

## Brief Report

ALLOIMMUNE THROMBOCYTOPENIA  
AFTER ORGAN TRANSPLANTATION

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**T**RANSPLANTED organs, particularly livers and kidneys, carry passenger lymphocytes that can transmit autoimmune diseases<sup>1</sup> or initiate alloimmune disorders<sup>2</sup> in the recipient. We recently treated three unrelated patients who each received an organ (two kidneys and a liver) from the same donor. In all three patients severe alloimmune thrombocytopenia developed as a result of antibodies against the HPA-1a (PLA<sup>1</sup>) alloantigen. In these three patients the thrombocytopenia was refractory to all medical maneuvers except the transfusion of HPA-1a-negative platelets. In one patient the thrombocytopenia contributed to death. In another, the thrombocytopenia was cured by splenectomy, and in the third patient the thrombocytopenia resolved after an episode of severe graft rejection.

## CASE REPORTS

## Organ Donor

The donor was a 57-year-old multiparous woman who had died of a cerebrovascular accident. She had no history of serious illness, and her three pregnancies were uneventful, with the most recent being more than 20 years before her death. She had never received a transfusion. Her platelet count was 567,000 per cubic millimeter just before her death. Table 1 shows the results of HLA typing of the donor and recipients.

## Patient 1

Patient 1 was a 35-year-old woman with a history of reflux nephropathy. Her platelet count was 270,000 per cubic millimeter before transplantation (Fig. 1). Renal transplantation was carried out without complications, and postoperatively she was treated with antithymocyte globulin, cyclosporine, azathioprine, and prednisone. On the fifth postoperative day, the antithymocyte globulin was discontinued. Four days later, the platelet count fell to

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**TABLE 1.** RESULTS OF HLA TYPING OF THE DONOR AND THE THREE TRANSPLANT RECIPIENTS.

SUBJECT	ORGAN TRANSPLANTED	HLA-A	HLA-B	HLA-C	HLA-DR
		HLA-A	HLA-B	HLA-C	HLA-DR
Donor	—	1, 2	8, 62	3, —	3, 4
Patient 1	Kidney	1, 2	8, 62	3, —	3, 13
Patient 2	Kidney	1, 2	8, 55	3, —	3, —
Patient 3	Liver	2, 29	58, 60	3, —	7, 13

2000 per cubic millimeter. The peripheral-blood smear showed only thrombocytopenia, and the bone marrow examination was normal. The severe thrombocytopenia persisted despite treatment with high-dose prednisone, high-dose intravenous immune globulin, transfusions of random-donor platelets, and plasma exchanges, including the use of staphylococcal protein A immunoadsorption columns. The cyclosporine and azathioprine were discontinued, but treatment with prednisone was continued at a dose of 30 mg per day for 30 days. There was no response to transfusions of random-donor platelets, but on three occasions the platelet count rose after the transfusion of HPA-1a-negative platelets. Fifty days after transplantation, a splenectomy was performed after a transfusion of HPA-1a-negative platelets. The platelet count returned to normal within two days (Fig. 1). The patient has remained well with a normal platelet count for over a year.

## Patient 2

Patient 2 was a 48-year-old man with polycystic kidney disease. Before kidney transplantation, his platelet count was 285,000 per cubic millimeter. He underwent renal transplantation with no complications and was treated postoperatively with cyclosporine-based immunosuppression. On day 8 after transplantation, his platelet count dropped to 2000 per cubic millimeter. The bone marrow examination was normal. On day 9, the patient underwent a hemicolectomy for a bowel perforation; a large intramural hematoma was found at the site of the perforation. The patient had no response to transfusions of random-donor platelets, treatment with high-dose prednisone and high-dose intravenous immune globulin, or plasma exchange. He died of multiorgan failure 21 days after transplantation.

