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Antibody-Mediated Rejection of Human Orthotopic Liver Allografts

A Study of Liver Transplantation Across ABO Blood Group Barriers

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A clinicopathologic analysis of liver transplantation across major ABO blood group barriers was carried out 1) to determine if antibody-mediated (humoral) rejection was a cause of graft failure and if humoral rejection can be identified, 2) to propose criteria for establishing the diagnosis, and 3) to describe the clinical and pathologic features of humoral rejection. A total of 51 (24 primary) ABO-incompatible (ABO-I) liver grafts were transplanted into 49 recipients. There was a 46% graft failure rate during the first 30 days for primary ABO-I grafts compared with an 11% graft failure rate for primary ABO compatible (ABO-C), crossmatch negative, age, sex and priority-matched control patients ($P < 0.02$). A similarly high early graft failure rate (60%) was seen for nonprimary ABO-I grafts during the first 30 days. Clinically, the patients experienced a relentless rise in serum transaminases, hepatic failure, and coagulopathy during the first weeks after transplant. Pathologic examination of ABO-I grafts that failed early demonstrated widespread areas of geographic hemorrhagic necrosis with diffuse intraorgan coagulation. Prominent arterial deposition of antibody and complement components was demonstrated by immunoflourescent staining. Elution studies confirmed the presence of tissue-bound, donor-specific isoagglutinins within the grafts. No such deposition was seen in control cases. These studies confirm that antibody mediated rejection of the liver occurs and allows for the development of criteria for establishing the diagnosis. (Am J Pathol 1988, 132:489–502)

THE EARLIEST demonstration that immune serum was capable of producing hepatic damage was reported by Pearce in 1904.[1] He found that when agglutinating serum from rabbits (sensitized by injection of dog red blood cells) was infused into dogs, hepatic infarcts, liver failure, and death were observed. Davidsohn et al[2] described a similar phenomenon in mice using hemolytic sera.

In the early years of renal transplantation, attempts to cross major ABO blood group barriers often, but not invariably, resulted in poor allograft survival.[3] This poor graft survival was due to antibody-mediated ("hyperacute") rejection. The pathophysiologic mechanisms underlying this phenomenon were described by Starzl and coworkers in the 1960s.[4–6] The precipitating event, namely, the binding of preformed isoagglutinins to the graft vasculature, which ex-

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presses the ABO antigens, results in complement activation, neutrophilic exudation, vessel damage (vasculitis), diffuse intraorgan thrombosis (ie, single organ diffuse intravascular coagulation), and consequent activation of the fibrinolytic system with hemorrhagic necrosis of the graft. That other presensitized states and various nonimmunologic causes of graft damage such as donor or recipient sepsis could cause a similar clinicopathologic syndrome was suggested, and termed a graft "Shwartzman" reaction.[4]

Liver allografts have been thought to be somewhat resistant to primary humoral or hyperacute rejection. The explanations offered for this observation include the dual vascularity of the liver, secretion of blocking antigen, and Kupffer cell removal of immune complexes.[7,8] Clinical experience[9,10] and recent animal studies[11,12] suggest that, while liver allografts may be resistant to hyperacute rejection, they may not be totally spared from such catastrophic events. To document the existence of this event in clinical material the authors retrospectively analyzed material relative to this important question.

There are many difficult factors to be considered when undertaking such an analysis. First, previous studies have failed to demonstrate primary humoral rejection of livers grafted into patients with a positive cytotoxic crossmatch.[13] Moreover, immunopathologic studies have relegated antibodies to a "minor" role in liver allograft rejection.[14] Furthermore, as was described for renal grafts, prior sepsis in the recipient or donor[4] and preservation injury[15] can produce a syndrome that is both clinically and pathologically similar to primary humoral rejection. Cellular rejection is easily recognizable on pathologic examination. It is characterized by a predominantly mononuclear portal tract inflammation with infiltration beneath the endothelium of portal veins and into bile ducts, which show evidence of damage.

An analysis of liver grafts transplanted across major ABO blood group barriers was undertaken because a state of presensitization is known to exist (isoagglutinins), a deleterious clinical course following such transplants[9] has been reported, and a likely cause for the decreased graft survival in such cases is antibody-mediated rejection.

Materials and Methods

Patient Selection

Index Cases

All patients who received an ABO incompatible liver graft (non-O to O, AB to non-AB, B to A or A to B) between 1981 and 1987 at the University of Pittsburgh were studied. These patients were subdivided into patients receiving primary ABO incompatible (ABO-I) grafts and patients receiving secondary or tertiary ABO-I grafts, where primary is the first graft and secondary the next, and so on. The reason for subdividing these patients was the fact that patients receiving secondary grafts experience a higher rate of complications that affect the analysis. Age, sex, and priority status of the primary ABO-I index cases were included in the analysis. Priority status refers to the condition of the patient before transplantation and is based on a numerical scoring system. Priority 1 is a patient at home without assistance, 2 is at home with assistance, 3 is hospitalized, 4 is hospitalized with complications, 5 is hospitalized in the intensive care unit (ICU), and 6 is ICU with ventilator support.

Control Cases-Group I

The first group of controls was selected on the basis of the time of initial transplantation by OLT number. All patients at the University of Pittsburgh receive a sequential OLT number. For example, if a pediatric patient received a primary ABO-I graft on 11-19-86 and was given OLT number 960, the immediate prior and consecutive *pediatric* patient (eg, OLT 959 and 961) receiving a primary ABO compatible (ABO-C) graft served as controls. Therefore, each index patient had two controls unless the ABO-I index patients were close or consecutive by OLT number. When this occurred a single case was used as a control for two index cases. The control patients were included in the analysis regardless of the availability, or the results of, the T warm lymphocytoxic crossmatches, which were performed using the standard complement dependent cytotoxicity assay. Crossmatches are performed routinely for kidney allograft recipients because when they are strongly positive, a high probability for hyperacute kidney rejection exists. The same is not true for liver allograft recipients. Age, sex, and priority status of the control patients were also included in the analysis.

The numerical OLT method of selecting control cases was chosen to compensate for variable handling of patients over a number of years. The most significant of the factors are the technical advances in the surgical procedure, the large number of new surgeons at a training institute such as Pittsburgh, and the use of venous bypass which has reduced the intraoperative use of blood products and therefore have less of an effect on the immune status of the recipient sera.

No controls were included for the secondary ABO-I grafts. These controls were omitted because of the difficulty in choosing appropriate cases when factors such as the cause of primary graft failure, priority sta-

tus, presence of sepsis, hypotension, and sensitization from the initial graft are taken into consideration.

Control Group II

The second group of controls was selected after an initial comparison of the index cases with OLT number control cases (Group I). It was found that primary ABO-I cases experienced a much higher incidence of early (<4 weeks) graft failure secondary to submassive or massive hemorrhagic necrosis, with/out vascular thrombosis. ABO-C, cytotoxic crossmatch negative patients, transplanted over the same period of time, who also lost their grafts within the first several weeks, were chosen. These were chosen as histo- and immunopathologic controls because it was hypothesized that all early graft failures may have a similar histo- and immunopathologic appearance, unrelated to prior sensitization. Cytotoxic crossmatch positive controls with early graft failure were excluded from the analysis, because presensitization may have contributed to graft failure and complicated the analysis.

