Transfusion-Associated Graft-vs-Host Disease
A Fatal Case Caused by Blood From an Unrelated HLA Homozygous Donor

Timothy E. Gorman, DO,1 Carmen J. Julius, MD,1 Rolf F. Barth, MD,1 A. Ng, MD,3 Melanie S. Kennedy, MD,1 Thomas W. Prior, PhD,1 James Allen, MD,2 and Larry C. Lasky, MD1

Key Words: Transfusion-associated graft-vs-host disease; TA-GVHD; Immunocompetent; GVHD; Autopsy; Blood irradiation; RBC transfusion; Open heart surgery

Abstract

Transfusion-associated graft-vs-host disease (TA-GVHD) is a rare complication of transfusion. We report fatal TA-GVHD in a 63-year-old coronary artery bypass patient of European descent after an RBC transfusion from an unrelated donor. The patient had mild lymphocytopenia and received 2 80-mg doses of methylprednisolone and 7 units of RBCs. On day 14 after the transfusion, he had fever, elevated liver enzyme levels, and a macular rash. Pancytopenia and bone marrow aplasia developed. On day 26, he had a massive gastrointestinal hemorrhage and died. At autopsy, histopathologic findings of the skin, liver, bone marrow, and gastrointestinal tract were consistent with TA-GVHD. One donor of the transfused RBCs (3 days old at transfusion) had a 1-way HLA match with the patient. A method using multiplex polymerase chain reaction is presented. This patient with TA-GVHD and mild immune suppression suggests that blood component irradiation guidelines may need to be reevaluated.
shared with a nonblood relative in the United States has been calculated to have an estimated range of risk of 1 in 17,700 to 39,000. Given this information, along with the fact that approximately 15 million cellular blood components are transfused annually, one would expect the incidence of TA-GVHD to be much higher. TA-GVHD undoubtedly is under-diagnosed and underreported. We describe a fatal case of TA-GVHD in the United States caused by blood from an unrelated and proven HLA homozygous donor.

**Case Report**

A 63-year-old white man with a 90-pack year tobacco history was admitted to the local hospital because of chest pain and was found to have coronary artery disease. He was a retired factory worker with a remote history of minimally invasive squamous cell carcinoma of the larynx for which curative local radiation therapy had been completed 13 months before admission. Two doses of methylprednisolone (80 mg) were given on admission for a suspected exacerbation of chronic obstructive pulmonary disease. He was anemic (hemoglobin, 8.5 g/dL [85 g/L]), most likely secondary to iron deficiency and peptic ulcer disease, with mild lymphocytopenia (630/µL [0.63 · 10⁹/L]), most likely due to nausea, vomiting, and abdominal pain. The physical examination revealed splenomegaly and an abdominal mass. The laboratory examination showed the following results: creatinine, 3.7 mg/dL (327 µmol/L); alkaline phosphatase, 200 U/L; hemoglobin, which was decreasing, 8.0 g/dL (80 g/L); and abnormal liver function test results, including aspartate aminotransferase, 1,231 U/L; alanine aminotransferase, 3,240 U/L; gamma-glutamyltransferase, 386 U/L; lactate dehydrogenase, 4,360 U/L; total bilirubin, 6.3 mg/dL (108 µmol/L); and direct bilirubin, 5.8 mg/dL (99 µmol/L). The patient’s amylase level was 477 U/L with a lipase level of 2,145 mIU/mL (2,451 U/L). The urinalysis results were unremarkable.

Serologic testing results for hepatitis A, B, and C were negative. Test results for antinuclear antibody, rheumatoid factor, and antimitochondrial antibodies were negative. Haptoglobin was normal, at 185 mg/dL (1.5 g/L); the rapid plasma reagin test was nonreactive, and testing for the HIV-1 antigen was negative. Serologic test results for cytomegalovirus IgG and IgM were positive. Examination results of the cerebrospinal fluid were unremarkable, and a chest radiograph revealed a left pleural effusion.

