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HETEROAGGLUTININS AND THEIR SIGNIFICANCE IN BABOON HEPATIC XENOTRANSPLANTATION

DARRELL J. TRIULZI,[1] ELIZABETH A. JOCHUM,[2] IGNAZIO R. MARINO,[3] AND THOMAS E. STARZL[4]

Institute for Transfusion Medicine, Transplant Division, and Transplant Institute, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania

The role of preformed xenoreactive antibodies in xenograft recipients is unknown. Humans and baboons possess red cell agglutinating activity associated with isohemagglutinins and heteroagglutinins (HA). We examined the role of HA in two patients who received ABO-identical baboon livers. Human antibaboon HA were assessed by correlating serial titers with studies for rejection. Serial direct antiglobulin testing (DAT) was used to detect baboon antihuman HA, potentially produced by transplanted passenger lymphocytes. Patient 1 survived 70 days. The human antibaboon HA titers remained essentially unchanged from preoperative values. Although hyperacute rejection did not occur, and there was only mild cellular rejection, liver function was suboptimal. Postreperfusion immunoglobulin and complement deposition and histologic changes suggested complement-mediated injury. DAT testing was negative except for passively acquired anti-A from transfusion. At autopsy there was marked bile stasis, but no rejection. Patient 2 survived 26 days with essentially unchanged HA titers until preterminal. Although there was no hyperacute rejection and only mild humoral rejection (without cellular rejection), suboptimal liver function and bile stasis were again noted. Postreperfusion immunoglobulin and complement deposition again suggested complement-mediated injury. DAT testing was negative. At autopsy there was no rejection. Human antibaboon HA do not appear to be associated with hyperacute or cellular rejection, but their role in the complement-mediated injury, suspected in both cases, cannot be definitively excluded. Baboon antihuman HA were not detected in either patient.

The relationship between xenoreactive antibodies and xenograft rejection remains unclear. Candidate antibodies include those which cause hemagglutination or lymphocytotoxicity (*1*). ABH antibodies have been associated with early loss of human hepatic allografts (*2*, *3*) and baboon renal (*4*) and cardiac (*5*) xenografts. Baboons do not express ABH antigens on their red cells (*6*, *7*) but these antigens have been demonstrated on baboon vascular endothelium (*8*). Non-ABH antibodies capable of agglutinating red cells from other species (heteroagglutinins) are present in both humans and baboons (*9*) and have been implicated in renal xenograft rejection (*4*). Passenger lymphocytes transplanted with the baboon liver represent a potential source of baboon antihuman heteroagglutinin (HA)* that could cause hemolysis or a positive direct antiglobulin test (DAT). Conversely, human sera contain antibaboon HA that could crossreact with baboon hepatocytes or endothelial cells causing rejection.

We report the results of red cell serologic studies to detect HA and their clinical correlation in two patients who underwent hepatic xenotransplantation using ABO-identical baboon donors.

[1] Address correspondence to Darrell J. Triulzi, M.D., Assistant Professor of Pathology and Medicine, University of Pittsburgh Medical Center, Institute for Transfusion Medicine, 3636 Blvd. Allies, Pittsburgh, PA 15213.

[2] Elizabeth E. Jochum, M.T. (A.S.C.P.), S.B.B., Institute for Transfusion Medicine, 3636 Blvd Allies, Pittsburgh, PA 15213.

[3] Ignazio R. Marino, M.D., Veteran's Administration Medical Center, 4 Falk, University of Pittsburgh Medical Center, Pittsburgh, PA 15213.

[4] Thomas E. Starzl, M.D., PhD., 4 Falk, University of Pittsburgh Medical Center, Pittsburgh, PA 15213.

* Abbreviations: DAT, direct antiglobulin test; HA, heteroagglutinin.