Patient Demographic Data

There were a total of 24 patients (5 adults, 19 children) who received primary ABO incompatible (ABO-I) hepatic grafts. Twenty-five patients (7 adults, 18 children) received ABO-I secondary, tertiary, or quartinary grafts. Two adult patients received two consecutive ABO-I grafts (secondary and tertiary). In total, 51 ABO-I grafts were transplanted into 49 patients.

The OLT number control cases (Group I) contained 38 patients (10 adults, 28 children) who received ABO compatible (ABO-C) primary grafts. The early graft-failure pathologic controls (Group II) consisted of ten patients who received ABO-C grafts who experienced early graft failure (<2 weeks).

A total of 62 cases (24 index and 38 group I control cases) were included for statistical analysis and will be referred to as the study group. The study group was primarily pediatric (average age for pediatric patients was 5.8 ± 4.8 years and for adults was 45.3 ± 9.0 years), with 48 (77%) under the age of 18 at the time of transplantation. Among the primary ABO-I patients, 83% were under the age of 18, compared with 74% of the controls ($P > 0.05$). The overall distribution of priority scores did not differ significantly between the primary ABO-I and control group I cases ($P > 0.05$).

Routine Pathologic Studies

All pathologic specimens from ABO-I grafts (primary and nonprimary), Group I, and II controls were reviewed. There were eight hepatectomy specimens, four autopsy livers, and 46 needle biopsy specimens available in the primary ABO-I group. Pathology specimens from all livers other than the ABO-I graft in a particular patient were omitted from consideration. Histologic sections were routinely stained with hematoxylin and eosin (H & E), trichrome, and periodic acid-Schiff with diastase digestion. Selected sections were stained for fibrin.

Particular attention was given to the histopathologic findings of platlet-fibrin thrombi, vasculitis, congestion, hemorrhage, neutrophilic infiltration, and ischemic necrosis.

Immunopathologic Studies

All failed allograft and autopsy tissues from ABO-I and from control group II that experienced early graft failure were stained for the presence of IgG, IgM, IgA, C1q, C3, C4, and fibrinogen using both a direct immunofluorescent and an indirect immunoperoxidase technique.[16]

Elution Studies

Frozen graft tissue (2 to 5 g) was minced by hand or through the use of a Kinematica Polytron tissue homogenizer. The tissue was then washed four times in 6% bovine albumin at 4 C. Heat elution was performed at 56 C for 15 minutes into 2 to 3 ml of albumin, then the tissue was centrifuged at 4500*g* for 5 minutes. The supernatant was tested by hemagglutination against A_1, B, and O red blood cells, with readings at immediate spin, 30 minutes at 37 C, and polyspecific antiglobulin. The immunoglobulin class of the eluted antibodies was determined by use of anti-IgG antiglobulin reagent and treatment with dithiothreitol to inactivate IgM. The last wash solution which tested negative for ABO antibodies served as a control. Testing was performed without knowledge of the donor and recipient ABO types.

Review of Operative and Clinical Course

A detailed review of pertinent clinical events was achieved by consultation with the surgical team involved in each of the cases combined with a review of the patients chart. Special attention was focused on other reasons for graft failure, including assessment of donor organ, operative complications, and other comorbid states (eg, sepsis, hypotension) that may have contributed to poor graft function or failure. Additionally, pertinent observations as to the early appear-

ance of the graft after reperfusion and the presence of postoperative bleeding were recorded.

Case Evaluation

The cases were evaluated after all available information including donor complications, preservation injury, technical or intraoperative complications, clinical course, routine and immune pathology, and elution studies were available. Because there are currently no criteria available for the diagnosis of primary humoral liver allograft rejection, the following classification was used: 1) *No apparent humoral event*—There was no clinically detectable graft malfunction, no pathology specimen, or insufficient evidence of pathologic changes in biopsy specimens. 2) *Probable humoral event*—There was clinical evidence of early (less than 1 week) graft malfunction, pathologic evidence of graft damage, tissue deposition of antibody and complement, but confounding influences that may have contributed to graft damage (eg, bacterial seeding of infarcted tissue, hypotension, prolonged preservation). 3) *Primary humoral rejection*—There was clinical evidence of early (less than 1 week) graft malfunction, graft failure, pathologic evidence of multiple graft infarcts, tissue deposition of immunoglobulin and complement, no evidence of confounding clinical or pathologic observations, and demonstration of donor-specific isoagglutinins in tissue eluates from the failed graft.

Results

Graft Survival Analysis

All patients were followed for a minimum of 30 days (up to 2141 days). Among control group I, 4 (11%) of grafts failed within 30 days, compared with 11 (46%) of the primary ABO-I cases ($P < 0.05$). The odds ratio of graft failure within 30 days of transplantation in strata defined by each of the independent variables (gender, age group, and priority status at the time of transplant) was calculated (data not shown). Odds ratios statistically significantly ($P < 0.05$) greater than 1, implying greater risk of early graft failure among those receiving incompatible grafts than among those receiving compatible grafts, were found for females, pediatric patients and for patients in the hospital (not in the ICU) at the time of transplantation.

A multivariate model, the logistic regression model, was used to determine whether there was an effect of ABO compatibility on graft failure, simultaneously adjusting for the effect of gender, age, and priority status. When the full model was fit, a statistically significant effect ($P < 0.05$) was found for ABO compatibility, but not for any of the other independent variables. The adjusted odds ratio was 11.8 with a 95% confidence interval of (2.5, 56.6). This implies that, adjusting for gender, age group, and priority status, the odds of a recipient suffering graft failure within the first 30 days of liver transplantation is about 12 times greater among those receiving ABO-I grafts than among those receiving ABO-C grafts. None of the other independent variables (age, sex, or priority status) examined were found to be statistically significant ($P > 0.05$), adjusting for case or control status.

Individual Case Analysis

A synopsis of the clinical, serologic, and routine and immune pathologic and elution studies of the primary ABO-I cases is shown in Table 1. A similar analysis of control group I and II cases is shown in Tables 2 and 3, respectively.

Index Cases

Follow-up to this writing has shown that ten (42%) of the primary ABO-I grafts are still functional. Fourteen patients (58%) have experienced graft failure and were retransplanted or expired. Four were classified as primary humoral rejection due to preformed isoagglutinins. Humoral mechanisms were thought to play a significant, if not the primary, role in graft failure in eight others. Recurrent hepatitis B was responsible for graft failure in one case. In the remaining case, although evidence of humoral involvement was present, graft failure was temporally too remote (147 days) from the operation to implicate humoral mechanisms as the primary reason for graft failure. No correlation was detected between the underlying liver disease and graft failure.

Only eight of the 27 (30%) ABO-I secondary or tertiary grafts are still functional. Nineteen of the grafts (70%) had to be retransplanted or the patient expired, with an average graft survival of 27 days. The causes of graft failure were quite similar to that seen in the primary ABO-I group, with a high incidence of hemorrhagic necrosis in the first several weeks, with or without vascular thrombosis. However, the incidence of infections, priority status, and other complicating clinical circumstances was higher (data not shown).