Therapy with broad-spectrum antibiotics and vasopressors for hemodynamic support was initiated. All bacterial, mycobacterial, fungal, and viral cultures, including serologic test results for parvovirus B19, were negative. During the next several days, the patient developed diarrhea, remained febrile, and had more encephalopathic symptoms and worsening coagulopathy. Renal function worsened, requiring replacement therapy with dialysis. The patient’s WBC count decreased to 200/µL (0.2 · 10⁹/L) with a cellular differential count of 2% (0.02) neutrophils and 98% (0.98) lymphocytes. He required multiple transfusions as his hemoglobin level continued to decrease; his nadir platelet count was 3 · 10⁹/µL (3 · 10⁹/L). A workup for disseminated intravascular coagulation and heparin-induced thrombocytopenia were negative. A skin biopsy of the patient’s rash revealed vacuolar dermatitis with a sparse lymphocytic infiltrate and abundant necrotic keratinocytes. A bone marrow biopsy revealed severe hypoplasia with less than 5% overall cellularity. Despite aggressive medical management, including broad-spectrum antibiotics, antifungal treatment, antiviral therapy, endotracheal intubation, mechanical ventilation, invasive hemodynamic monitoring, and administration of numerous blood products, the patient’s condition continued to deteriorate. On the 26th day after transfusion, the hemoglobin level decreased abruptly, from 9.5 g/dL (95 g/L) to 5.0 g/dL (50 g/L), and the patient died. The working clinical differential diagnosis of this patient before death was a severe drug reaction vs a viral-induced hemophagocytic syndrome. The morning of the patient’s death, *Aspergillus flavus* was found in his sputum specimen. Consent for a complete autopsy was granted.

**Methods**

A pathologist suspected TA-GVHD, and HLA typing was performed on the patient at The Ohio State Medical Center from peripheral-blood lymphocytes before his death. The first 4 of the 7 RBC unit donors consented to HLA typing, which was performed by the American Red Cross. Serologic HLA typing was performed using the
National Institutes of Health method of microlymphocytotoxicity testing. Molecular HLA typing was done using the sequence-specific primers polymerase chain reaction method. 

DNA fingerprinting was performed by multiplex polymerase chain reaction analysis using 10 different microsatellite markers. The autoradiographic banding patterns at each locus were compared to identify unique alleles present in the spleen and lymph node that were absent in the myocardium. Paraffin-embedded fixed tissues from the patient’s myocardium, lymph nodes, and spleen were used for DNA extraction. Histologically, the myocardium showed no infiltration of lymphocytes and, therefore, was used as the control tissue. The lymph node and spleen tissue were chosen for evaluation with the anticipation that they would have the greatest potential yield of foreign lymphocytes (DNA). Unique microsatellite alleles from the lymph node or spleen, different from the control tissue (myocardium), would indicate the presence of foreign DNA within the patient.

**Results**

Pathologic Findings

At autopsy, the patient was severely icteric with a maculopapular rash on his trunk and extremities with partial desquamation of his trunk. A skin biopsy on day 4 of hospitalization and at autopsy showed a vacuolar dermatitis with a mild lymphocytic infiltrate and abundant necrotic keratinocytes. These findings are consistent with GVHD. The portal triads within the liver showed scattered lymphocytes invading bile duct epithelium with atypia. The gastrointestinal tract was filled with a cast of clotted blood from duodenum to sigmoid colon. Microscopically, the gastrointestinal tract revealed almost complete mucosal denudation with prominent mucosal hemorrhage, reactive epithelial changes, and focal apoptosis. The bone marrow was markedly hypocellular with an overall cellularity of less than 5%. The marrow spaces were replaced by numerous macrophages with scattered lymphocytes and erythroid precursors. These histopathologic findings were consistent with TA-GVHD. No viral cytopathic changes were seen, and immunohistochemical stains for cytomegalovirus and herpesvirus in the skin, liver, gastrointestinal tract, and bone marrow were negative. In addition, other autopsy findings included severe lymphoid depletion of the lymph nodes and spleen and diffuse alveolar damage and early Aspergillus flavus pneumonia of the lungs. There was no evidence of recurrent carcinoma of the larynx.

**HLA Typing**

HLA typing was performed on the patient and the first 4 RBC transfusion donors. The patient’s HLA type was A1, A–, B8, B– DRB1 *03XX* 15XX; DQB1 *0602, 0201/2. One of the 4 RBC donors was found to have an extended homozygous HLA type for which the patient was heterozygous at the class II region (donor 1, A1, A–, B8, B– DRB1 0301/4, –; DRB3*; DQB1 *0201/2). Even in the class II region, the donor and recipient shared DQB1 *0201/2. The implicated donor was male, and the blood was 3 days old at transfusion.

