Red cell antibody problems in 1000 liver transplants

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Abstract

Liver transplant patients frequently require large amounts of blood. The frequency and nature of their red cell (RBC) antibody problems were examined. Records were reviewed in 496 adults and 286 children undergoing 1000 consecutive transplants. Twenty-two percent of adults and 14 percent of children had RBC alloantibodies. Antibodies of potential clinical significance were found before transplant in 6.3 percent of adults and 1.0 percent of children; despite immunosuppression, they appeared 1 to 5 weeks after transplant in an additional 7.5 and 5.2 percent respectively. These antibodies probably represented secondary immune responses. Of 58 transplant patients with prior potentially significant antibodies, 8 required 7 to 110 units of antigen-untyped blood after 8 to 28 units of antigen-negative blood; of these patients, one had subsequent hemolysis. Positive direct antiglobulin tests in 24 percent of adults and 10 percent of children were most often thought to be due to nonspecific adsorption of IgG. Anti-recipient ABO antibodies developed in 22 of 60 (37%) evaluable ABO-unmatched grafts; 13 cases had associated hemolysis. In all, 36 percent of adults and 20 percent of children had diverse RBC antibody problems. Resolution of these problems is an important part of the laboratory support necessary for a liver transplantation program.

The Blood Bank plays an important role in the supportive care of liver transplant patients before, during, and after surgery. Before transplant for end-stage liver disease, patients are often transfused for bleeding due to portal hypertension, esophageal varices, deficient coagulation factors, thrombocytopenia, or other surgery. After transplant, many patients need further transfusions and some require retransplantation. The liver transplant program at the University of Pittsburgh has the world's largest such experience, and since the program's inception in 1981, its overall perioperative and postoperative blood use has been carefully documented.[1–5] These patients have ample opportunity for red cell (RBC) alloimmunization.

The purpose of this study was to examine all RBC antibody problems encountered in 1000 consecutive liver transplants performed in our center from February 1981 to February 1987. Previously, as part of an examination of the relationship of the HLA system to RBC alloimmunization,[6] we reported a 9.5 percent frequency of potentially significant RBC alloantibodies in 263 adults. We present here our cumulative experience in both adults and children with regard to 1) the overall frequencies of RBC antibodies; 2) the onset and duration of significant RBC antibodies relative to transplant immunosuppression; 3) the cases in which insufficient compatible blood was available for surgery; and 4) the frequencies and causes of

positive direct antiglobulin tests (DATs) in these patients, including an update of our previous series of ABO antibodies of graft origin.[7] We have found that 36 percent of adults and 20 percent of children undergoing liver transplantation have one or more RBC antibody problems at some point during their hospital course.

Materials and Methods

We examined retrospectively the records in our Central Blood Bank transfusion service for 782 patients undergoing 1000 consecutive liver transplants from February 1981 to February 1987. Children and adults underwent transplantation at Children's Hospital of Pittsburgh and Presbyterian-University Hospital, respectively. Previous transfusion histories were usually unavailable owing to referral from distant hospitals. Patient diagnoses, surgical and anesthetic techniques, and overall outcomes have been described.[8,9] Immunosuppression consisted of cyclosporine, corticosteroids, and adjunctive rabbit antilymphocyte globulin or monoclonal OKT3 antibody.[10,11] The average posttransplant hospitalization was 4 to 6 weeks. When patients received potentially incompatible blood at transplant or had positive DATs due to alloantibodies or graft-origin ABO antibodies, their charts were reviewed for evidence of concurrent hemolysis, that is, for otherwise unexplained decreases in hematocrit and increases in serum indirect bilirubin.

Our antibody screen employed two cells with an autocontrol and was read at immediate-spin, after 30 minutes at 37° C in saline, and then with antiglobulin reagent. In 1986, the antiglobulin reagent was changed from a polyspecific reagent to anti-IgG and the autocontrol was replaced by a DAT. Until 1985, positive autocontrols, associated with positive DATs due to IgG sensitization, led to elution studies in all cases; since then elution studies have been done only when transfusions were given in the past month, when the transfusion history was unknown, when possible hemolysis was reported, or when ABO antibodies were suspected. Crossmatch procedures were similar to those for the screen until 1985, when only immediate-spin crossmatches were used for patients without evidence of irregular antibodies. Antibodies were identified with untreated and ficin-treated RBC panels.