MATERIALS AND METHODS

Patients. The case histories for these patients have been described in detail elsewhere (*10, 11*). Briefly, Patient 1 was a 35-year-old man with end-stage liver disease due to hepatitis B virus. The patient was also HIV 1–positive without evidence of immune deficiency. The patient's blood group was A-positive and he received a liver from a group A baboon. The red cell antibody screen was negative but the patient did have lymphocytotoxic antibodies against baboon donor lymphocytes (positive lymphocytotoxic crossmatch).

Patient 2 was a 62-year-old man with end-stage liver disease due to hepatitis B. The patient's blood group was B-positive and he received a liver from a group B baboon. His red cell antibody screen was negative but the patient did have lymphocytotoxic antibodies against baboon donor lymphocytes (positive lymphocytotoxic crossmatch).

Patient testing. Serum and EDTA samples were obtained on both patients preoperatively and daily postoperatively for at least 3 weeks and then weekly. The serum titers of human antibaboon HA were determined on each sample. A DAT using antihuman IgG and antihuman C3 (Ortho Diagnostics, Raritan NJ) was performed weekly. Immune hemolysis was defined as a decrease in hemoglobin with a rise in indirect bilirubin and a positive DAT.

Determination of antibaboon HA titers. Patient serum was serially diluted and incubated with a 3–5% suspension of washed red cells from the baboon donor at room temperature and antiglobulin (Coomb's) phase. Titers were repeated on patient serum treated with dithiothreitol to inactivate IgM antibody. Each of these samples was tested in parallel with a preoperative serum sample. The last tube showing macroscopic or microscopic reactivity was considered the endpoint.

ABO determination of baboons. Preparation of baboon serum: Complement was inactivated by heat-treating baboon serum at 56°C for 60 min. Heated baboon serum was then adsorbed with washed human group O red blood cells at 4°C for 2 to 18 hr to remove baboon antihuman HA. Reverse typing: Adsorbed baboon serum was tested against washed human A1, B, and O cells using 2 drops of serum per drop of cells. O cells were used as a control to monitor the removal of antihuman HA.

RESULTS

Patient 1: clinical course. The patient was treated with an immunosuppressive regimen that included FK506, steroids, prostaglandins, and cyclophosphamide. Surgery was tolerated well and the patient was eating and walking within 5 days. A transient increase in plasma hemoglobin was noted on day 2 without evidence of immune hemolysis. There was no other clinical or laboratory evidence of hemolysis until late in the course (day 65) when the patient developed sepsis complicated by disseminated intravascular coagulation.

Intraoperative liver biopsy after reperfusion revealed sinusoidal neutrophilia with 3^+ IgG and 3^+ IgM sinusoidal deposition and focal C3 deposits (Figs. 1 and 2). Subsequent biopsies showed only mild evidence of cellular rejection and decreasing immunoglobulin and complement deposition with virtual disappearance by day 24 (Figs. 3 and 4). By day 55, minimal sinusoidal deposition of IgG and IgM (no C3) was again present, which increased in the final biopsy (Table 1). Despite the lack of histologic evidence of rejection, suboptimal xenograft function was observed. Biliary canalicular enzymes were markedly elevated and serum albumin levels were persistently less than 2 g/dl. The patient died on day 70 of a subarachnoid hemorrhage due to invasive aspergillosis. At autopsy there was no evidence of cellular rejection, but the entire biliary tree was filled with inspissated bile.

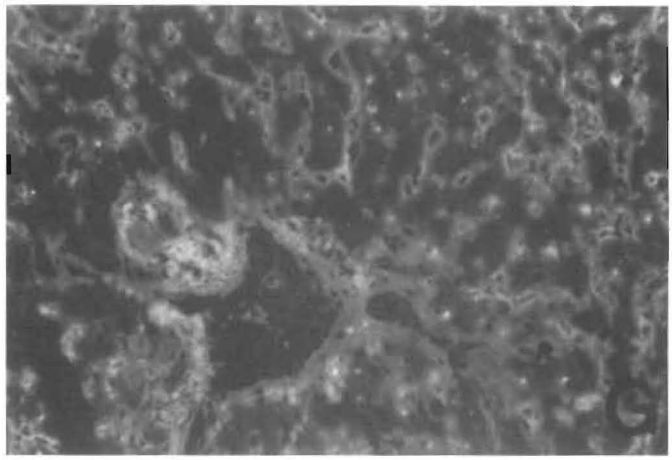

FIGURE 1. Postreperfusion liver biopsy in patient 1 showing 3+ sinusoidal staining of IgG (20×).