**DNA Fingerprinting**

Results of the DNA fingerprinting using a multiplex of 10 different microsatellites resulted in an identical DNA fingerprint at every loci. Since each of these microsatellite markers contains multiple alleles, and the tissue DNA was heterozygous at approximately 80% of the loci, the results are consistent with the absence of foreign DNA. A representative pattern that shows identical alleles in the control heart tissue, spleen, and lymph node samples is shown.

**Discussion**

A maculopapular skin rash, fever, diarrhea, liver dysfunction, and eventual bone marrow aplasia developed in this patient 14 days after transfusion of nonirradiated RBCs from a nonrelated HLA homozygous donor. The clinical time frame, laboratory findings, autopsy findings, and HLA
typing results all support the diagnosis of TA-GVHD. Other potential causes of the patient’s clinical syndrome were excluded. His mild lymphocytopenia and the 2 doses of methylprednisolone (80 mg) given on admission for chronic obstructive pulmonary disease were the only evidence of immune suppression. An argument could be made that given the high degree of 1-way HLA match with the RBC donor, the mild degree of immune suppression would not have mattered. Our results, as well as the results of others, suggest that shared class 1 HLA haplotypes are critical for the development of TA-GVHD. The transfused lymphocytes recognized the patient as foreign; however, the patient recognized the transfused lymphocytes as self. The transfused lymphocytes engrafted within the host and initiated TA-GVHD. Undoubtedly, the shared extended haplotype also was important, since most cases of TA-GVHD occurring in
immunocompetent patients have been reported in genetically homogeneous populations.5 The RBC donor and patient in this case shared an extended HLA haplotype that was discrepant only at the class II region. Only a few other reported cases of TA-GVHD demonstrate only a class II HLA mismatch.5 There is only a small percentage of reported TA-GVHD cases in which extensive HLA typing of donor and patient has been performed. The present case of TA-GVHD in a patient with only mild immune suppression joins a handful of other cases in the English literature caused by blood from random HLA homozygous donors.6–9

Our case illustrates the degree of difficulty in diagnosing this disease. If a patient develops a skin rash, diarrhea, liver failure, and bone marrow aplasia shortly after blood transfusion, TA-GVHD should be considered in the differential diagnosis. Being familiar with TA-GVHD manifestations, disease symptomatology, clinical time course, and related histopathologic changes will greatly aid in the diagnosis of this disease. Perhaps with greater awareness and clinical suspicion, the reported incidence of this disease may increase. Although antemortem diagnosis using the methods we have described would be even more important, the autopsy is a valuable way to prove or disprove a potential TA-GVHD case.

The negative DNA fingerprinting result was not surprising. The number of foreign lymphocytes in the tissue sections was very small, especially from the markedly lymphoid-depleted tissue from the spleen and lymph node. The clinical picture, histopathologic findings, and HLA typing results indicate that this patient had TA-GVHD. The negative DNA fingerprint supports the degree of difficulty in diagnosing this disease but provides a method for possible molecular documentation of future cases of TA-GVHD.

Given the poor prognosis of this disease and its almost universal fatality, efforts must be placed on prevention. Using properly irradiated blood components would prevent this disease; however, without completely knowing which patients are at risk, all transfused cellular blood products would have to be irradiated for adequate prevention. The cost and logistics of such a task and the radiation effects of shortening the shelf life of the blood supply and increasing plasma potassium in RBC units make the value of widespread irradiation questionable given the uncertain benefits for such an apparently rare disease. Even using leukocyte depletion filters before transfusion will not prevent this disease.14,15 This case illustrates the need for further prospective studies to determine the true prevalence and clinical significance of this disease. Nevertheless, perhaps the data from prospective studies along with reported TA-GVHD cases like this one may suggest that current cellular blood product irradiation practice guidelines may need to be reassessed.

From the Departments of 1Pathology and 2Internal Medicine, The Ohio State University, and 3The American Red Cross, Central Ohio Region, Columbus, OH.


Address reprint requests to Dr Lasky: Division of Transfusion Medicine, Dept of Pathology, The Ohio State University, E310 Doan Hall, 410 W 10th Ave, Columbus, OH 43210-1228.

References