OLT# Control Cases (Group I)

Twenty-four (63%) of the primary ABO-C OLT# control grafts are presently functioning. However, in contrast to the ABO-I primary index cases, the aver-

Table 1—Clinicopathologic Summary of ABO Incompatible Primary Liver Allografts*

OT#	Age (yrs)	Sex	Donor ABO	Recip ABO	Graft status	Graft surv	Clinical profile	Pathology findings	IPEX† IF	Eluate react	Summary
193	10.1	F	A	B	Funct	2060	Portal vein anastomotic, bile duct stricture	Portal vein thrombectomy	NA	NA	No apparent humoral event
196	36.9	F	A	B	Fail	9	Graft necrosis, fungemia	Early graft damage with progressive infarcts	Pos	NA	Probable humoral rejection
240	16.8	M	AB	A	Fail	10	Graft necrosis, ?vasospasm	Hemorrhagic necrosis of liver, no vascular thrombosis	Pos	Anti-B	Primary humoral rejection
445	2.0	M	B	A	Funct	993	Smooth course	No tissue	NA	NA	No apparent humoral event
475	9.3	M	A	B	Fail	147	Jaundice, biliary sludge	Large duct necrosis with biliary sludge	Wk Pos	ND	Possible humoral component involved
536	0.9	F	A	B	Funct	664	Smooth course	HBV infection of graft	ND	ND	No apparent humoral event
547	2.7	M	A	B	Fail	17	Poor early function	Early "preservation" injury, followed by progressive graft infarction and candida	Pos	NA	Probable humoral rejection
597	0.9	M	AB	A	Fail	7	Reexplored, found cyanotic, mottled and infarcted liver	No tissue available	NA	NA	Probable humoral rejection
600	2.8	M	A	B	Fail	14	Poorly early function	HAT with candida seeding of liver	Wk Pos	ND	Possible humoral rejection
614	6.0	F	A	B	Fail	27	Poor early graft functioning. Angiograms showed arterial "spasm"	Progressive graft infarction with neutrophilia	NA	NA	Probable humoral rejection
615	2.8	M	A	B	Fail	13	Reexplored, found enlarged cyanotic liver with ruptured capsule	Progressive injury with hemorrhagic necrosis and neutrophilia	Pos	Anti-A	Primary humoral rejection
686	0.3	F	B	O	Funct	428	Poor early graft functioning	"Preservation" injury	ND	ND	No apparent humoral event
689	4.3	F	A	B	Fail	7	Graft necrosis, fistula technical problems	HAT with hemorrhagic necrosis of liver and bacterial overgrowth	ND	ND	Probable humoral rejection
721	10.3	F	B	A	Fail	6	Unexplained graft necrosis	Hemorrhagic necrosis of graft with intrahepatic thrombi	Pos	Anti-B	Primary humoral rejection
829	60.6	M	B	O	Fail	141	Recurrent HBV	Recurrent hepatitis B	ND	ND	No apparent humoral event
871	0.1	M	A	O	Funct	203	HAT but graft still functional	Severe "preservation" injury with bile duct necrosis	ND	ND	No apparent humoral event
948	5.4	M	A	O	Funct	365	Poor early functioning	"Preservation" injury	ND	ND	No apparent humoral event
960	0.6	F	AB	A	Funct	102	Poor early functioning	Focal necrosis with neutrophilia	ND	ND	No apparent humoral event
1011	31.9	F	B	O	Funct	149	Poor early functioning	Mild cellular rejection	ND	ND	No apparent humoral event
1016	45.0	M	B	O	Fail	41	Graft necrosis of hilum at 1 week with HAT	Progressive graft infarction and HAT	Pos	ND	Probable humoral rejection
1093	1.1	F	AB	A	Fail	4	Unexplained graft necrosis	Severe hemorrhagic necrosis with arteritis	Pos	Anti-B	Primary humoral rejection
1113	10.6	F	B	O	Funct	35	Smooth course	NA	NA	NA	No apparent humoral event
1118	3.2	F	A	O	Funct	31	Smooth course	NA	NA	NA	No apparent humoral event
1121	10.2	F	AB	A	Fail	5	Graft necrosis	Multiple infarcts with HAT	Pos	ND	Probable humoral rejection

* All patients had a negative lymphocytoxic crossmatch or crossmatch results were not done.

† IPEX/IF = Immunoperoxidase and immunofluorescent results for immunoglobulin and complement.

HAT, hepatic artery thrombosis; HBV, hepatitis B virus, Fail, failed; Funct, functioning; NA, not available; ND, not done; Surv, survival (days).

Table 2—Clinicopathologic Summary of ABO Compatible Primary Liver Allografts (Control Group I)*

OLT#	Age (yrs)	Sex	Donor ABO	Recipient ABO	Graft status	Graft survival (days)	Cause of graft failure or patient death
192	10.5	M	A	A	Function	2141	
195	44.6	M	O	O	Failed	439	Sepsis, recurrent CAH-B
197	39.0	M	O	O	Failed	905	Chronic rejection
201	4.5	M	O	O	Failed	33	HAT with liver abscesses
239	5.9	F	A	A	Function	1773	
246	2.4	M	A	A	Failed	26	Adenovirus and chronic rejection
444	3.4	F	A	A	Function	926	
446	5.2	F	A	A	Function	924	
472	7.8	F	A	AB	Failed	672	Chronic vascular rejection, duct obstruction
478	5.9	F	O	O	Function	853	
535	1.2	F	A	A	Function	699	
537	2.6	M	O	O	Failed	119	HAT with hilar necrosis
546	10.3	M	O	O	Function	678	
551	4.1	F	A	A	Function	667	
594	0.9	M	O	O	Failed	29	HAT with hilar necrosis, abscesses, CMV
604	9.6	M	A	A	Failed	86	Hilar abscess with vein-abscess fistual and hematoma
610	6.6	M	A	A	Function	589	
619	11.8	F	O	O	Failed	54	Perioperative stroke, lymphoma in liver at autopsy
678	1.5	F	O	O	Failed	41	Aorta-enteric fistula with sepsis
692	4.9	F	O	O	Function	473	
720	0.8	F	A	A	Failed	275	HA stenosis, sepsis from cholangitis
732	7.4	M	O	O	Function	418	
827	49.3	M	O	O	Function	305	
830	53.0	M	AB	AB	Function	247	
870	16.1	M	B	B	Failed	10	Uncontrolled rejection
874	2.2	F	O	O	Function	253	
946	54.8	F	O	O	Function	169	
949	16.1	M	B	B	Function	165	
958	2.8	M	A	A	Function	104	
964	5.0	M	O	O	Failed	71	HAT with organ infarction
1010	36.1	F	A	A	Function	151	
1012	37.1	M	A	A	Function	148	
1013	37.7	M	O	O	Function	76	
1017	53.4	F	A	A	Function	73	
1088	7.8	M	A	A	Function	58	
1096	13.4	M	O	O	Failed	3	Ischemic necrosis, technical problem at arterial anastomosis
1112	6.8	M	A	A	Function	35	
1122	54.6	F	O	O	Function	189	

* All patients had a negative T-warm lymphocytotoxic crossmatch or results were not available except for OLT# 619 who had a positive crossmatch.
CAH-B, chronic active hepatitis B; CMV, cytomegalovirus; Function, functional graft; Fail, graft failure; HAT, hepatic artery thrombosis.

age survival of failed grafts in this group was 197 days. Additionally, the causes of graft failure differed significantly from that seen in the primary ABO-I group (see Tables 1 and 2).