All transplant candidates were typed and screened at our center on initial evaluation and usually on each subsequent admission. At surgery, which typically took place on 4 to 8 hours' notice, 20 units of RBCs were initially crossmatched for adults and 10 units for children. For patients with potentially significant RBC antibodies, blood lacking the offending antigens (antigen-negative) could be sought with our blood center computer system (Model 3000, Hewlett-Packard, Corvallis, OR), which has provisions for seeking known donor phenotypes in the current inventory (System software, Blood Center of Southeastern Wisconsin, Milwaukee, WI). RBCs and fresh-frozen plasma were generally used in equal amounts until 1986, when the blood inventory for adults was changed so that the most common types, groups B Rh+, O, and A, could usually receive their first 20 units as modified platelet-poor whole blood. The organ donor's ABO type was noted on the recipient's blood bank record.

Statistical comparisons employed the chi-square test or Fisher's exact test with significance at $p < 0.05$.

Results

We analyzed RBC antibody problems for 496 adults receiving 616 liver transplants and for 286 children receiving 384 transplants. The overall frequencies of clinically significant and insignificant RBC antibodies in adults and children are shown in Table 1. The most common potentially significant antibodies (n = 130) were anti-K (n = 45), -E (40), -D (14), and -Jk^a (9). Fifty-two patients had one potentially significant antibody, 25 had two, 8 had three, and 1 had

four. The overall frequency of potentially significant antibodies in adults, 13.7 percent, is somewhat higher than that in our earlier report (9.5%),[6] mainly because the frequency has risen in recent years. The 1985 to 1987 frequency in adult patients was 16.9 percent. The most common clinically insignificant antibodies (n = 92) were cold agglutinins (n = 31), high-titer low-avidity antibodies (20), and anti-M, -Lu[a], and -Bg (7 each).

We found, or knew of, RBC antibodies of potential clinical significance prior to the first transplant in 31 adults (6.3%) and 3 children (1.0%) (Table 1). In 24 of these 34 cases, we identified the antibodies before the day of transplant; other centers informed us of two patients in whom no antibodies were detected by us. Another 37 adults (7.5%) and 15 children (5.2%) developed such antibodies after their first transplant; blood usage in these transplants averaged 9 units of RBCs in children and 19 in adults, which was no different than usual.[4] Posttransplant antibodies were all found 1 to 5 weeks later, except for three patients in whom antibodies (anti-K [n = 3] and -E [n = 1]) were found at 7 weeks to 4.5 months after transplant. Eleven percent of retransplants were preceded by potentially significant RBC antibodies in both adults (13/120) and children (11/98); these totals include third transplants in 20 adults and 14 children and fourth transplants in 1 adult and 1 child.

The frequency of potentially significant RBC antibodies was analyzed according to patient sex and diagnosis. Females had higher rates than males in both pediatric (8 vs. 4%) and adult (15 vs. 11%) patients. The latter difference was due to primary biliary cirrhosis (PBC), which occurs mainly (90%) in women and accounts for 40 percent of our adult female transplant patients. Twenty percent of women with PBC had potentially significant RBC antibodies, compared to 12 percent of non-PBC women. On the other hand, adults who underwent transplantation for hepatic neoplasms (7% of our adult patients) had a low rate (3%) of potentially significant RBC antibodies. None of these differences reached statistical significance. No other diagnoses exhibited any trends different from the overall pediatric or adult rates.

In our general hospital population, we previously studied the frequency of loss of alloantibodies over time, and found that 29 percent of 170 potentially clinically significant antibodies, were undetected on screenings done at least one month later. [12] In liver transplant patients analyzed similarly, 14 of 28 (50%) such antibodies were lost before surgery, and 9 of 14 (63%) antibodies present at surgery were lost afterward (both values, p < 0.01, compared to the previous study). Our earlier data suggested that children had a higher rate of antibody loss; this would help explain why we did not detect antibodies until soon after transplant, when they were presumably restimulated, in 15 of our 18 significantly alloimmunized pediatric patients.

There were 58 transplant patients in whom significant antibodies were known previously. Because of urgent need, eight of these patients required RBCs or whole blood that had not been fully typed for the antigen(s) in question. This blood was issued after 8 to 28 units of antigen-negative RBCs were given. These eight cases are detailed in Table 2. Four of these patients survived the hospitalization. The number of antigen-positive units given was estimated from the expected phenotype frequencies of the untyped blood. Patient 1 was the only one to receive a large number (18 units) of antigen-negative RBCs at the end of the case. Only Patient 4 had evidence of subsequent hemolysis due to anti-Fy[a] that was found 17 days postoperatively, after the hematocrit had dropped from 39 to 22 percent over 1 week without bleeding. This 14-year-old girl received the fewest untyped units but was also the only one of the group with the full combination of antibody present at surgery, antigen-untyped blood given at the end of the case, and long-term survival.

