Donor anti-Jk^a causing hemolysis in a liver transplant recipient

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BACKGROUND: Hemolytic transfusion reactions have been observed in recipients of ABO- and/or D-mismatched marrow, peripheral blood, and solid organs. Passenger lymphocyte syndrome occurs when immunocompetent donor lymphocytes transferred during transplantation produce alloantibodies against host antigens.
CASE REPORT: The first case of a delayed, anti-Jk^a-mediated hemolytic reaction in a liver transplant recipient, caused by passenger donor lymphocytes, is reported here. A 43-year-old man underwent liver transplantation. Six weeks later, the patient underwent a second liver transplant. On Day 10 of the second transplant, clinical hemolysis ensued; anti-Jk^a was detected. The patient's DAT became positive, and anti-Jk^a was eluted from his RBCs. On Day 35 of the patient's second transplant, 3 weeks after the last blood transfusion, the patients' DAT was still weakly positive with anti-Jk^a in the eluate. Six months later, serum antibody screening was negative, but the DAT was still weakly positive. The patient's RBCs tested Jk(a+), whereas the second donor's RBCs were Jk(a–).
CONCLUSION: This is the first documentation of clinically significant hemolysis caused by anti-Jk^a, produced by passenger lymphocytes transferred from the donor's liver to the transplant recipient.

ABBREVIATION: PLS = passenger lymphocyte syndrome.

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Hemolytic transfusion reactions are well-described in recipients of mismatched transplants. They are common in recipients of marrow or PBPC ABO- and D-incompatible grafts,[1-3] where recipient lymphocytes produce antibodies that cause hemolysis of donor RBCs, as in major incompatibility, or by minor incompatibility, where donor antibodies are infused and/or lymphocytes that produce antibodies against recipient's RBCs are transferred. This latter phenomenon is known as passenger lymphocyte syndrome (PLS).

Alloimmune hemolysis caused by an ABO or D mismatch has also been described in solid-organ trans plant recipients:[4] Hemolysis after renal,[5-7] lung,[8] or heart–lung,[9-11] as well as liver transplants[12-14] has been described; most cases[5,6,8,9,12-15] have involved transplants with minor ABO incompatibility between donor and recipient, and the hemolysis was attributed to PLS.

The occurrence of clinically significant hemolysis caused by alloantibodies to minor blood group antigens other than ABO and/or D in a transplant recipient is rather uncommon. Hemolysis caused by anti-Jk^a has been recently described in a marrow recipient[15] but has not yet been definitely documented in a solid-organ recipient.[4]

We report here a delayed, anti-Jk^a-mediated hemolytic reaction that was caused by passenger donor lymphocytes in a liver transplant recipient.

A 43-year-old man was a victim of second-degree burns (caused by petroleum-based thinner in May 2000) on 45 percent of his body surface area. During hospitalization, he received multiple antibiotics and blood components. Fulminant hepatic failure developed in July 2000. Screening tests for CMV, Epstein-Barr virus, HCV, HBV, and HAV were all negative. Cholostatic hepatitis was found in a needle aspiration. As bilirubin values rose to 40 mg per dL, the patient underwent cadaveric liver transplantation on August 7, 2000. Both donor and recipient were group O, D+.

Two weeks after the first transplant, necrosis of the hepatic artery developed and was surgically corrected. One month later, on September 11, 2000, an aneurysm of the hepatic artery was found, and a surgical exploration revealed a necrotic vessel. The patient underwent a second cadaveric liver transplant. The donor was HBV core

antigen positive and her blood was typed as group O, D+. Her serum antibody screen before death was negative.

Antibody detection tests performed on the recipient's serum before the second liver transplantation were all negative. He received a total of 30 units of packed RBCs from early August 2000 until September 22, 2000.

Hb levels were stable at approximately 10 g per dL, and serum LDH was normal (<320 mg/dL) until 10 days after the transplant.

On September 22, Day 10 of the second transplant, the patient's Hb level dropped to 7.1 g per dL, his LDH rose to 660 mg per dL, and the bilirubin level reached 9.6 mg per dL (8.5 mg/dL direct), whereas his other liver enzymes were improving. A liver biopsy excluded rejection. Serum antibody detection testing revealed a positive screen caused by an anti-Jk^a at 2+. The patient's DAT became positive, and anti-Jk^a was eluted from his RBCs. From that point on, only Jk(a−) RBCs were transfused. The clinical hemolytic reaction gradually subsided, despite the presence of antibodies in vitro.