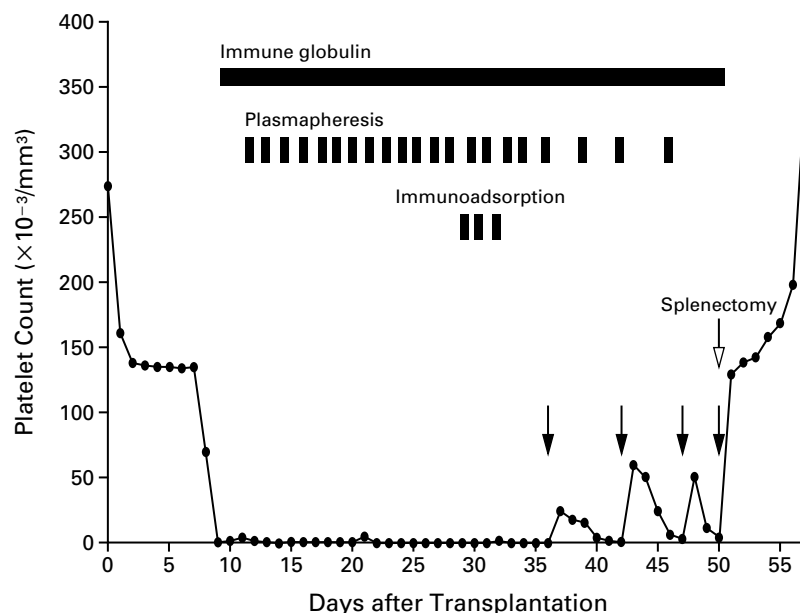
## Patient 3

Patient 3 was a 43-year-old woman with end-stage primary biliary cirrhosis. She had a platelet count of 113,000 per cubic millimeter before undergoing liver transplantation. There were no complications of transplantation, and she was treated with tacrolimus and prednisone postoperatively. Two days after transplantation the platelet count was 34,000 per cubic millimeter. There was no response to transfusions of random-donor platelets, and on day 11 the platelet count was 12,000 per cubic millimeter. The patient had an episode of acute rejection, with an increase in alkaline phosphatase. There was no response to corticosteroids, but the episode of rejection resolved with treatment with antithymocyte globulin. There was an increase in the platelet count that coincided with the rejection episode and its treatment. The platelet count reached 98,000 per cubic millimeter by day 15 after transplantation. The patient was discharged on day 38 with a platelet count of 125,000 per cubic millimeter, and her condition has remained stable for more than a year.

## METHODS

## Radioimmunoprecipitation Assay and Typing of Platelets

Serologic evaluation of the patients' antiplatelet alloantibodies was performed with a radioimmunoprecipitation assay.<sup>3</sup> Platelets



**Figure 1.** Changes in the Platelet Count in Patient 1 after Kidney Transplantation.

Therapeutic interventions included transfusions of HPA-1a–negative platelets (arrows), intravenous immune globulin, plasmapheresis, and the use of staphylococcal protein A immunoabsorption columns.

from HPA-1a–positive and HPA-1a–negative subjects were radiolabeled with iodine-125 and incubated with serum immunoglobulin from the patients, the donor, or control subjects and adsorbed onto protein A–Sepharose (LKB-Pharmacia, Baie d’Urfe, Que., Canada). In the case of Patient 1, serum samples obtained before and after transplantation were tested. Radiolabeled bound proteins were eluted and analyzed by sodium dodecylsulfate–polyacrylamide–gel electrophoresis and autoradiography. Samples of serum with HPA-1a antibodies, serum with HPA-5a antibodies, and normal serum were used as controls.<sup>3</sup>

HPA-1a typing of the donor, the three recipients, and the donor’s family was performed with the use of the polymerase chain reaction (PCR) and restriction–fragment–length polymorphism analysis.<sup>4</sup> After amplification (Perkin–Elmer Cetus, Norwalk, Conn.), the PCR product was incubated with 2 U of the HPA-1a–specific restriction enzyme *Sca*FI and analyzed by agarose–gel electrophoresis.

#### Detection of Chimerism

Evidence of sequences of donor DNA was assessed in DNA obtained from Patient 1 with an HLA-specific nested PCR assay.<sup>5</sup> The donor’s HLA type differed from that of the recipient only at the DR locus: the donor had an HLA-DR4 allele, whereas Patient 1 did not. DNA was isolated from peripheral-blood cells from the donor and Patient 1 and from cells obtained at splenectomy in Patient 1. DNA was amplified first with a set of primers that was generic for the second exon of all HLA-DRB1 alleles and then with a set of nested primers specific for the HLA-DR4 allele.<sup>6</sup> The products were analyzed by agarose–gel electrophoresis.