Early Graft Failure/Pathology Controls (Group II)

Ten patients receiving ABO-C primary grafts, who had a negative T warm cytotoxic crossmatch experienced graft failure within the first two weeks. Eight grafts failed from hepatic artery or iliac graft thrombosis or mural dissection, which according to the operating surgeon, was explainable on technical grounds. One patient had no portal vein for anastomosis, requiring an alternative venous anastomosis that was unsuccessful. The cause of failure in the remaining patient was determined to be the result of preservation injury.

Routine Gross and Light Microscopic Pathology

Index Cases

The majority of the pathologic samples were taken as a result of the early onset of graft malfunction. Pre- and postimplantation biopsies were available in five patients. The following description is a summary of the pathologic findings present in the primary ABO-I livers.

Preimplantation samples generally showed minimal to no pathologic alterations, other than mild focal

Table 3—Clinicopathologic Summary of Group II Controls* (Early Graft Failures)

OLT#	Graft survival (days)	Clinical events	Pathology findings	IF ipex	Eluate react.	Summary
220	4	No recipient portal vein available for anastomosis	Bridging periportal necrosis, no microvascular lesions	Neg	Neg	Technical problem with portal vein anastomosis with ischemic graft injury
271	4	Technical problem with arterial anastomosis	Periportal hemorrhage, focal central necrosis steatosis	Neg	ND	Ischemic graft injury from technical problem at arterial anastomosis
273	9	Portal vein thrombosis and rejection	Portal vein thrombosis severe cellular rejection and necrosis	Neg	ND	Portal vein thrombosis, probably technical and severe cellular rejection
284	5	Primary nonfunction	Extensive periportal necrosis, no vascular thrombosis identified	Neg	ND	Injury probably the result of preservation
498	5	Technical problem with arterial anastomosis with HAT	HAT with multiple infarcts and bacterial seeding	Neg	ND	Technical problem at arterial anastomosis
522	4	Primary nonfunction	Necrotic arterial wall with focal with little reaction with infarcts	Neg	ND	Technical, arterial wall necrotic at anastomosis
716	12	Technical problem at arterial anastomosis	Arterial wall dissection, bacterial seeding and diffuse necrosis	Neg	Neg	Technical problem resulting in arterial dissection and bacterial seeding of liver
8081	8	Difficult arterial anastomosis	HAT, moderate acute cellular rejection, central ballooning	Wk pos	Neg	Technical arterial problem with HAT and cellular rejection
836	15	Arterial thrombosis	HAT with hilar necrosis/abscess	Neg	ND	HAT, probably technical
1068 technical.	2	Iliac vascular graft thrombosis	Multiple subcapsular Infarcts, arterial thrombosis, hilar necrosis	Neg	ND	Arterial thrombosis

* All patients had a negative T-warm lymphocytotoxic crossmatch, except for OLT 808, which was weakly positive.
HAT, hepatic artery thrombosis; IF/ipex, Immunofluorescence/immunoperoxidase; ND, not done; Neg, negative.

hepatocellular swelling. Samples taken 2 to 6 hours postimplantation showed a rather impressive clustering of neutrophils, fibrin deposition, and red blood cell sludging in the sinusoids (Figure 1). This was seen in association with focal hemorrhage into the space of Disse, focal hepatocellular cytoaggregation, or single cell acidophilic necrosis. Biopsy samples 1 to 2 days later continue to showed the above changes, but small clusters of hepatocytes with coagulative necrosis, increased red cell congestion, and hemorrhage were seen also. Small portal arteries may or may not show fibrinoid degeneration. A mild neutrophilic portal exudate may begin to appear, with focal duct proliferation as signs of regenerative attempts. At this stage, the histologic changes can be difficult if not impossible to separate from those of prolonged preservation injury (unpublished observation). Thereafter, a progressive patchy geographic hemorrhagic infarction of the organ ensues. The early progression of the changes may or may not be detected in biopsies because of sampling problems, but once the process is widespread, coagulopathy, submassive or massive necrosis and hepatic failure manifest, necessitating retransplantation.

An examination of failed ABO-I grafts revealed enlarged (up to twice the preimplantation weight) hemorrhagic organs, mottled with random areas of necrosis, with or without large vessel thrombosis. The capsule had actually ruptured in OT# 615. Microscopically, focal fibrinoid necrosis, inflammatory vasculitis, and thrombosis of some medium and small-sized arteries was seen in only four cases (Figures 2 and 3). More prevalent arterial and venous findings included plump reactive endothelial cells with lumenal platelet and neutrophil sludging, focal fibrin layering around their circumference or fibrin masses extending in a flamelike fashion into the lu-

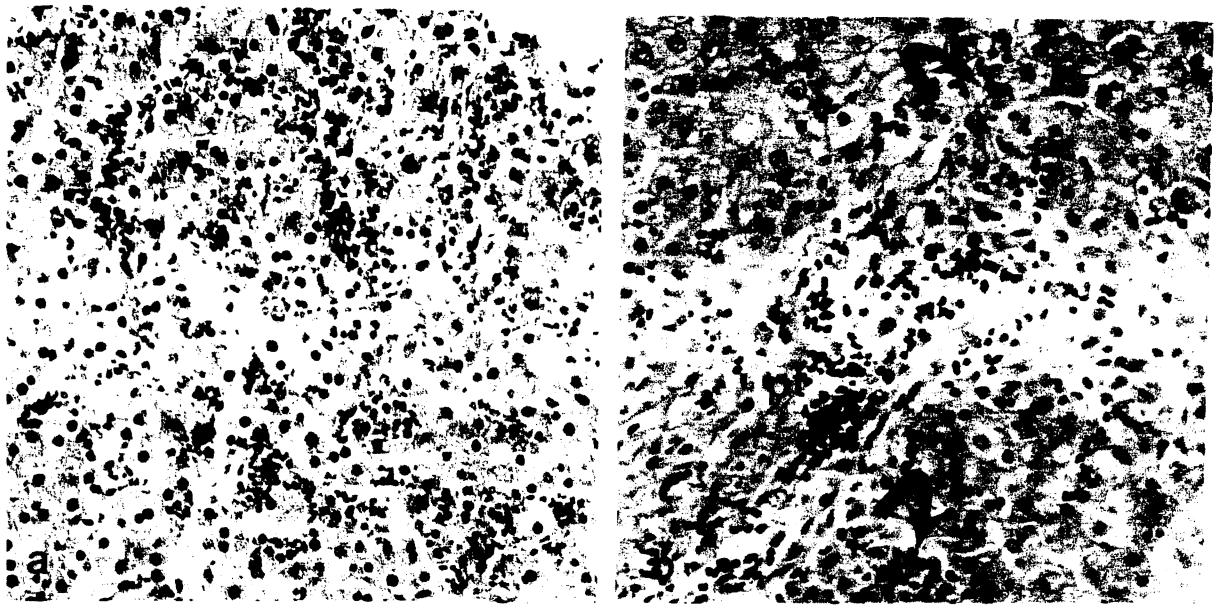

Figure 1—Histologic appearance of liver biopsy specimens from ABO-I grafts taken early after reperfusion. **a**—Postimplantation biopsy taken approximately 2 to 4 hours after reperfusion, with diffuse sinusoidal neutrophilic and red blood cell congestion. (H & E, ×300) **b**—Biopsy after 1 day, note the small areas of coagulative necrosis in the lobule (arrows), mild portal neutrophilia, and red blood cell congestion. (pt, portal tract, H & E, ×300) A biopsy on day 5 showed progressive infarction. Findings in the failed allograft specimen (OLT 721) are illustrated in Figures 4, 5, 6A, 6B.

mens from an attachment to a partially disrupted vascular wall (Figure 4). The parenchyma in all cases showed severe and widespread geographic areas of hemorrhagic necrosis intermixed with neutrophils, often with no particular zonal distribution (Figure 5). The hepatic cords often were compressed and the hepatocytes necrotic, leaving ghosts of the normal architecture and cells.

