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Fatal Graft-Versus-Host Disease in a Patient With Lymphoblastic Leukemia Following Normal Granulocyte Transfusions

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A woman with lymphoblastic lymphoma was treated with combination chemotherapy. She subsequently became febrile while granulocytopenic and was given unirradiated granulocyte transfusions from normal, unrelated donors. She recovered, but 12 days later noted the onset of progressive skin rash, hepatic dysfunction, diarrhea and pancytopenia and, 22 days after her last granulocyte transfusion, died of gram negative septicemia. Histologic examination of multiple tissues including the skin, liver, and intestinal tract showed changes characteristic of acute graft-versus-host disease (GVHD). Y-chromatin analysis of the patient's peripheral blood just before death indicated

the presence of male cells. HLA typing of lymphocytes and skin fibroblasts from the patient and lymphocytes from the family and granulocyte donors was also consistent with engraftment of cells from one of the male granulocyte donors. This donor most likely was homozygous for one of the patient's haplotypes, perhaps facilitating engraftment of his cells and subsequent development of transfusion-induced acute GVHD. Until more precise guidelines can be established, we recommend that all cellular blood products given to patients receiving intensive chemotherapy be irradiated with 1500 rad.

GRAFT-VERSUS-HOST DISEASE (GVHD), a well-described complication of allogeneic marrow transplantation, has also been reported following transfusion of nonirradiated blood products to patients with compromised immunologic function. GVHD has been described in neonates who received intrauterine or exchange transfusions for hemolytic disease of the newborn,¹ in children with congenital immune deficiency,² or progressive vaccinia necrosum³ treated with multiple transfusions of leukocyte-rich plasma or whole blood, in patients with leukemia or lymphoma given granulocyte transfusions from either normal donors⁴⁻⁷ or patients with chronic myelogenous leukemia,^{8,9} and most recently, in patients with neuroblastoma or Hodgkin's disease receiving transfusions of packed red cells only.^{10,11}

This report describes the engraftment of allogeneic cells and the occurrence of fatal GVHD, proven by histology, HLA typing and cytogenetics, in a patient with lymphoblastic lymphoma who had received intensive chemotherapy and granulocyte transfusions during a period of granulocytopenia and infection.

This case and the other cases cited above reinforce the need for irradiation of blood products administered to immunocompromised patients in order to prevent the development of GVHD.

CASE REPORT

An 18-yr-old woman presented with a mediastinal mass and pleural effusion and was found to have diffuse lymphoblastic lymphoma. Bone marrow biopsy revealed extensive replacement by immature lymphoid cells. She was treated using the Memorial Hospital LSA2-L2 protocol¹² and received cyclophosphamide, 2000 mg intravenously once, vincristine, 2 mg intravenously weekly, prednisone, 80 mg p.o. daily, and methotrexate, 10 mg intrathecally once. Thirteen days later when her peripheral counts had recovered, she was given adriamycin, 48 mg, intravenously twice on consecutive days and continued on prednisone. One week later, she had persistent spiking fevers and a granulocyte count of less than 100/ μ l. She received 5 daily transfusions of granulocytes collected on the Haemonetics 30 from different normal, unrelated donors. The granulocytes were not irradiated. She recovered clinically and hematologically (WBC = 4800/ μ l with 74% granulocytes, hematocrit = 28%, and platelet count = 275,000/ μ l), and was discharged.

Two weeks after the last granulocyte transfusion, she was admitted with a 2-day history of fever to 40°C, severe myalgias and arthralgias, slight sore throat, and 6-8 loose stools per day. Physical exam revealed diffuse erythema and edema of the face and upper thorax, conjunctival injection and diffuse abdominal tenderness with some distention, and diminished bowel sounds. WBC was 3600/ μ l with 67% granulocytes, hematocrit 25%, and platelet count 42,000/ μ l. Bone marrow aspirate and biopsy showed 20%-30% of normal cellularity without evidence of lymphoma. Tests of liver function showed elevation of bilirubin (2.8 mg/dl), alkaline phosphatase (407 IU), SGOT (1375 IU), LDH (3740 IU), and GGT (455 IU).

