph node biopsy) were of a different HLA genotype (A3; Bw35, w38, w4, w6; Cw4), which was inappropriate for parental HLA haplotypes but identical to the HLA phenotype of one of the blood donors. The HLA-DR typing of the patient’s family and of the blood donor demonstrated that the patient and the donor probably were HLA-DR identical (DRw5/DRw6), although no B lymphocytes could be obtained from the patient for direct DR typing. We are currently irradiating all blood products administered to patients with Hodgkin’s disease receiving intensive treatment. Further observations will be necessary to determine whether transfusions to other cancer patients with immunodeficiency states should be restricted to irradiated blood products.

A CUTE graft-versus-host disease (GVHD) is an immunologically mediated syndrome manifested by skin rash, liver function abnormalities, and gastrointestinal tract disturbances caused by transplanted allogeneic immunocompetent lymphocytes. Some degree of GVHD occurs in 70% of patients receiving HLA-identical allogeneic bone marrow transplants. It has also been reported following blood transfusion in infants with various primary immunodeficiency syndromes, after intratertiary and exchange blood transfusion to infants for hemolytic disease of the newborn, and after granulocyte transfusion to patients with hematologic malignancies. This report documents by HLA typing and skin biopsy the occurrence of fetal GVHD following the transfusion of packed red blood cells in a patient with Hodgkin’s disease undergoing treatment with combination chemotherapy and radiotherapy.

CASE HISTORY

An 18-yr-old female was first seen at Memorial Hospital in May 1978 with a 1-mo history of lymph node enlargement. Physical examination was remarkable for bilateral supravacular adenopathy and a palpable spleen. Lymph node biopsy revealed Hodgkin’s disease, mixed cellularity type. Chest x-ray demonstrated bilateral hilar and mediastinal adenopathy, and enlarged para-aortic lymph nodes were seen on a lymphangiogram. Bone marrow aspiration and biopsy, complete blood count (CBC) and platelet count, and serum hepatic biochemistry studies were all normal. Her clinical stage was thus IIIA, and she was begun on a combined chemotherapy and radiotherapy program that involves the administration of MOPP (nitrogen mustard, vincristine, procarbazine, and prednisone) and ABVD (adriamycin, bleomycin, dacarbazine, and vinblastine) in alternate monthly cycles (month 1–4 and 6–9) and local radiation to original areas of bulky disease (month 5). After 4 cycles of chemotherapy, she received radiation therapy, 2000 rads tumor dose to mantle, para-aortic and splenic portals. At the resumption of her next cycle of chemotherapy her CBC and platelet count were normal. Two weeks later, her Hb was 6.5 g/dl, platelet count 143 \times 10^{12}/cumm, and WBC 5200/cumm, and she was transfused with 2 U of packed red blood cells. She was hospitalized 8 days following the transfusion with an erythematous rash and a fever. Her admission physical exam was remarkable only for a temperature of 39°C and a confluent erythematous maculopapular rash on her face and upper trunk. Her Hb was 9.9 g/dl, platelet count 153 \times 10^{12}/cumm and WBC 4100/cumm with a “left shift” in the differential count. Her serum bilirubin was 1.3 g/dl, glutamic oxaloacetic transaminase (SGOT) 110 U/liter (normal up to 25 U/liter), LDH 421 U/liter, and alkaline phosphatase 102 U/liter (normal 31–82). Numerous cultures of her blood and urine and serologic studies for hepatitis B surface antigen (HBsAg) and cytomegalovirus antibody titers were all negative. By the third day, the skin rash involved her entire body. A skin biopsy from her left leg (outside the RT portal) disclosed early acute graft-versus-host reaction (Fig. 1), and she was begun on prednisone 100 mg/day. By the fifth day, severe pancytopenia had developed, and bone marrow and lymph biopsies were markedly hypocellular. She remained febrile with temperatures of 39°–40°C throughout the rest of her hospitalization, despite treatment with broad spectrum antibiotics. On the tenth hospital day, bilateral pulmonary infiltrates appeared that gradually progressed. The erythematous rash became exfoliative without vesiculation. Diarrhea never developed, and the sole gastrointestinal tract manifestation of GVHD was severe oral mucositis. She became increasingly jaundiced with her serum bilirubin rising to 25 g/dl. On the fourteenth hospital day, intravenous cyclophosphamide 3 g/day was begun in an attempt to abort the GVHD. Progressive hypoxia supervened, and she died on the seventeenth hospital day. Permission for autopsy was refused.