Control Cases (Groups I and II)

Biopsy and failed graft specimens from group I, showed little resemblance to the findings described for the ABO-I cases. The only exception is that a few of the biopsies obtained in the first several postoperative days contained the focal sinusoidal neutrophilic clusters, focal congestion and single acidophilic necrosis of hepatocytes. The severity of this change was less than that seen in the ABO-I group.

Failed grafts from control group II with arterial thrombosis and hilar abscesses, demonstrated intraparenchymal areas of necrosis with abscess formation, which appeared reminiscent of the necrosis seen in the ABO-I livers. Necrotizing or inflammatory cell vasculitis was not found, however, nor was there significant staining for immune deposits.

Cases not included in this study but reviewed for comparison in which there was donor or recipient sepsis or both, or prolonged preservation injury showed similar changes to those seen in the biopsies obtained from the ABO-I grafts in the first several days. Immune staining in these cases were negative.

Immunoflourescence/Immunoperoxidase

ABO-I Cases

All cases thought to represent primary humoral rejection or probable humoral rejection demonstrated the presence of focal but intense deposition of IgM and C1q, with lesser amounts of C3, usually in arterial walls, from hepatectomy specimens (Figure 3). Focal IgG deposits were found in only one case. Patchy sinusoidal and venous staining also could be seen but it was much less impressive than the deposits in the arteries, which reflects intrahepatic distribution of the ABO antigens.[17] Ultrastructural studies revealed electron-dense deposits in the vasculature with overlying fibrin (Figure 6). In general, the arterial deposits were not uniformly distributed throughout the liver. In fact, some subcapsular sections were negative, while those taken closer to the hilar demonstrated impressive arterial deposition. Sequential staining of a biopsy at day 6 in OT 196 showed diffuse sinusoidal, arterial and venous deposition of IgM, IgG, and C3, with little cellular infiltrate. The autopsy specimen 2 days later showed only focal IgM and C1q, with multiple geographic hemorrhagic infarcts.

Control Cases (Group II)

Deposition of IgM and IgG in arterioles was seen in one case, with a weakly positive cytotoxic crossmatch.

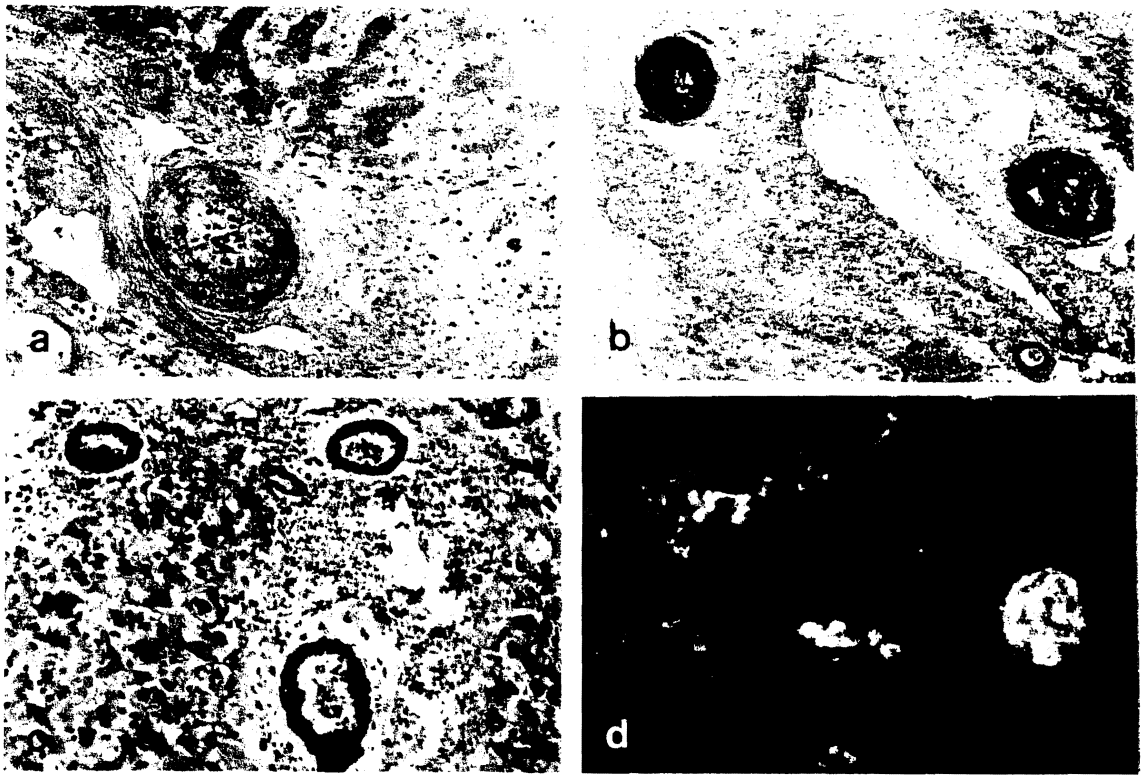

Figure 2—Failed allograft (OLT 1093) 4 days after transplantation. **a**—Note the necrotic and thrombosed hepatic arteries in a portal triad. The cellular detail is lost because the liver is infarcted and hepatocyte nuclei no longer visible (H & E, ×120) **b**—The thrombi and the entire thickness of the arterial wall stained intensely with PAS after diastase digestion. (PAD-D, ×120) This contrasts markedly with the H & E which was hemorrhagic and infarcted but did not catch the eye. **c**—The thrombi and the arterial walls demonstrated deposition of IgM. The C1q stain was identical. (immunoperoxidase IgM, ×120) **d**—The thrombi and the arterial walls demonstrated deposition of C1q. The IgM was identical. (immunoflourescence, C1q, ×120)