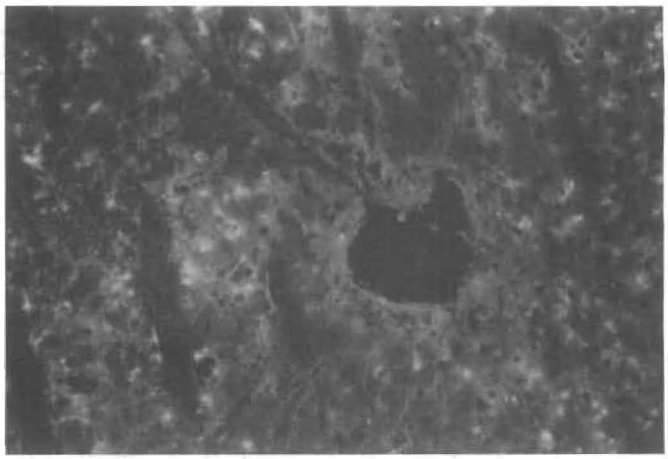

FIGURE 2. Postreperfusion liver biopsy in patient 1 showing 3+ sinusoidal staining of IgM (20×).

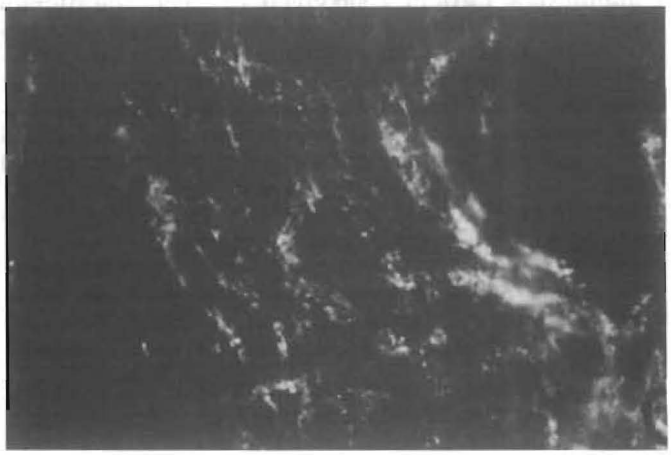

FIGURE 3. Day 24 liver biopsy in patient 1 showing weak sinusoidal staining of IgG (20×).

Patient 1: serologic data (Table 1). The human antibaboon HA titers in both neat and dithiothreitol (DTT)-treated serum remained essentially unchanged from preoperative values. Titers with dithiothreitol-treated sera were consistently

TABLE 1. Patient 1 HA titers and hepatic immunoglobulin deposition

Date	Patient sera vs. baboon RBC		DTT-treated sera vs. baboon RBC		Liver biopsy—Ig staining[a]	
	RT	AGT	RT	AGT	IgG	IgM
Preop.	1:4	1:32	1:1	1:2		
Intraop.[b]					+++	+++
POD 3[c]	1:2	1:64				
POD 7	Neg	1:128	Neg	Neg		
POD 12	Neg	1:64			++	++
POD 14	Neg	1:64	Neg	Neg		
POD 24	Neg	1:64	Neg	Neg	+	+/−
POD 35	Neg	1:32	Neg	Neg		
POD 55	Neg	1:16	Neg	Neg	++	+
POD 61	Neg	1:32	Neg	Neg		
POD 65					+++	++
POD 69	1:4	1:1				
POD 70	Patient died					

[a] Sinusoidal staining.
[b] Postreperfusion.
[c] POD, postoperative day; DTT, dithiothreitol; RT, room temperature; AGT, antiglobulin phase.