She was given packed red blood cells, platelets, steroids, and antibiotics, but her clinical status did not improve. Facial plethora and edema progressed and a generalized maculopapular rash developed. Pancytopenia (WBC = 600/ μ l, platelets = 26,000/ μ l) worsened and repeat marrow aspirate and biopsy showed markedly decreased cellularity (10% of normal). Diarrhea continued and liver function deteriorated (bilirubin = 5.8 mg/dl, alkaline phosphatase = 583 IU, and SGOT = 1044 IU). She died of gram negative septicemia 35 days after her last chemotherapy, 22 days after her

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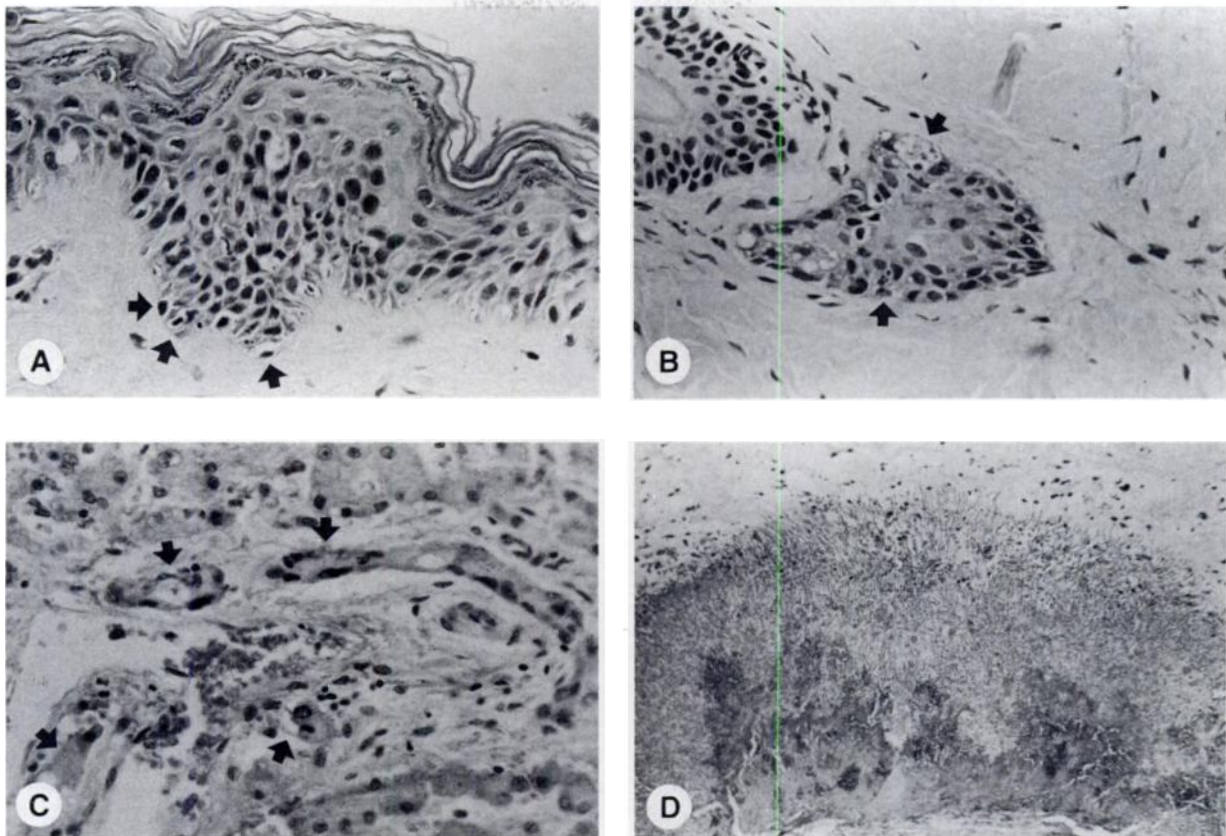


Fig. 1. Histologic evidence from skin, liver, and intestine supporting diagnosis of graft-versus-host disease. (A) Skin biopsy 22 days after last granulocyte transfusion showing necrotic basal cells in epidermis (arrows) and minimal mononuclear cell infiltrate; (B) hair follicle from same biopsy showing necrotic basal cells (arrows) with cytoplasmic swelling and minimal lymphocytic infiltrate; (C) liver section from autopsy showing minimal lymphocytic infiltration, marked atypia and degeneration of small bile ducts (arrows); and (D) section of ileum showing total replacement of mucosal surface by pseudomembrane composed of fibrin, blood, and both yeasts and pseudohyphae of *Candida albicans*.

last granulocyte transfusion, and 10 days after the onset of her terminal illness.