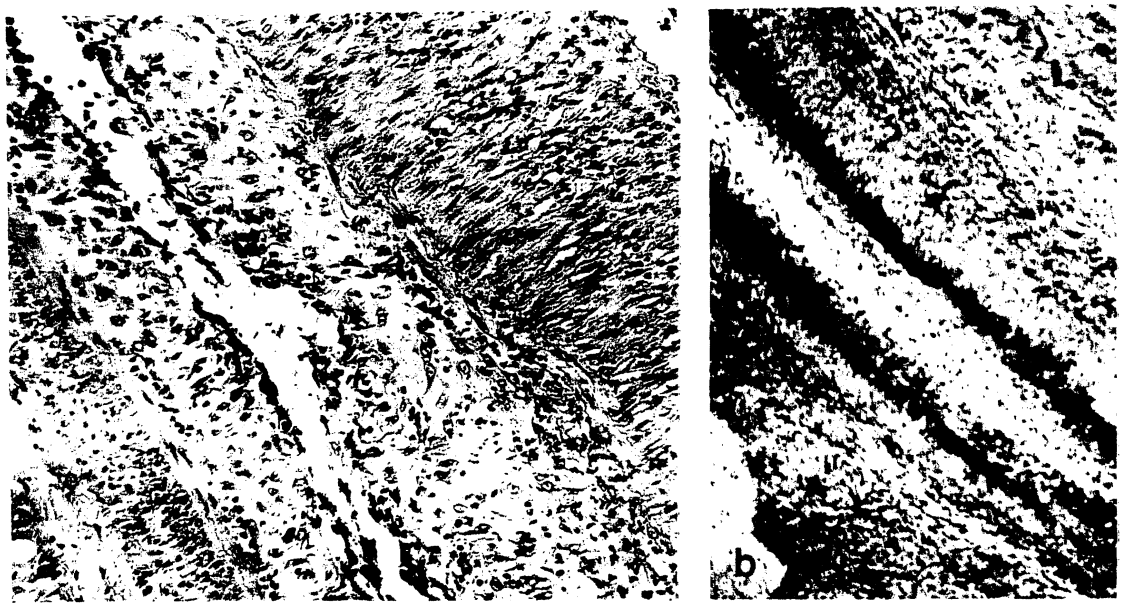

Figure 3—Hepatectomy specimen from failed nonprimary ABO-I graft (17 days posttransplant), most of which showed changes identical to the primary ABO-I grafts. **a**—Note the severe proliferative and neutrophilic arteritis (H & E, ×450) with deposition of IgM in the arterial wall (**b**, immunoperoxidase IgM, ×300). Staining for C1q and C3 were also present.

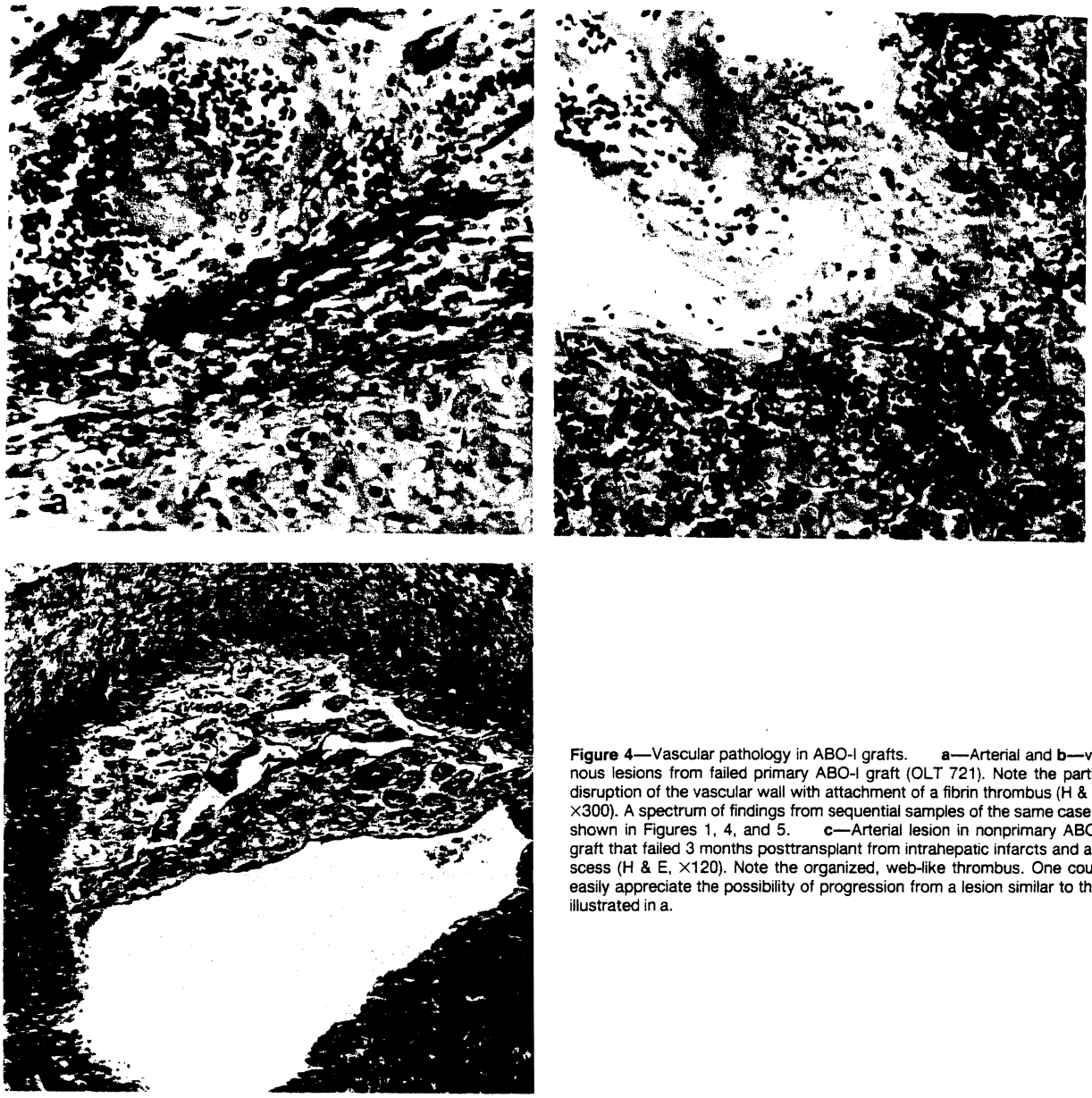

Figure 4—Vascular pathology in ABO-I grafts. **a**—Arterial and **b**—venous lesions from failed primary ABO-I graft (OLT 721). Note the partial disruption of the vascular wall with attachment of a fibrin thrombus (H & E, ×300). A spectrum of findings from sequential samples of the same case is shown in Figures 1, 4, and 5. **c**—Arterial lesion in nonprimary ABO-I graft that failed 3 months posttransplant from intrahepatic infarcts and abscess (H & E, ×120). Note the organized, web-like thrombus. One could easily appreciate the possibility of progression from a lesion similar to that illustrated in a.

All other cases were uniformly negative. Sinusoidal staining for C1q and fibrinogen were positive frequently, particularly in and around the central vein region, in this group and in the ABO-I group. This was considered to be nonspecific as a result of leakage through the damaged sinusoidal wall or Kupffer cell phagocytosis. Although not included in this study, several cases of IgM leakage into the wall of medium-sized arteries of grafts with thrombosis at the arterial anastomosis, as a result of technical problems, has been seen. In general, however, the deposits were less intense, and were accompanied by the leakage of other serum components such as fibrinogen (unpublished observation). In addition, elution studies failed to demonstrate donor-specific antibodies.