FIGURE 4. Day 24 liver biopsy in patient 1 showing disappearance of sinusoidal IgM staining (20×).

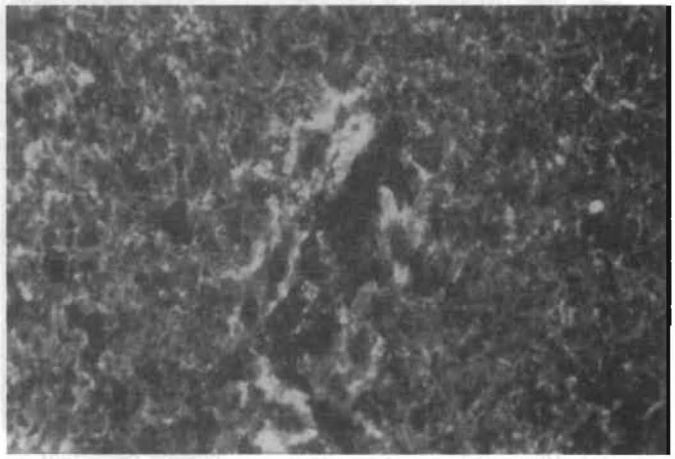

FIGURE 5. Postreperfusion liver biopsy in patient 2 showing 3+ sinusoidal staining of IgG (20×).

negative postoperatively, indicating that the antibody was predominantly IgM. The DAT was transiently positive on days 7–18 due to anti-A passively acquired from a group O platelet transfusion. The patient's red cell antibody screen remained negative throughout hospitalization.

Patient 2: clinical course. The patient was treated with an immunosuppressive regimen that included FK506, steroids, prostaglandins, and cyclophosphamide. Surgery was complicated by acute renal failure and the patient remained icteric and comatose postoperatively. A transient increase in plasma hemoglobin was noted on day 4 and 9 without evidence of immune hemolysis.

As in patient 1, the intraoperative liver biopsy after reperfusion revealed sinusoidal neutrophilia with 3^+ IgG and 3^+ IgM sinusoidal and focal complement deposition (Figs. 5 and 6), but no hepatocellular necrosis or vascular thrombosis suggesting hyperacute rejection. Subsequent liver biopsies on days 4, 8, and 12 showed a marked decrease in sinusoidal IgM staining but persistent IgG staining suggestive of mild humoral rejection (Table 2, Figs. 7 and 8). There was no evidence of cellular rejection. Although there was only mild humoral rejection, suboptimal graft function was manifested by increasingly severe bile stasis histologically and persistent hypoalbuminemia (2 g/dl). During the third postoperative week, sepsis and peritonitis developed as a result of an intestinal anastomotic leak. Despite repair of the leak and

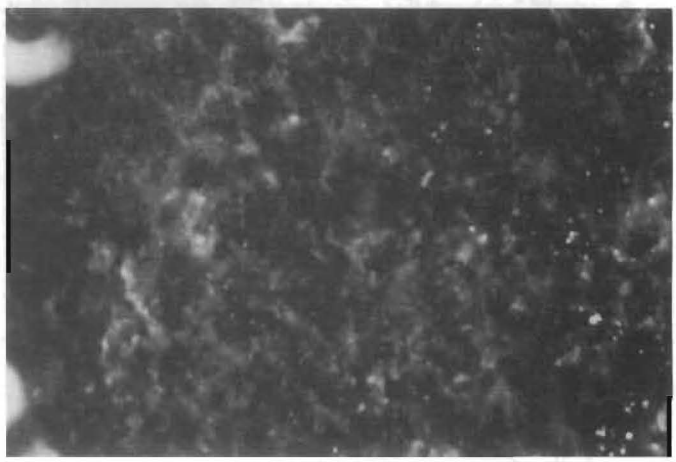

FIGURE 6. Postreperfusion liver biopsy in patient 2 showing 3+ sinusoidal staining of IgM (20×).