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MATERIALS AND METHODS

**HLA Studies**

HLA-A, B, and C typing was performed on peripheral blood lymphocytes and DR typing on B lymphocytes by standard two-stage microcytotoxicity techniques using a battery of well defined typing sera, as previously described. Skin fibroblasts from the patient were cultured for 3 wk in RPMI 1640 medium containing 20% fetal calf serum and antibiotics. They were subsequently typed for HLA-A, B, and C antigens by both direct (complement-dependent microcytotoxicity) and indirect (absorption/inhibition) procedures appropriate for cultured cells.

**Skin Biopsy (Fig. 1)**

Histologic examination of the skin showed dilated vessels in the upper dermis, a sparse perivenous lymphoid infiltrate, mild papillary edema, vacuolar alteration, and scattered lymphocytes along the dermoepidermal interface. Necrotic keratinocytes in the form of eosinophilic bodies were present in the hydropic areas. The stratum corneum had a normal “basket weave” appearance.

**HLA Studies**

The results of HLA typing of the patient’s lymphocytes during the acute GVHD and typing of her skin fibroblasts are shown in Table 1. The HLA genotypes of the patient’s parents, two siblings, and the HLA phenotype of one of the blood donors are also shown.

The patient’s peripheral blood lymphocytes were clearly of inappropriate HLA phenotype. Not even a minor population of cells positive for the parental A-locus antigens, A1 or A2, could be detected. The patient’s HLA phenotype, on the other hand, corresponded exactly to that found for one blood donor. The HLA phenotype of the peripheral blood lymphocytes was A3;Bw35,w38,w4,w6;Cw4, while the phenotype of the fibroblasts was A2,A3;B15(w4),w44(w4). One of the blood donors was available for HLA typing, and analysis of his lymphocytes also showed A3;Bw35,w38,w4,w6;Cw4, which was identical to that found in the patient. The patient was, therefore, chimeric. Lymph node lymphocytes were also HLA typed and found to be of the same phenotype as this particular blood donor. The frequency of this HLA phenotype in the white population is $2.27 \times 10^{-6}$. It is therefore highly probable that the patient was engrafted with immunocompetent lymphocytes from the blood donor, who had the same HLA phenotype as the one found in the patient.

The HLA-DR typing of purified B lymphocytes was performed in the patient’s family and the blood donor (Table 1). Attempts to isolate the B lymphocytes from the patient’s peripheral blood and lymph node were unsuccessful on several occasions. By extrapolation from the DR-typing in the family, it can be concluded that the patient should be DRw5/DRw6 (Table 1), which is identical to the DR-typing of the blood donor.

**Cytogenetics**

Dividing cells were absent among the PHA-stimulated lymphocytes so that karyotypic analysis could not be performed. Fluorescent Y-chromatin was not detected in interphase lymphocytes stained with quinacrine dihydrochloride, although it was found in all the lymphocytes examined from the blood donor.