Elution Studies

Eluates of tissue from failed primary ABO-I allografts that were thought to represent primary humoral rejection all showed reactivity against donor ABO antigens (N = 4). Analysis of the reactivity using dithiothrietol and anti-IgG antiglobulin reagent gave results characteristic of IgM antibody class, identical to that found within the tissues.

Eluates of three primary ABO-C early graft failures (group II controls), were negative for isoagglutinins.

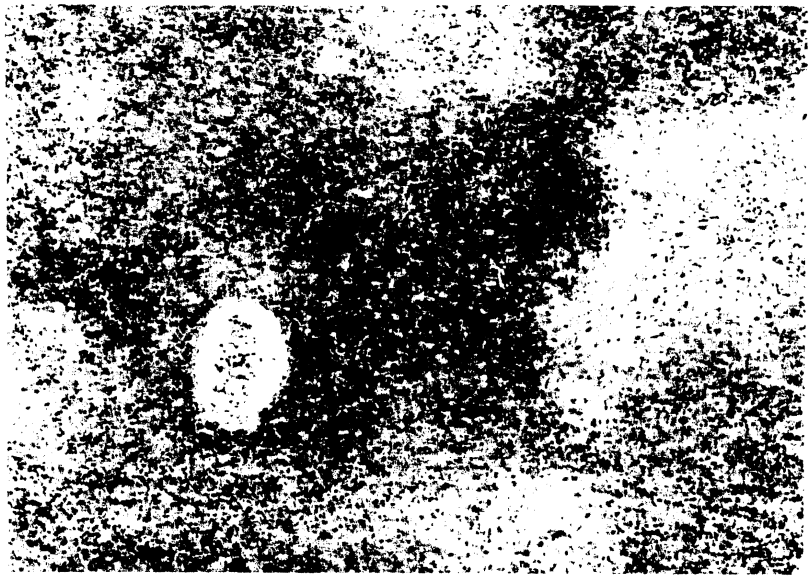

Figure 5—Low power (H & E, ×100) view of failed ABO-I graft showing diffuse hemorrhagic necrosis. A portal tract, with little to no inflammation is seen at the middle right of the photograph.

Eluates were not studied in cases classified as probable humoral rejection.

Clinical Observations

A clinical diagnosis of "hyperacute" rejection of a liver allograft, that is, a graft that after initial adequate reperfusion, becomes cyanotic, mottled, flaccid, and fails to produce bile, was not made in this group of patients. The primary ABO-I grafts in each case were reperfused adequately and produced bile when inspected by the operating surgeon before abdominal closure. The only clinical clue to future complications in the immediate reperfusion period was difficulty in achieving hemostasis. In retrospect this may have been due to activation of the fibrinolytic and kinin cascades as a response to extensive intrahepatic coagulation. The events of the first several days after transplantation were more revealing.

During this period, the cases of primary humoral rejection most often had a relentless rise of serum transaminase values. An initial reperfusion value for ALT and AST in the 400 to 500 IU range on day 1 followed by values ranging from 2000 to 10,000 IU on subsequent days signaled a catastrophic event. Angiograms and percutaneous liver biopsies were performed to rule out arterial thrombosis or other identifiable causes of graft malfunction. Arterial angiography in one case was particularly striking. It demonstrated a markedly narrowed arterial tree, thought to be the result of diffuse arterial spasm. Biopsies in general showed the changes described above and were initially interpreted as "preservation injury." The rise in transaminases was followed by severe bleeding and hepatic failure, necessitating re-exploration, retransplantation or both. The appearance of the organs at retransplantation was similar to the appearance of kidneys undergoing "hyperacute" rejection. They were enlarged, mottled, and cyanotic. The gross intraoperative appearance of one of the grafts (OLT# 615) was particularly impressive. The organ was massively enlarged, cyanotic, had a capsular rupture, and was bleeding from the surface.

This clinical course is somewhat different from that seen in organs damaged from prolonged preservation. In general, initial reperfusion transaminase values are high (1000 to 2000 IU) in preservation injury, but show a downward trend over the next several days rather then a relentless upward progression, as was seen in the primary ABO-I grafts with humoral rejection.

ABO Profiles of Primary ABO Incompatible Grafts

The profiles of the primary ABO-I grafts are shown in Table 4. A noticeable difference in the survival rates of O recipients who received non-O grafts was evident. The majority (six of ten) of the primary ABO-I that remained functional were from this combination. The same trend is true of nonprimary ABO-I grafts in that six of eight of the functional grafts were non-O donors to O recipients.

Discussion

The results of this study show that liver allografts are susceptible to primary humoral rejection when major ABO blood group barriers are violated. The di-

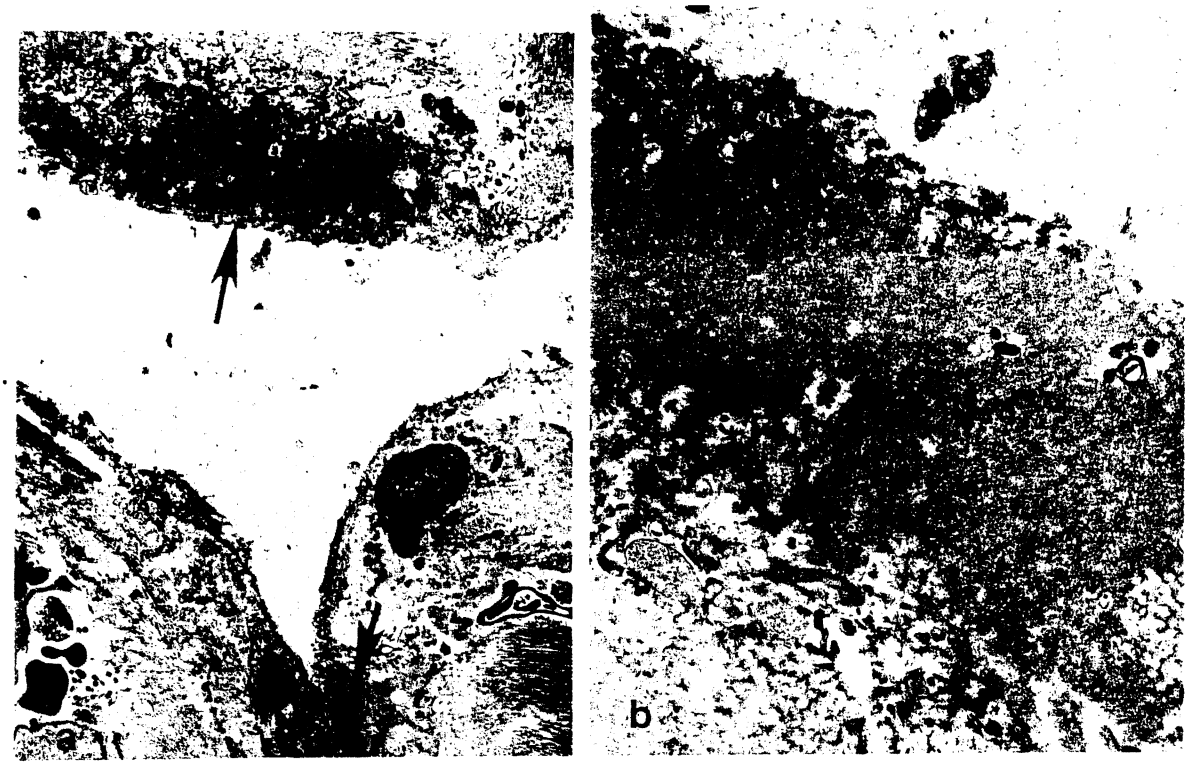

Figure 6—Ultrastructural appearance of immune deposits in vein wall from failed ABO-I graft. **a**—Note the electron dense deposits with thin overlying layer of fibrin (arrows, ×5000). **b**—Higher power detail of electron-dense deposits (×15,000).