Table 1 shows the frequency of positive DATs in adults and children. Of the 782 patients, 146 (18.6%) had positive DATs. As 69 of these latter patients had nonreactive eluates, it was

thought that a major cause of their positive DATs was the nonspecific adsorption of IgG onto the RBCs. Five eluates reacted with all RBCs tested, and in 18 cases eluates were not prepared when few clinically significant results were expected (see Methods).

In the 14 cases in which alloantibodies were found by elution, we sought evidence of hemolysis in 13; one patient's chart could not be located. Ten patients had delayed antibody responses to K (n = 6), E (3), Jk^a (3), and M (1), but only one adult had clinical hemolysis, which was due to anti-Jk^a. This patient had two transplants in 3 days and then a week later had a 2-day hematocrit drop from 34 to 19 percent, a rise in serum indirect bilirubin from 4 to 17 mg per dl, and no detectable serum haptoglobin. Hemolysis also occurred in the pediatric patient who received Fy(a)-incompatible RBCs (Patient 4 above).

The transplantation of ABO-unmatched grafts, with the potential to produce ABO antibodies of graft origin, declined from 17.8 percent of all transplants in 1981 to 19847 to 4.8 percent in 1986 and 1987; such grafts have fared worse than ABO-matched ones,[13] Twenty-two of 60 (37%) evaluable ABO-unmatched grafts produced definite or probable anti-recipient ABO antibodies, as originally defined.[7] Of the 22 grafts producing antibodies in 20 patients, 3 could not be evaluated for hemolysis because of concurrent gastrointestinal hemorrhage or prior use of group O RBCs. Thirteen of the other 19 (68%) antibodies caused hemolysis, with an average hematocrit drop of 18 percentage points (standard deviation, 6%; range, 11–30%). We have never observed such antibodies in group A-to-B or group B-to-A liver transplants, but graft survival is poor in these patients because of ABO incompatibility.[14]

In all, 36 percent of adults and 20 percent of children had at least one RBC antibody problem.

Discussion

Patients undergoing liver transplants often are heavily transfused. The overall frequencies of RBC alloantibodies of potential clinical significance in our study—14 percent in adults and 6 percent in children—are analogous to those reported in other conditions such as gastrointestinal bleeding (8%) and chronic renal failure (7–10%).[6,15] Obtaining a type and screen has been a helpful part of our pretransplant evaluation. Of 58 total transplants in which the patient had significant antibodies, only 10 cases (8 first transplant, 2 second) were not known to us until the day of surgery.

Blomqvist et al.[16] found clinically significant RBC antibodies in 8 of 18 (44%) adult liver transplant patients. In the first 83 liver transplant patients (nearly all adults) at the Mayo Clinic, 12 (14%) had significant antibodies before operation and 5 (6%) formed such antibodies afterward.[17]

Many potentially significant antibodies in our study were first detected by us after transplantation, despite the immunosuppressive regimen involved. For the following reasons, we suspect that most of these antibodies represented secondary immune responses. First, as we have noted with regard to graft-origin ABO antibodies,[7] cyclosporine is less effective against secondary than primary immune responses. Second, nearly all of these posttransplant antibodies were found within 5 weeks, in contrast to primary Rh isoimmunization, in which anti-D generally does not appear for 2 to 6 months. Third, in another study we have found that primary anti-D formation was detected infrequently after multiple Rh-incompatible RBC transfusions in organ transplant patients.[18] Some of these posttransplant antibodies may have been detected elsewhere previously and subsequently lost before transplant. Hence, the availability of information from the referring hospital would be desirable. However, the clinical impact of these posttransplant antibodies was limited with regard to delayed hemolysis. We excluded the case of anti-M because it is not usually clinically significant. This left nine patients who had alloantibodies of potential clinical significance eluted from circulating RBCs, but

only one of the eight evaluable cases had clinical hemolysis. Hyma et al[19] observed a case of immune hemolysis due to multiple patient alloantibodies appearing 9 days after liver transplant.