On October 26, 2000, Day 35 of the patient's second transplant, 3 weeks after the last blood transfusion, the patients' DAT was still weakly positive with anti-Jk^a in the eluate. Six months later, on April 2001, the serum antibody screening was negative, but the DAT was still weakly positive with a nonreactive eluate.

MATERIALS AND METHODS

Reticulocyte isolation

The patient's whole blood was drawn in EDTA. RBCs were separated by centrifugation and were washed three times with normal saline. The washed RBCs were transferred to a capillary, and reticulocytes were separated by centrifugation. The capillary was cut in two unequal parts, and the content from both parts was flushed with saline. The percentage of reticulocytes in both preparations was measured by an automated counter (Coulter Gen.S, Coulter Electronics, Brea, CA).

Immunohematologic monitoring

ABO and Rh blood group typing were performed with the standard tube agglutination method by using commercially available reagents according to the manufacturer's instructions (Gamma Biologicals, Houston, TX). For antibody detection and antibody identification, gel agglutination was used (DiaMed-ID Microtyping System, DiaMed AG, Cressier, Switzerland) with either the LISS and/or IAT or the one-stage enzyme method (Papain, DiaMed). A DAT on RBCs and reticulocytes was performed by the gel agglutination method (DC-Screening I, DiaMed-ID).

Eluates from RBCs were prepared by acid elution with a kit (DiaCidel, DiaMed). Kidd phenotyping of RBCs and reticulocytes was performed by gel agglutination (DiaMed) with anti-Jk^a- and anti-Jk^b-containing gel cards. Rh typing was performed with the tube agglutination method by using MoAb specific for C, c, E, and e (Gamma Biologicals).

RESULTS

Both the recipient and the second donor were typed as group O, D+. The donor was typed as Jk(a−b+). The sample used had been taken 2 days prior to the donor's death and had been stored for 14 days before phenotyping. The serologic phenotype of the recipient's RBCs before and after transplantation could not be determined because of previous transfusions, resulting in a mixed-field population. To identify the recipient's Kidd phenotype, reticulocytes enriched to a 38.3-percent fraction were isolated as described previously here. The recipient's reticulocytes were clearly typed as Jk(a+b−). An overview of the serologic blood group typing in the second donor and the recipient is presented in Table 1.

The results of antibody screening performed on the patient's pretransplantation and early posttransplantation samples were negative. Antibody screening became positive on Day 10 of the second transplant, revealing anti-Jk^a. The DAT, performed with the gel agglutination method, at that time gave strongly positive results (IgG, 2+; IgM, 1+; and C_3d, 3+), and no mixed-field agglutination was seen. Anti-Jk^a was confirmed by acid elution. On Day 35, anti-Jk^a was still detectable, and the DAT was weakly positive (IgG, 1+; C_3d, 1+). Six months later, no antibodies were detected, but the DAT was still weakly positive with no reactive antibody detectable in the eluate. The immunohematologic findings are summarized in Table 2.

Transfused RBCs were all ABO and D identical (group O, D+). Jk(a−) RBC units were provided when the antibody was detected. All transfused RBCs were WBC reduced by bedside filtration. The patient received immunosuppressive therapy that consisted of corticosteroids, Tacrolimus, and Mycomofetyl fenolate.

DISCUSSION

We present here a case of a liver transplant recipient who developed a delayed hemolytic transfusion reaction

TABLE 1. Second donor and recipient RBC phenotypes

	ABO	Rh	Jk^a	Jk^b
Second donor	O	D+C+c+e+e+	−	+
Patient				
Before liver transplantation	O	D+C+c+e+e+	Mixed field	NT
After liver transplantation				
Day 10 (RBC)			Mixed field	NT
Day 11 (reticulocyte)			+	−
Day 230 (RBC)			+	−

TABLE 2. Immunohematologic findings during the course of the second liver transplantation

Days after liver transplantation	DAT	Antibody detection	Antibody specificity	Eluate
1	Negative	Negative		
10	Positive (IgG 2+, IgM 1+, C_3d 3+)	Positive	Anti-Jk^a	Anti-Jk^a
35	Positive (IgG 1+, C_3d 1+)	Positive	Anti-Jk^a	Anti-Jk^a
230	Positive (IgG 1+)	Negative		

caused by passenger lymphocytes from the donor. The patient, typed group O, D+, and Jk^a+, received a second liver transplant from a Jk(a−) donor. Despite immunosuppressive treatment, the patient developed a hemolytic reaction, as evidenced by a drop in the Hb and an increase of LDH levels at 11 days after transplantation. The transfusion reaction was self-limited and subsided when all further units given to the patient were Jk(a−).