agnosis, however, should be limited to cases in which the following tetrad of findings are all present: 1) early graft failure (usually within 2 to 4 weeks), with no alternative clinical or pathologic explanation; 2) consistent routine light and immunoflourescent microscopic findings (see results); 3) demonstration of a presensitization state in the recipient, and 4) presence of donor-specific antibodies in an eluate from the failed graft. Strict adherence to these criteria will prevent overdiagnosis, because a similar clinical course and hemorrhagic necrosis of the graft may occur in a variety of settings.

Previously, the diagnosis of "hyperacute" or antibody-mediated rejection of liver grafts has been hindered for several reasons. First, as in renal transplantation, a sufficient number of cases is necessary for comparison analysis. Second, there are a multitude of factors that can contribute to early graft failure such as "preservation injury," donor or recipient cardiovascular instability or sepsis, and operative difficulties. Third, the pathologic correlates of the above mentioned factors have not been described well.

Table 4—ABO Profiles of Donor/Recipient for Primary ABO-I Grafts According to Graft Status

Donor to recipient combination	Total no. grafts	Functional grafts	Failed grafts
non-O to O	8	6	2
A to B	9	2	7
B to A	2	1	1
AB to A	5	1	4
AB to B	–	–	–
TOTAL	24	10	14

Presently, we are in a better position to critically analyze the causes of early graft failures with the expectation of reasonable conclusions due to the following factors: 1) improved graft survival with fewer early graft failures as a result of advances in operative techniques, patient selection, and immunosuppressive protocols; 2) better understanding of the pathologic correlates of other graft complications, and 3) a sufficient number of cases are available to compare graft survival and the spectrum of pathologic findings.

This combined clinicopathologic analysis has led to the conclusion that liver grafts are susceptible to early graft failure as a result of primary antibody mediated damage. Based on the data presented, thinking had to be redirected as to the immunopathologic mechanisms that may be responsible for liver allograft rejection because antibodies were previously thought to play only a minor role. Unlike kidney and heart grafts, the term "hyperacute" rejection is probably not the

best term for this phenomenon because it was not immediately recognizable by the operative surgeon shortly after reperfusion. A more generically applicable term is primary humoral rejection. The reasons for this difference are probably the same as those offered for explaining the seeming resistance of liver allografts to the phenomenon. The documentation of such a diagnosis requires a detailed analysis of each case because other factors mentioned can also lead to a similar biologic phenomenon.

Early allograft failure secondary to hemorrhagic necrosis may be seen as a consequence of damage before or during preservation,[18] ischemic ("shock liver") organs[19] with or without vascular thrombosis, or when there is coexistent sepsis (particularly gram negative) in the donor or recipient. The observation that preservation injury, hypoxic, and septic- or endotoxin-induced liver damage resembles primary humoral rejection is not surprising if one considers the underlying pathophysiology. The primary insult in each of these disorders is endothelial cell damage,[18–20] resulting in release of tissue thromboplastins and exposure of underlying collagen. Vasospasm, platelet plugging, and initiation of the intrinsic and extrinsic clotting cascades and the subsequent activation of fibrinolytic and kinin systems ensues. This single-organ DIC-syndrome may result in systemic bleeding complications, as was seen in many of the patients. While the initial triggers of the single organ Shwartzman or DIC reaction may vary, the subsequent cascade of events that result in tissue damage are probably similar. The key to determining the cause of graft failure when such a situation is encountered is to identify the initial event.

The study of ABO-I grafts was instrumental in providing the major clue to the triggering event of the single organ Shwartzman reaction. The authors had already discovered that graft survival was significantly worse when crossing ABO barriers,[9] that the patient (by definition) is presensitized, and that humoral rejection was a likely cause of the poorer survival. The further documentation of clinical events, pathologic correlates, isoagglutinin deposits and elution studies was needed to confirm these suspicions and to confidently separate the grafts with humoral rejection from those with other causes of failure.

Difficulties in establishing a pathologic diagnosis of suspected humoral rejection can be encountered. These arise when severe tissue damage is accompanied by nonspecific leakage of immunoglobulin and complement, particularly in subcapsular infarcts, or because of the sometimes patchy distribution of immune deposits in antibody mediated rejection. Careful interpretation of the immunoflourescent findings for distribution (arterial) and specificity (selective deposition) will assist in the differentiation of nonspecific leakage from antibody mediated rejection. The observation of the focal nature of the deposits has several possible explanations. Antibody-mediated damage may be the initial trigger of the cascade of events early after transplantation, but becomes degraded and not detectable when searched for in the failed graft, days or weeks later. This scenario was seen in OLT 196 (see results). Also, the heaviest deposition may occur in vessels that are first exposed to the circulation and explain the greater likelihood of finding deposits in the perihilar vessels in some of the cases.

Therefore, the diagnosis of humoral rejection may be difficult to establish before organ failure and resection. Clinical cues that signal the onset of this phenomenon include dramatic elevations of hepatic enzymes in the first postoperative week combined with bleeding and progressive liver failure, all in the absence of any explanation by other factors. Documentation then requires tissue sampling (wedge biopsy is optimal). Histologic findings that should make one suspect antibody mediated rejection are outlined in the results section. Immune staining for antibody and complement components should be performed and, if positive, followed by tissue eluate analysis, which can be done on a small frozen-tissue sample. The final diagnosis must be based on a complete clinicopathologic analysis, as was done in this study, and fulfill the criteria offered at the beginning of the discussion section.

An interesting trend was noted in the donor and recipient ABO profiles of surviving ABO-I grafts. Twelve of the 16 ABO-I grafts that are still functional were from non-O donors to O recipients. Although the numbers are small and the implications serious, crossing the ABO barrier in this direction may be safer than with other combinations. Hypothetically, this observation is consistent with the immunoglobulin class response patterns seen in the rejected organs. The immune response of group A and B subjects to ABO antigens is predominantly IgM in nature, whereas group O subjects respond with IgG antibodies.[21] In addition, children 5 to 15-years-old, who may be the most likely candidates to receive ABO incompatible grafts because of reduced donor availability, also have the highest levels of IgM ABO antibodies of any age group,[22] a finding consistent with the observation that the pediatric primary ABO-I cases presented with the most striking findings. This observation may or may not have significance for T warm lymphocytotoxic antibodies, which are IgG in nature.

Finally, because it was found that humoral rejection of liver allografts is possible when ABO barriers are violated, a search must be made for the conse-

quences of ignoring other presensitized states. A detailed analysis of each case with strict adherence to the criteria outlined is essential, however.

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