

570

suggestive evidence that circular EBV-DNA molecules are replicated by cellular DNA polymerases. In vitro EBV-transformed cell cultures are known to be resistant to acyclovir.<sup>13,16,24</sup> This observation and our clinical experience suggest a need to develop anti-EBV agents directed against viral targets other than the DNA polymerase.

In conclusion, we have developed a simple gel technique that may be useful in the discrimination of permissive from nonpermissive EBV-induced lymphoproliferative disorders. Such studies may help physicians to choose between antiviral chemotherapy and traditional cytotoxic chemotherapy in the treatment of such disorders.

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### ISOHEMAGGLUTININS OF GRAFT ORIGIN AFTER ABO-UNMATCHED LIVER TRANSPLANTATION

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THE increasing success of liver transplantation in recent years has provided an experimental model to study and document the hepatic synthesis of many plasma proteins.<sup>1-5</sup> The normal hepatobiliary tract has not been regarded as a major source of antibody,<sup>6,7</sup> aside from the enteric IgA secreted from plasma into the biliary tree.<sup>8</sup> Liver transplantation affords the opportunity to study the production of antibody to red cells. Recipient ABO incompatibility to the donor (a mismatched transplant, e.g., a group A liver transplanted into a group B recipient), although not absolutely contraindicated in liver transplantation, is avoided when possible. However, ABO-unmatched transplants (defined as a group O liver transplanted into a non-group O recipient or a group A or B liver to an AB recipient) are used frequently. We report the short-term occurrence of anti-recipient ABO antibody after eight unmatched transplants. In five cases there was evidence of hemolysis. No such antibodies have been seen in over 180 ABO-matched transplants at

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our center. These antibodies were most probably produced by donor lymphocytes transplanted in or with the livers.

### METHODS

Serologic studies were performed according to standard methods.<sup>9</sup> Serum isohemagglutinins were tested without intubation, after 37°C incubation for 30 minutes, and with broad-spectrum antiglobulin reagents.

Six patients with ABO-unmatched liver transplants and confirmed anti-recipient antibody production were studied prospectively. Blood samples were drawn at least weekly for direct and indirect antiglobulin testing, crossmatching, and elutions. The hematocrit, serum bilirubin, clinical status, and transfusions were closely monitored. Two other confirmed cases (in Patients 5 and 7) were found on review of all ABO-unmatched liver transplantations performed at our center. Confirmed cases of isohemagglutinin production were defined as those in which passive transfer of antibody could be ruled out — i.e., either no unmatched plasma-containing blood products were given at surgery (five cases) or, if they were, immediate postoperative specimens were negative for antibody (Patients 1A, 1B, and 6) — and there was serologic or clinical confirmation of the antibody's presence by repeat testing or signs of hemolysis. Cases were designated as possible if the antibody was first detected after the second postoperative day but the above criteria were not met in full.

Liver transplantation was performed according to established techniques, including cyclosporine immunosuppression.<sup>10,11</sup> Little or no plasma was transferred in the grafts because the livers were copiously perfused with preservative solution and then, just before anastomosis, with saline.<sup>12</sup> Our transfusion practices have been described elsewhere.<sup>13</sup>

### RESULTS

One hundred seventy-one patients underwent a total of 225 orthotopic liver transplantations at the University of Pittsburgh from February 26, 1981, to January 20, 1984. Unmatched livers were transplanted in 40 instances (18 per cent). In 33 of these, a non-group O patient (16 group A, 15 group B, and 2 group AB patients) received a group O liver. The other seven unmatched transplants were in group AB patients who received group A (five) or group B (two) livers.

Table 1 summarizes the prevalence of anti-recipient ABO antibodies in the 40 unmatched transplants. Such antibodies were demonstrated in 8 of 29 evaluable cases (28 per cent). In addition, anti-recipient ABO antibodies were possibly present in five other transplants (17 per cent). In 13 cases no antibody was seen in specimens tested during 7 to 21 days after surgery. In three instances, antibody was detected one day after unmatched blood components had been given at surgery but all subsequent specimens were negative; these findings were attributed to the transient presence of passively acquired antibody. Eleven patients were not evaluable because of early death (four), the preoperative presence of anti-A<sub>1</sub> in group A<sub>2</sub> patients (two), or the absence of blood-bank specimens during the first 7 to 21 days (five).

Table 2 shows the clinical and serologic characteristics of the eight ABO-unmatched patients in whom the presence of anti-recipient ABO antibody was confirmed. When studied with monospecific antiglobulin reagents, all patients had complement and five had IgG on their red cells. When studied with polyspecific

Table 1. Prevalence of Anti-Recipient ABO Antibody in 40 Consecutive ABO-Unmatched Liver Transplants.