Because the investigations described were a normal part of patient care, approval by an ethics committee and specific informed consent were not required.

#### RESULTS

Patient 1 did not have detectable antiplatelet antibodies in her serum before kidney transplantation.

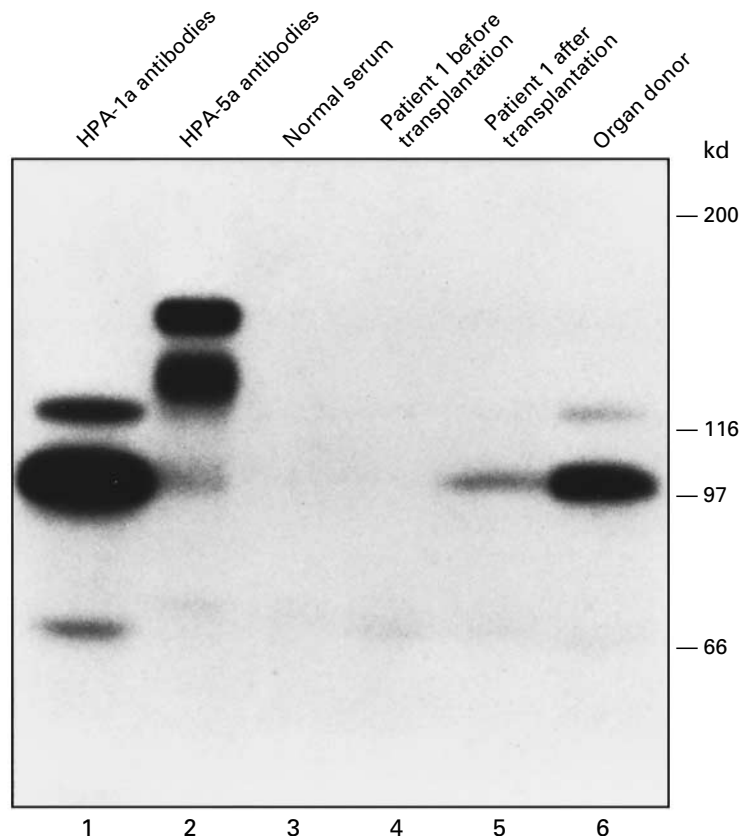
After transplantation, antibodies against glycoprotein IIIa on HPA-1a–positive platelets (Fig. 2), but not on platelets positive for HPA-1b (PLA<sup>2</sup>), were found in her serum (data not shown). Serum obtained from Patient 2 after transplantation also contained HPA-1a alloantibodies that were detectable by the radioimmunoassay. No pretransplantation serum samples were available from this patient. The organ donor herself had antiplatelet antibodies against HPA-1a in her serum. In the case of Patient 3, no serum sample was available before transplantation or during the episode of thrombocytopenia.

DNA typing revealed that the three recipients were homozygous for HPA-1a and that the donor was homozygous for HPA-1b. Two children of the donor were heterozygous (HPA-1a/HPA-1b), and a third was homozygous for HPA-1b.

The results of nested PCR with a set of primers specific for the HLA-DR4 allele showed that DNA from the donor was present in the spleen of Patient 1, but not in peripheral blood (Fig. 3).

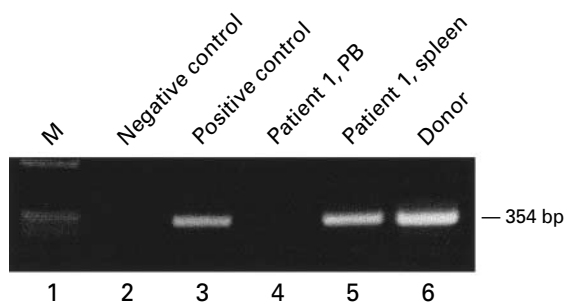
#### DISCUSSION

The transplantation of solid organs invariably results in the transmission of passenger leukocytes to the recipient. These immunologic stowaways usually do not cause complications, but graft-versus-host disease<sup>2</sup> and autoimmune thrombocytopenia<sup>1</sup> have been described after liver transplantation. In contrast, alloantibodies against red cells are not uncommon after the transplantation of ABO-mismatched liver and