Histological Findings (Fig. 1)

Skin biopsy from the dorsum of the hand taken 20 days after the last granulocyte transfusion showed infiltration of lymphocytes with individual cell necrosis of basal layer cells. These changes are characteristic, but not diagnostic, of acute GVHD of the skin.¹³ Autopsy 22 days after transfusions confirmed these findings in the palms, sole of the feet, and chest skin, and in the epithelial surface of the tongue, cervix, vagina, sublingual ducts, lip, and conjunctival mucosa. The liver exhibited a moderate degree of diffuse atypia of small bile ducts characteristic of acute GVHD.¹⁴⁻¹⁶ The esophagus showed denudation of surface epithelium with *Candida* superinfection and damaged submucosal glandular epithelium. The cecum, jejunum, and ileum showed nearly total loss of epithelium and replacement by a fibrinous pseudomembrane containing gram negative rods and yeast forms of *Candida*. A heavy infiltrate of reactive lymphocytes was found in the submucosa. Lymph nodes and spleen showed moderate lymphoid depletion, edema, and necrosis of lymphocytes. There was no evidence of residual tumor in the fibrotic, edematous thymus, or elsewhere. Bone marrow was approximately 10% of normal cellularity. Myeloid, erythroid, and megakaryocytic elements were present with an increase in stromal histio-

cytes and iron. The lungs were severely hemorrhagic, consolidated, and overgrown with large numbers of bacteria. No viral inclusions were identified, although viral cultures were positive for cytomegalovirus. *Clostridia sp.* grew from both lungs and *E. coli* grew from the liver, spleen, and kidneys.

Laboratory Data

Peripheral blood samples were obtained from the patient 1 and 2 days before her death, from her family and from 3 of the normal granulocyte donors.

Cytogenetic preparations of peripheral blood and bone marrow from the patient failed to yield metaphases. Y-chromatin analysis of unstimulated mononuclear cells in the peripheral blood showed 80 of 100 interphase nuclei to have a prominent fluorescent body, consistent with male cells. Each of the 3 male granulocyte donors demonstrated greater than 80% Y-chromatin in mononuclear cells from peripheral blood.

HLA-A and B typing were performed according to the standard NIH procedure.¹⁷ HLA-DR (B cell) typing and determination of HLA-D antigens using homozygous typing cells were performed as previously described.^{18,19} Results are depicted in Table 1. When mononuclear cells obtained from the patient's peripheral blood were typed for HLA-A and B, only two antigens, A1 and B8, were

Table 1. HLA Typing of Patient, Family Members and Granulocyte Donors

Individual	Sex	HLA Phenotype				HLA Genotype
		A Locus	B Locus	DR Locus	D Locus	
Patient	F					
Lymphocytes	M*	1	8	†	†	†
Fibroblasts	†	1	8	†	†	†
Father	M	1, w31	5, 8	3	w1, w3	A1, B8, DR3, Dw3/Aw31, B5, DR-, Dw1
Mother	F	26, 29	12	7	w7	A26, B12, DR-, D-/A29, B12, DR7, Dw7
Sister 1	F	1, 29	8, 12	3, 7	w3, w7	A1, B8, DR3, Dw3/A29, B12, DR7, Dw7
Sister 2	F	1, 26	8, 12	3	w3	A1, B8, DR3, Dw3/A26, B12, DR-, D-
Granulocyte donor						
No. 1	F		ND	†		
No. 2	F		ND	†		
No. 3	M	1, 32	7, 8	†		
No. 4	M	1	8	3	w3	
No. 5	M	3, 11	14, w44	†	†	

*Only male cells were detected in the peripheral blood.

ND = not determined.

detected. A sufficient number of cells could not be obtained from the patient for HLA-D or DR study.

Only the antigens A1,B8,DR3, and Dw3 were detected on lymphocytes from granulocyte donor 4. Thus the most likely source of the circulating male, A1,B8 positive cells in the patient was granulocyte donor 4. Homozygosity of granulocyte donor 4 for A1,B8, and DR3 is likely, but could not be proven by family study since his father was deceased. Cells of granulocyte donor 4, however, were able to stimulate cells from each parent and both sisters of the patient to respond in MLC (relative response = 43%–50%).

Skin fibroblasts were cultured in Waymouth's medium with 20% fetal calf serum and antibiotics. They were subsequently typed for informative antigens by absorption. Aliquots of antisera recognizing A1,B8,Aw31, and B5 were absorbed with 1×10^6 , 0.5×10^6 , and 0.25×10^6 fibroblasts from the patient. Absorbed and unabsorbed sera were diluted 1:1, 1:2, 1:4, 1:8 and tested in cytotoxicity against normal lymphocytes positive for A1,B8,Aw31, or B5. The patient's fibroblasts absorbed A1 and B8 antibodies, but not Aw31 or B5. Thus, the patient presumably inherited the paternal A1,B8,DR3,Dw3 haplotype.