Eight patients with previously detected, potentially significant RBC antibodies were transfused urgently with RBCs untyped for the offending antigen(s). Delayed clinical hemolysis occurred in one patient. There are a number of possible reasons that most of these transfusions did not cause complications. Four of these patients had lost their previous antibodies by the time of surgery. Circulating antibodies in the others were presumably washed out to a large extent by 8 to 27 initial units of antigen-negative RBCs and other fluids. The corticosteroid regimen may have suppressed postoperative extravascular immune RBC destruction. Also, two patients died early, before hemolysis might have ensued. Saving some antigen-negative units for the end of surgery is desirable and was attempted in several of our cases, but the proper timing proved difficult to estimate. Thompson et al.[20] suggested that when sufficient antigen-negative blood cannot be obtained for patients with multiple antibodies, consideration should be given first to avoiding antibodies that can fix complement and thus potentially cause intravascular hemolysis. Intraoperative blood salvage can also be of benefit in transplant cases with difficult RBC antibodies.[21]

Positive DATs were due to a number of reasons but were thought most commonly to be due to nonspecific adsorption of IgG onto the RBCs. Toy et al.[22] described an association of positive DATs, nonreactive eluates, and elevated serum immunoglobulins in patients with liver disease or infection; they suggested that the IgG was nonspecifically adsorbed onto the RBC membrane. In our ABO-unmatched transplants, the occurrence of graft-origin ABO antibodies to the recipient's RBCs declined recently, both because fewer such cases were done and because the frequency of antibody appearance in 1986 and 1987 decreased to 1 in 10 cases. In 20 ABO-unmatched liver transplant patients of Jenkins et al.,[23] 4 had hemolysis and 2 others had possible antibodies. Steininger et al.[24] noted hemolysis in 2 of 11 patients. On the other hand, Angstadt et al.[25] and Badosa et al.[26] observed hemolysis after all nine ABO-unmatched hepatic grafts in their combined series. IgG allotyping has demonstrated the donor origin of such antibodies from liver and renal transplants.[27,28] We found no non-ABO antibodies of graft origin and no Rh immunization attributable to Rh-incompatible grafts, both of which have been observed after renal transplantation.[29–33]

The extensive blood needs of liver transplant patients are frequently complicated by a diverse range of RBC antibody problems. Resolution of these problems is an important part of the laboratory support necessary for a liver transplantation program.

Acknowledgments

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Table 1

Frequencies of RBC antibody problems, in 782 liver transplant patients receiving 1000 liver transplants

Antibody problem [*]	Adults (n = 496)				Children (n = 286)			
	Before transplant	After transplant	Total	Percentage	Before transplant	After transplant	Total	Percentage
Alloantibodies	60	50	110	22.2	16	24	40	14.0
Significant	31	37	68	13.7	3	15	18	6.3
Insignificant	42	20	62	12.5	13	9	22	7.7
Other[†]	0	1	1	0.2	1	1	2	0.7
Positive DAT	44	74	118	23.8	5	23	28	9.8
Nonreactive eluate	25	34	59	11.9	4	6	10	3.5
Panreactive eluate	3	1	4	0.8	1	0	1	0.3
No eluate	14	4	18	3.6	0	0	0	0.0
Alloantibody	1	8	9	1.8	0	5	5	1.7
Delayed	1	7	8		0	4	4	
Anti-D in Rh+	0	1	1		0	1	1	
Graft ABO antibody	0	13	13	2.6	0	7	7	2.4
Passive ABO antibody	0	10	10	2.0	0	3	3	1.0
Other[‡]	1	8	9	1.8	0	2	2	0.7
All problems	99	80	179	36.1	20	37	57	19.9

[*] Patients with multiple problems are counted in each individual category.

[†] Anti-D from Rh immune globulin (1 child) or of uncertain transient nature in 2 Rh+ patients who are also included below in DAT alloantibodies.

[‡] IgG and C3 (n = 3), C3 (n = 6; 4 adults and both children), or not tested monospecifically (n = 2).

Table 2

Patients with previous significant RBG alloantibodies receiving antigen-untyped blood at liver transplant

| | | Units of RBCs | | | | | After transplant | | |
| | | Initial | | | | | | | |
Patient	Antibodies	Ag-Neg*	UT†	Estimated Ag-Pos	Final Ag-Neg	Total used	Antibody in eluate	Clinical hemolysis	Patient survival
1	c, E	27	110	86	18	155	Anti-E	N‡	Died 26 days
2	c, E, Fy^b	10	74§	64	–	84	N	N	Died 6 days
3	E, (K, Jk^a)‖	8	36	30	–	44	N	N	Died 7 days
4	c, E, Fy^a	18	7¶	4	–	25	Anti-Fy^a	Y**	Y
5	(E)	28	50	15	–	78	N	N	Y
6	(E, K, Jk^a)	10	35††	29	–	45	B (passive)	N	Y
7	(K)	26	11	1	4	41	N	N	Y
8	(K)	20	11	1	4	35	N	N	Died 48 days

*
Antigen-negative.

†
Antigen-untyped.

‡
No.

§
51 c(−), E(−).

‖
( ): Previously present but not detected at surgery.

¶
c(−), E(−).

**
Yes.

††
10 E(−).