TABLE 2. Patient 2 HA titers and hepatic immunoglobulin deposition

Date	Current pt sera vs. baboon RBC		DTT-treated sera vs. baboon RBC		Liver biopsy—Ig staining[a]	
	RT	AGT	RT	AGT	IgG	IgM
Preop.	1:4	1:32	1:4	1:16		
Intraop.[b]					+++	+++
POD 3[c]	Neg	1:16				
POD 4					+++	+/−
POD 8	Neg	1:64	Neg	1:1	++	+/−
POD 12	Neg	1:64			+++	+/−
POD 15	Neg	1:32	Neg	1:1		
POD 22	1:32	1:256	1:1	1:256		
POD 23	1:64	1:512				
POD 24	1:16	1:256				
POD 25	1:32	1:1024	1:2	1:64		
POD 26	Patient died					

[a] Sinusoidal staining.
[b] Postreperfusion.
[c] POD, postoperative day; DTT, dithiothreitol; RT, room temperature; AGT, antiglobulin phase.

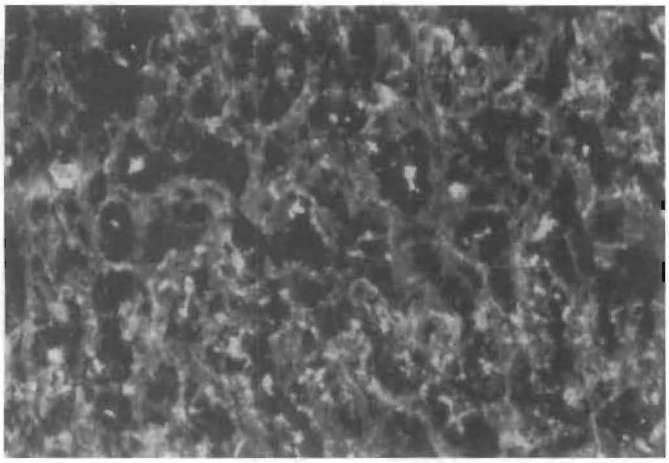

FIGURE 7. Day 4 liver biopsy in patient 1 showing persistant 3+ sinusoidal staining of IgG (20×).

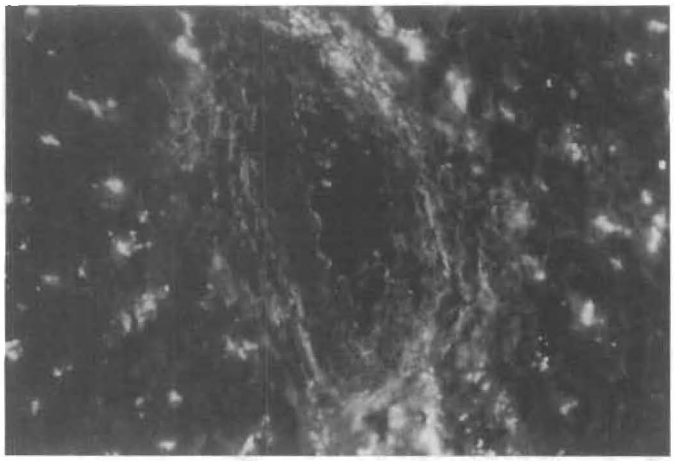

FIGURE 8. Day 4 liver biopsy in patient 2 showing marked decrease in sinusoidal IgM staining (20×).

antibiotic therapy the patient died on day 26. Postmortem examination of the liver showed diffuse necrosis with vascular thrombosis but no evidence of cellular rejection.

Patient 2: serologic data (Table 2). The human antibaboon HA titer was predominantly IgM and remained at or near preoperative levels until the terminal phase of life when it rose to a titer of 1:1024 at the antiglobulin phase. Both neat and DTT-treated sera showed a similar rise in titer, suggesting that IgG antibaboon HA levels were also increased. The DAT and the patient's red cell antibody screen remained negative throughout hospitalization.