PREVALENCE OF ANTIBODY	BLOOD GROUPS OF DONOR → RECIPIENT		TOTAL
	O → Non-O	A or B → AB	
	no. of recipients		
Antibody formation	7	1	8
Possible antibody	5	0	5
No antibody	12	1	13
No antibody except passive	2	1	3
Not evaluable	7	4	11
Total no. of transplants	33	7	40

antiglobulin reagents, freeze-thawing or heat-induced eluates from the patients' red cells gave 1+ to 3+ reactions with other red cells of the same ABO group. In three of the transplants from group O donors, anti-A, B was present in eluates in addition to anti-A or anti-B alone in the serum. Reactions of the patients' serum samples against appropriate red cells ranged from weak to 2+ by indirect antiglobulin testing. The reaction strength did not correlate with the degree of hemolysis. Serum samples from two patients (1A and 3) were treated with dithiothreitol to inactivate IgM; both had residual activity when tested with antiglobulin reagents, indicating the presence of IgG antibody. No other irregular red-cell antibodies were detected with group O screening cells, except for one instance of anti-Kell in serum.

There was evidence of hemolysis in five cases. The changes in hematocrit shown in Table 2 occurred over two to three days, well after the period of perioperative transfusion requirements (see below), and were not associated with hemorrhage. Concurrent increases in total and indirect serum bilirubin were also noted. Rejection could not be ruled out as a factor contributing to the elevation in total bilirubin levels, but it is not known to cause sudden anemia.

Figure 1 shows the period when the anti-recipient ABO antibodies were present. The antibodies were first detected 8 to 16 days after transplantation and were last detected at 11 to 41 days. Hemolysis, when it occurred, began 5 to 8 days after surgery and lasted for 7 to 19 days. In Figure 1, the final red-cell transfusion of the episode is used to indicate the end of hemolysis. In two cases complement was still present on the red cells for prolonged periods after the ABO antibody was undetectable.

Five of these eight grafts have survived for 5 to 16 months; repeat transplants were necessary after herpesvirus and cytomegalovirus hepatitis in Patients 1A and 3 and after rejection in Patient 7. All patients except Patient 3 have survived.

### DISCUSSION

In each of the eight cases reported here, circulating and red-cell-bound isohemagglutinins to the patient's own A or B antigen were detected 8 to 16 days after transplantation of a liver from an ABO-unmatched

Table 2. Patients in Whom Anti-Recipient ABO Antibody Was Detected.

PATIENT No.	AGE/SEX	DIAGNOSIS	ABO GROUPS		ANTIBODY IN ELUATE	DIRECT ANTI-GLOBULIN TEST *		INDIRECT ANTI-GLOBULIN TEST †			CHANGE IN HEMATOCRIT
			DONOR	PATIENT		IgG	C	IS	37°C	AGT	
1A ‡	26/M	Wilson's disease	O	B	To B	-	+	1+	W	W	From 42-24%
1B ‡			O	B	To B:A.B	-	+	W	N	W	None
2	19/F	Non-A, non-B hepatitis	O	B	To B	+	+	N	1+	1+	From 41-28%
3	28/M	Sclerosing cholangitis	O	B	To B:A.B	+	+	4+	3+	2+	From 38-23%
4	27/M	Hepatitis B	O	B	To B	+	+	N	W	W	GI hemorrhage
5	2/M	α <sub>1</sub> -Antitrypsin deficiency	O	A <sub>2</sub>	To A <sub>1</sub>	-	+	N	N	W	From 44-20%
6	52/M	Alcoholic cirrhosis	O	A <sub>1</sub> §	To A:A.B	+	+	N	N	W	None
7	5/F	α <sub>1</sub> -Antitrypsin deficiency	A	AB	To B ¶	+	+	1+	2+	1+	From 42-12%

\*The direct antiglobulin test is specific for IgG or complement (C).

†For the indirect antiglobulin test with immediate spinning (IS), 37°C, and antiglobulin testing (AGT). W denotes weak and N negative.

‡1A and 1B refer to the second and third transplantations in Patient 1. Group O red cells were given at the third transplantation.

§A<sub>1</sub> typing was performed on reticulocyte-enriched red cells 48 days after surgery.

¶Anti-Kell was also present in serum, but not in a red-cell eluate.

donor. Evidence of hemolysis was present in five of the seven patients. The antibodies persisted for 2 to 4½ weeks. Passive transfer of antibody from the liver itself or from transfusions was ruled out. The findings in Patient 5, in theory, may represent hemolytic anti-A<sub>1</sub> alloimmunization in a group A<sub>2</sub> patient, but this is extremely rare.<sup>14</sup>

We conclude that these antibodies most probably originate from B lymphocytes that are transplanted in or with the donor liver and then respond to the recipient's ABO antigens in a type of graft-versus-host reaction. A large number of lymphocytes may accompany

the liver in the lymphatics<sup>15,16</sup> and lymph nodes.<sup>17</sup> The 8-to-16-day latency period and the frequently observed IgG component of the antibodies are consistent with a secondary immune response from previously primed donor B lymphocytes. In addition, cyclosporine does not blunt secondary immune responses as much as primary ones.<sup>18,19</sup> Proof that these antibodies are of donor origin could be obtained by immunoglobulin allotyping, but in our patients this is often precluded by plasma transfusions before and at surgery.