**Figure 2.** Results of Radioimmunoprecipitation Assays for Serum Antiplatelet Alloantibodies against HPA-1a.

The donor had antibodies against HPA-1a (PL<sup>A1</sup>) in her serum (lane 6), as did Patient 1 after transplantation (lane 5) but not before transplantation (lane 4). Samples of serum with HPA-1a antibodies (lane 1), serum with anti-HPA-5a antibodies (lane 2), and normal serum (lane 3) were used as controls.



**Figure 3.** Results of Nested PCR Analysis with Primers Specific for the HLA-DR4 Allele.

HLA-DR4 DNA from the donor was present in spleen (lane 5) from Patient 1 but not in peripheral blood (PB) (lane 4). Lane 1 shows a lane marker (M); lane 2, DNA from a negative control; lane 3, DNA from a positive control; and lane 6, DNA from the donor.

kidney grafts; however, the resulting hemolysis is usually self-limited.<sup>7</sup> We identified severe, life-threatening thrombocytopenia in two recipients of kidney transplants that was caused by antiplatelet alloantibodies against HPA-1a, which were produced by passenger lymphocytes from the donor. The donor was homozygous for HPA-1b. A third patient who received a liver transplant from the same donor also had severe thrombocytopenia presumably owing to the same mechanism; however, this patient was not evaluated for antiplatelet alloantibodies.

Alloimmune thrombocytopenia is an uncommon, but not rare, cause of thrombocytopenia. Alloantibodies against platelet-specific alloantigens, in particular HPA-1a, can cause severe thrombocytopenia, which may require treatment with high-dose intravenous immune globulin and plasma exchange.<sup>8,9</sup> Neither of the kidney-transplant recipients we studied had a response to these therapies. Patient 2 died of multiorgan failure and bowel perforation related to a he-

matoma in the bowel wall. This patient, like Patient 1, had severe thrombocytopenia that was not controlled by standard immunosuppressive therapy. The platelet count in the liver-transplant recipient (Patient 3) paradoxically increased during a severe episode of rejection, suggesting that the donor's B lymphocytes that were producing the antiplatelet antibodies were also rejected. The high degree of HLA compatibility between the donor and the recipients may have prevented rejection of the donor's B cells in the kidney-transplant recipients, even after immunosuppressive therapy was stopped.

The platelet count in Patient 1 returned to normal after splenectomy, which cured the alloimmune thrombocytopenia. This treatment has not been used for alloimmune hemolytic anemia after transplantation of ABO-incompatible grafts. After splenectomy and the recovery of the platelet count to normal, there were no detectable antiplatelet alloantibodies in Patient 1. This observation suggests that B lymphocytes migrated from the donor kidney to this patient's spleen, where some of them continued to produce the HPA-1a antibodies that were found in the donor's serum. The detection of DNA of donor origin in the patient's spleen supports this interpretation.

The donor had HPA-1a alloantibodies in her serum despite the passage of more than 20 years since her last pregnancy. The production of antiplatelet alloantibodies for such a long time has been reported previously.<sup>9</sup> The donor's HLA type — DR3, B8, A1 — is associated with a high risk of peripartum allo-sensitization to the HPA-1a alloantigen.<sup>10,11</sup> Although none of the donor's children reported a history of neonatal alloimmune thrombocytopenia, not all women who produce HPA-1a alloantibodies have clinically affected children.<sup>10,11</sup> In transplantation-mediated alloimmune thrombocytopenia, we have demonstrated that antibodies of donor origin are directed against recipient platelet alloantigens. These features distinguish transplantation-mediated alloimmune thrombocytopenia from a similar disorder, post-transfusion purpura, in which HPA-1a-negative recipients generate antiplatelet antibodies after receiving an HPA-1a-positive blood transfusion.