DISCUSSION

Preformed naturally occurring antibodies in xenograft recipients have been postulated to account for hyperacute rejection seen in some, but not all, species combinations (*1*). Hyperacute rejection appears to be the most severe manifestation of a spectrum of "humoral" complement activation syndromes that may be antibody mediated (classical pathway) or non–antibody mediated (alternate pathway) (*11*). A "slow-motion" version of this humoral process has been described in allografts and appears to be characteristic of concordant xenograft transplants (*12*). Previously reported baboon-to-human renal transplant recipients experienced early graft loss but not hyperacute rejection despite the presence of HA and isohemagglutinins (*4*). Similarly, hyperacute rejection was not observed in the only baboon-to-human cardiac transplant recipient despite the presence of both preformed isohemagglutinins and HA (*5*).

The potential clinical significance of human antibaboon HA was assessed by following the titer and correlating the results with liver biopsy studies for rejection. The significance of ABH isohemagglutinins was not evaluable in our two patients because ABO-identical baboon donors were selected. HA directed at baboon red cells were detected preoperatively in both patients. There was little change in HA titers in either patient except for a preterminal rise in patient 2. Liver biopsy immunofluorescent staining showed prominent sinusoidal deposition of immunoglobulin and complement immediately after reperfusion of the xenograft in both patients. In patient 1 sinusoidal IgG and IgM deposition markedly decreased in subsequent biopsies and then reappeared late in the hospital course. In patient 2 IgM deposition markedly decreased but IgG persisted. The lack of correlation between HA titers and observed changes in

sinusoidal staining for IgG and IgM could be due to binding of only the highest-affinity HA antibodies to the graft or to binding of non-HA antibodies such as lymphocytotoxic or endothelial antibodies. The disappearance of lymphocytoxic antibodies, which were present in both patients prior to transplant, may be due to adsorption of these antibodies to the graft.

Despite the early deposition of immunoglobulin and complement in the grafts, histologic evidence of hyperacute rejection was not present. These findings support the observations in baboon renal transplantation that HA do not mediate hyperacute rejection. However, suboptimal graft function must still be explained. Both patients demonstrated histological and serological evidence for complement activation injury (*11*). This injury may represent a manifestation of a "slow-motion" humoral rejection as described above (*12*). Antibody-dependent complement activation via a subset of HA antibodies or via non-HA antibodies (i.e., lymphocytotoxic, endothelial cell) cannot be completely excluded. The hemagglutination technique used in this study is limited in that it reflects only gross changes in HA and does not detect antibodies with specificities not expressed on red cells. It is also possible that the complement injury was mediated through alternate pathway activation (antibody-independent pathway).

Passenger lymphocytes transplanted with human hepatic grafts can produce both ABH and non-ABH red cell–directed antibodies capable of causing hemolysis (*13*, *14*). Passenger lymphocytes transplanted with the baboon liver are a potential source of baboon antihuman HA since both baboon donors as well as all 11 other baboons tested (data not shown), possess HA directed against human red cells. Production of these antibodies was assessed by following the results of serial direct antiglobulin testing and clinical or laboratory evidence of hemolysis. The lack of a positive DAT (except for a transient passively acquired anti-A) in either patient suggests that either these antibodies were not produced or were produced in undetectable amounts. The fate of the transplanted baboon lymphocytes capable of producing the HA is unknown. ABH antibodies produced by passenger lymphocytes from ABO-unmatched human hepatic allografts are detected in 40–60% of cases and are typically transient, only persisting for several weeks (*13*, *14*). The transient early hemolysis observed in both patients did not appear to have an immune etiology and remains unexplained. The late hemolysis in patient 2 was thought to be associated with a consumptive coagulopathy.

In summary, human antibaboon HA do not appear to be associated with hyperacute rejection or cellular rejection, but their role in the complement-mediated injury, suspected in both cases, cannot be definitively excluded. Baboon antihuman HA were not detected in either patient. The presence of HA and/or hemolysis should be assessed in future xenograft recipients to evaluate their potential clinical significance.

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