Production of anti-recipient red-cell antibodies has also been associated with spleen,<sup>20,21</sup> kidney,<sup>22-28</sup> and lung<sup>29</sup> transplantation. All except one report<sup>25</sup> involved ABO antibodies. ABO-unmatched splenic grafts have induced severe hemolysis. Patients with renal grafts, like our patients, have had short-lived, usually hemolytic antibodies appearing one to two weeks after transplantation. Designation of these antibodies as "autoantibodies"<sup>22,24-29</sup> tends to obscure their true pathogenesis.

Unless the supply of donor organs increases to allow uniform ABO matching, ABO-unmatched liver transplantation is likely to continue. To date, 20 per cent of our group A recipients, 56 per cent of our group B recipients, and 90 per cent of our group AB recipients have received ABO-unmatched livers. Our current transfusion policy in such cases is to use recipient-type blood components initially and to monitor the patients for anti-recipient ABO antibodies. If they appear, donor-type packed red cells are given when needed throughout the period when the antibody is present. During our prospective study, these red cells were washed to remove blood-donor antibodies, but the small amount of antibody in the plasma of packed red cells is unlikely to be harmful to the recipient. Plasma, if needed, should be of the recipient's ABO type; the soluble ABO antigens therein may neutralize some of the ABO alloantibody.<sup>14</sup>

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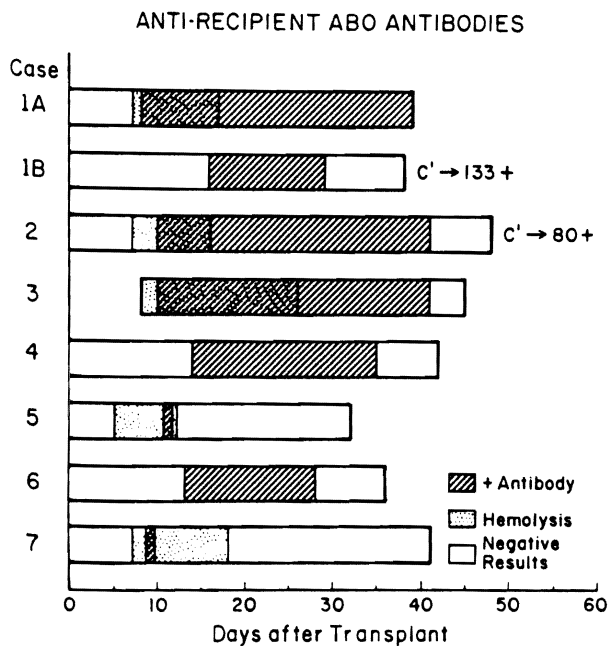


Figure 1. Presence of Anti-Recipient ABO Antibodies and Hemolysis after ABO-Unmatched Liver Transplantation.

C' indicates the persistence of complement on red cells in the final samples obtained at 133 and 80 days.

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## CASE RECORDS OF THE MASSACHUSETTS GENERAL HOSPITAL



### Weekly Clinicopathological Exercises

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### CASE 44-1984

#### PRESENTATION OF CASE

A 15-year-old girl was admitted to the hospital because of a question of chronic active hepatitis.

She was well until 1½ years earlier, when she began to experience fatigue, malaise, and arthralgia in the

elbows and knees. One year before admission a physician made a diagnosis of infectious mononucleosis and prescribed prednisone, without improvement. Eight months before entry orthodontic braces were fitted, and she began to notice bleeding gums. Five months before admission anemia was found. A physician made a diagnosis of mycoplasma infection, but the administration of iron and an antibiotic had no effect. The results of tests of liver function were reported to be normal.

One month before entry she complained of "sinus headaches." Laboratory studies revealed abnormalities in tests of liver function, and she was admitted to another hospital. Examination revealed scleral icterus, lymphadenopathy, and hepatosplenomegaly. Neurologic examination was negative; no Kayser-Fleischer rings were observed on slit-lamp examination. The hematocrit was 33 per cent. The prothrombin time was 17.5 seconds, with a control of 12 seconds. The bilirubin was 2.2 mg per 100 ml (38  $\mu$ mol per liter). The aspartate aminotransferase (SGOT) was 591 U per milliliter (4.73  $\mu$ mol · sec<sup>-1</sup> per liter), the glutamic pyruvic transaminase (SGPT) 257 U, and the alkaline phosphatase 47 U. The serum copper was 120  $\mu$ g per 100 ml (19  $\mu$ mol per liter) (normal, 100 to 200  $\mu$ g [20 to 30  $\mu$ mol]). The ceruloplasmin was 0.17 g per liter (normal, greater than 0.23 g). In a 24-hour specimen