Although maternal-fetal HPA-1a incompatibility occurs in 2 percent of pregnancies among white women, the formation of alloantibodies is distinctly uncommon, and in most cases, it is restricted to women with the HLA-DR3 antigen (our donor was positive for HLA-DR3).<sup>10,11</sup> This strong HLA association suggests that the antibody response against HPA-1a in the

donor was dependent on helper T cells.<sup>9,13</sup> However, the production of these antibodies in the recipients may not have required HPA-1a-specific helper T cells, because the immune response had already been initiated in the donor. Moreover, the recipients were treated with cyclosporine or tacrolimus and, in one case, antithymocyte globulin, all of which strongly inhibit T-cell function.<sup>14</sup>

In summary, we identified three cases of severe alloimmune thrombocytopenia that were caused by antibodies produced by passenger B cells in organs (liver and kidney) from an HPA-1a-mismatched donor. Transfusion with HPA-1a-negative platelets caused a substantial increase in the platelet count in one transplant recipient, and splenectomy was curative in one patient. A diagnosis of transplantation-mediated alloimmune thrombocytopenia must be considered in patients with severe thrombocytopenia after organ transplantation.

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## REFERENCES

1. Friend PJ, McCarthy LJ, Filo RS, et al. Transmission of idiopathic (autoimmune) thrombocytopenic purpura by liver transplantation. *N Engl J Med* 1990;323:807-11.
2. Burdick JE, Vogelsang GB, Smith WJ, et al. Severe graft-versus-host disease in a liver-transplant recipient. *N Engl J Med* 1988;318:689-91.
3. Smith JW, Hayward CP, Horsewood P, Warkentin TE, Denomme GA, Kelton JG. Characterization and localization of the Gova/b alloantigens to the glycosylphosphatidylinositol-anchored protein CDw109 on human platelets. *Blood* 1995;86:2807-14.
4. Unkelbach K, Kalb R, Santoso S, Kroll H, Mueller-Eckhardt C, Kiefel V. Genomic RFLP typing of human platelet alloantigens Zw(PIA), Ko, Bak and Br (HPA-1, 2, 3, 5). *Br J Haematol* 1995;89:169-76.
5. Knoop C, Andrien M, Defleur V, et al. Detection of blood chimerism after lung and heart-lung transplantation: the superiority of nested as compared with standard polymerase chain reaction amplification. *Transplantation* 1994;58:1335-8.
6. Bein G, Gläser R, Kirchner H. Rapid HLA-DRB1 genotyping by nested PCR amplification. *Tissue Antigens* 1992;39:68-73.
7. Gregoire JR. Immune hemolytic anemia after renal transplantation secondary to ABO-minor-mismatch between the donor and recipient. *J Am Soc Nephrol* 1993;4:1122-6.
8. Mueller-Eckhardt C, Kiefel V, Grubert A. High-dose IgG treatment for neonatal alloimmune thrombocytopenia. *Blut* 1989;59:145-6.
9. Goldman M, Filion M, Proulx C, Chartrand P, Decary F. Neonatal alloimmune thrombocytopenia. *Transfus Med Rev* 1994;8:123-31.
10. Blanchette VS, Chen L, de Friedberg ZS, Hogan VA, Trudel E, Decary F. Alloimmunization to the P1<sup>A1</sup> platelet antigen: results of a prospective study. *Br J Haematol* 1990;74:209-15.
11. Williamson LM, Hackett G, Rennie J, et al. The natural history of fetomaternal alloimmunization to the platelet-specific antigen HPA-1a (PIA1, Zwa) as determined by antenatal screening. *Blood* 1998;92:2280-7.
12. Waters AH. Post-transfusion purpura. *Blood Rev* 1989;3:83-7.
13. MacLennan IC, Gulbranson-Judge A, Toellner KM, et al. The changing preference of T and B cells for partners as T-dependent antibody responses develop. *Immunol Rev* 1997;156:53-66.
14. Suthanthiran M, Morris RE, Strom TB. Immunosuppressants: cellular and molecular mechanisms of action. *Am J Kidney Dis* 1996;28:159-72.