DISCUSSION

That fatal GVHD can occur after transfusion of blood products is not in doubt, as reported previously¹⁻¹¹ and confirmed in the present report. GVHD in recipients of allogeneic marrow grafts has been described extensively, and our patient showed the typical clinical and histologic features of GVHD involving skin, liver, and gut with terminal widespread infection. In contrast to acute GVHD in allogeneic marrow graft recipients,^{15,20} marrow hypocellularity is characteristic of acute GVHD resulting from transfusions since the hematopoietic cells are of host origin and are targets of the GVH reaction.²¹

Nevertheless, the clinical diagnosis of transfusion-induced GVHD is difficult. Infection is an almost invariable component that obscures the basic underlying pathophysiology. Disseminated viral syndromes can create a similar picture of pancytopenia, skin, liver, and gastrointestinal disease with death from

secondary infections. Ultimately the diagnosis of GVHD rests on confirmation of engraftment and on the presence of characteristic, albeit not diagnostic, histologic findings in the skin, liver, and intestine.^{13-16,22,23}

The risk of developing GVHD following blood product transfusion is unknown but may be higher than apparent from the case reports in the literature since the clinical and pathologic features of GVHD have become widely recognized only in the past several years. In addition, there may be mild or transient cases that go undetected. As more aggressive treatment protocols are developed for patients with malignant disease and as both granulocyte and platelet transfusions are more widely utilized, the number of patients with GVHD secondary to blood products may increase.

Engraftment of donor cells in the present case may have been facilitated by the compatibility that existed between the patient and granulocyte donor 4, i.e., the absence of excess HLA antigens on the donor cells. The detection of HLA-A1 and B8 on the patient's fibroblasts strongly suggests that she inherited the paternal A1,B8,DR3,Dw3 haplotype and only HLA A1,B8,DR3,Dw3 could be detected in granulocyte donor 4. Similarly, in a case recently reported by Dinsmore et al., engraftment of donor lymphocytes may have been facilitated by compatibility between patient and donor for HLA-DR.¹¹ These two cases suggest that patient-donor compatibility for portions of the major histocompatibility complex may increase the risk of GVHD, particularly in immunologically compromised patients.

Because of the uncertain incidence and course of transfusion-induced GVHD, recommendations for its prevention are difficult to formulate. Certain patients, including children with severe immune deficiency and

marrow transplant recipients, appear to be at high risk to develop GVHD. In chemotherapy recipients, however, variable degrees of immunosuppression, histocompatibility relationships, and cell dose make assessment of risk extremely difficult. Until these problems are solved, the most prudent approach appears to be irradiation of all blood products given to patients receiving intensive chemotherapy.

Irradiation of blood products with a commercially available cesium source (Gammacell 1000 Irradiator, Atomic Energy of Canada, Ltd., Ottawa) can be performed with minimal inconvenience, time, and relatively modest expense, but the dose of irradiation necessary to eliminate the risk of GVHD is uncertain. We choose 1500 rad more than a decade ago based on early studies of irradiation effects on lymphocytes.⁸ Irradiation of blood products with 1500 rad was adequate to markedly inhibit proliferation of lymphocytes²⁴ without impairment of red cell, platelet or granulocyte function^(references 25 and 26 and unpublished observations). Furthermore, irradiation of canine peripheral WBC with 1500 rad was adequate to eliminate their effectiveness in promoting allogeneic engraftment in recipients of histoincompatible marrow.²⁷ Nevertheless,

there are no data to substantiate that this dose of irradiation eliminates the risk of GVHD. Other investigators feel higher doses of irradiation are needed. Button, for example, recommends the administration of 5000 rad, based on greater inhibition of PHA-stimulated blastogenesis of peripheral blood lymphocytes by 5000 than 1500 rad without detrimental effects on stored RBC or platelets.²⁸

Contraindications to irradiation are theoretical, while the benefit, however infrequent, to the patient who would otherwise have developed GVHD may be lifesaving. We therefore recommend that all patients who are receiving aggressive therapy for malignant disease or are otherwise severely immunocompromised receive only irradiated (1500 rad) cellular blood products.

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