This case is unusual, being the first report of a hemolytic transfusion reaction caused by passenger lymphocytes in a solid-organ transplant with a mismatch other than ABO.

Immune hemolysis occurs occasionally after allogeneic transplantation, most often after BMT or a peripheral blood progenitor cell transplant. A few mechanisms might be responsible: recipient-derived antibodies reactive against donor antigens are observed most often in transplants where a major ABO incompatibility exists, for example, when the recipient has group O RBCs and the donor has group A RBCs. Most of these reactions are immediate and are caused by lymphocytes already cognizant of the antigen.[1,4,16] They persist until replacement of recipient RBCs by donor cells occurs. Delayed hemolysis by this mechanism may occur if very high titers of anti-A or anti-B persist in the recipient.[1] The probability of a positive DAT in such cases is as high as 40 percent.[17]

A hemolytic reaction may also be observed shortly after transplant in cases of minor incompatibility, where donor lymphocytes are transferred through the transplant and produce antibodies to the recipient's RBC antigens, for example, a group O donor and a group A recipient.

The complexity and diversity of posttransplantation hemolytic reactions are beyond the scope of this report, but suffice it to mention that they occur in 10 to 78 percent of the cases.[18,19] A combination of the latter two mechanisms has been reported in the same patient.[20]

A subclass of the minor-mismatch type of immune reaction is the PLS, where donor lymphocytes produce antibodies to the recipient antigens, but in a delayed manner. This phenomenon can hardly be explained by the passive transfer of serum antibodies during the time of transplant[3] but is the result of proliferating lymphocytes from the graft. Some maintain that it is a form of GVHD.[21,22]

In BMT and/or PBPC transplants, delayed hemolysis caused by passenger lymphocytes usually occurs before engraftment and has been attributed to the production of antibody by rapidly proliferating immunocompetent passenger lymphocytes that are transferred with the graft. Delayed appearance of antibodies to non-ABO antigens by this mechanism has been reported in bone marrow and PBPC transplant recipients: anti-D,[3] anti-Le,[23] and anti-Jk^a,[15,24,25] but hemolysis is infrequent. Antibodies to the MNS and Kidd system antigens have been reported most often.[17]

When hemolysis is clinically observed, it is usually apparent between Days 5 and 15,[2] is often abrupt in onset, and is sometimes severe, and it subsides when the recipient's incompatible RBCs are replaced, because of production of donor RBCs in BMT and/or PBPC engraftment.[1] It was noted to possibly occur more severely in recipients of PBPC transplants than in bone marrow recipients because of the larger numbers of lymphocytes in the graft.[26,27]

Interestingly, it has been pointed out[17] that antibodies to non-ABO antigens appear more often in ABO-mismatched PBPC transplants and/or BMT than in ABO-compatible transplants.

In most reported cases of bone marrow and/or PBPC transplants with non-ABO antibodies, however, there was no overt hemolysis, whereas in the case report by Leo et al.,[15] the patient developed clinical hemolysis caused by anti-Jk^a, similar to our patient.

Solid-organ transplantation complicated by hemolysis has been documented after heart−lung (70%), liver (30-40%),[28-30] kidney (17%), and bowel (9%) transplantation.[4] All are cases of ABO incompatibility. Hemolysis is the most common in minor mismatches and is often immediate and severe enough to require hemodialysis or exchange transfusion.[1] However, clinically significant hemolysis in solid-organ transplantation where the donor and recipient were matched for ABO but were incompatible for non-ABO antigens has not been documented.

An unresolved question is this: How long are donor lymphocytes transferred in grafts capable of producing antibodies? Hows et al.[3] reported that ABO antibodies are transient and are absent 3 months after transplantation, whereas anti-D has persisted for up to 1 year. There are no data regarding non-ABO- and/or Rh system antibody-producing lymphocytes. Subpopulations of WBCs, that is, T cells, myeloid, as well as B cells, were found in the circulation of immunocompetent transfusion recipients up to 1.5 years after transfusion,[31] whereas mixed chimerism between donor cells and recipient kidney and blood cells was found up to 23 years;[32] donor cells were detected after 25 years in the recipient of an intrauterine transfusion.[33]

In our case, the donor was tested antemortem for unusual serum RBC antibodies, and none were found. This may further suggest that the antibody was produced by immunocompetent lymphocytes transferred to the recipient during organ transplantation.

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