**DISCUSSION**

Acute GVHD developed in this patient 8 days after the transfusion of 2 U of packed red blood cells from separate donors. The major manifestations were a

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**Table 1. Results of the HLA Studies**

<table>
<thead>
<tr>
<th>Sample Identification</th>
<th>HLA-A,B,C Antigens</th>
<th>HLA-DR Antigens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient’s lymphocytes</td>
<td>A3;Bw35,w38,w4,w6;Cw4</td>
<td>DRw5/DRw6*</td>
</tr>
<tr>
<td>Patient’s skin fibroblasts</td>
<td>A3,Bw44(w4)/A2,B15(w4)</td>
<td>DRw5/DRw5</td>
</tr>
<tr>
<td>Father</td>
<td>A2,B17(w4)/A3,Bw44(w4)</td>
<td>DRw5/DRw6</td>
</tr>
<tr>
<td>Mother</td>
<td>A1,Bw35(w6),Cw4/A2,B15(w4)</td>
<td>DR-/DRw6</td>
</tr>
<tr>
<td>Sister</td>
<td>A2,B17(w4)/A2,B15(w4)</td>
<td>DRw5/DRw6</td>
</tr>
<tr>
<td>Brother</td>
<td>A3,Bw44(w4)/A1,Bw35(w6);Cw4</td>
<td>DRw5/DRw6</td>
</tr>
<tr>
<td>Unrelated male blood donor</td>
<td>A3;Bw35,w38,w4,w6;Cw4</td>
<td>DRw5/DRw6</td>
</tr>
</tbody>
</table>

*DR alloantigens were determined by extrapolation from family haplotypes derived from direct typing of B cells, since fibroblasts do not express these antigens.
rash, jaundice, and bone marrow aplasia. The diagnosis of GVHD was established by the demonstration of chimerism on HLA typing and by the characteristic skin biopsy.\textsuperscript{15} Donor lymphocytes may circulate in the blood for up to 1 wk following blood transfusions.\textsuperscript{16} Such studies were, however, done in patients who received large amounts of fresh blood, and host as well as donor lymphocytes were detected. The HLA typing of our patient was done 14 days after the transfusion of blood that had been stored for at least 10 days, and no host lymphocytes were found.

The occurrence of GVHD in adults following blood transfusions is extremely rare. Only two reports have previously described GVHD in a patient with Hodgkin's disease following a blood transfusion.\textsuperscript{17,18} Hodgkin's disease is associated with immunodeficiency, particularly involving cell-mediated immunity.\textsuperscript{19} In concert with the radiation and chemotherapy, this immunodeficiency state probably permitted the engraftment of donor lymphocytes in our patient. Although the patient and the blood donor were phenotypically different for at least three HLA-A, B, and C antigens, it is likely that they were identical at the HLA-DR locus. The HLA-DR compatibility may have enabled the donor lymphocytes to survive in this immunosuppressed host and, subsequently, induce the graft-versus-host reaction. Preliminary data from renal transplantation using DR matching indicate that DR compatibility may result in prolonged graft survival.\textsuperscript{20}

The inability to detect the Y body in the patient's lymphocytes and their presence in the donor's lymphocytes is difficult to explain. For unknown reasons, the patient's own lymphocytes may have been detected by quinacrine staining but not by HLA-typing. However, the HLA data did document that chimerism was present.

Since there is no satisfactory therapy for GVHD, the emphasis has been on prevention. Storage of blood does not remove the potential for GVHD as was demonstrated in this patient. Based on in vitro observations of lymphocyte sensitivity to radiation,\textsuperscript{21} irradiated blood is transfused when GVHD may be anticipated. Doses of 1500–3000 rads apparently cause no impairment of red cell, granulocyte, or platelet function.\textsuperscript{3,22} Because of our experience with this patient and with several other patients with Hodgkin's disease in whom the diagnosis of GVHD was suspected but never documented, we are currently irradiating (2000 rads) all blood products administered to patients with Hodgkin's disease who are treated with chemotherapy and radiation therapy. Acute GVHD has been reported following transfusion in patients with acute leukemia,\textsuperscript{6–8} neuroblastoma,\textsuperscript{23} Hodgkin's disease,\textsuperscript{17,18} and non-Hodgkin's lymphoma.\textsuperscript{4} Whether transfusions should be restricted to irradiated blood in other cancer patients with immunodeficiency states, resulting from the underlying disease and/or the therapy, awaits further observations.

\textbf{ACKNOWLEDGMENT}

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