American Journal of Pathology, Vol. 142, No. 5, May 1993

Liver Allograft Rejection in Sensitized Recipients

Observations in a Clinically Relevant Small Animal Model

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A sequential analysis of liver allograft rejection in sensitized rats using immunopathological and ultrastructural microscopy is described. Lewis rats were primed with four ACI skin grafts and challenged with an arterialized ACI orthotopic liver allograft 14 to 17 weeks later. The sensitization resulted in a mix of IgG and IgM lymphocytotoxic antibodies at a titer of 1:512 at the time of transplantation. Specificity analysis of pretransplant immune sera revealed a predominance of IgG anti-class I major histocompatibility complex (RT1) antibodies with a minor IgG fraction showing apparent endothelial cell specificity (non-RT1). This level of sensitization was associated with accelerated graft failure in 3 to 5 days from mixed humoral and cellular rejection. Sequential analysis of serial posttransplant graft biopsies revealed diffuse vascular IgG deposition and platelet thrombi in portal veins and periportal sinusoids within 3 minutes after reperfusion. This was followed by endothelial cell hypertrophy and vacuolization, periportal hepatocyte necrosis, arterial spasm, focal large bile duct necrosis, and hilar mast cell infiltration and degranulation. However, the liver allografts did not fail precipitously and hyperacute rejection was not seen. Kupffer cell phagocytosis of the sinusoidal platelets began as early as 30 minutes posttransplant and by 24 hours, the platelet thrombi had decreased. Cholangioles appeared focally at the edge of the limiting plates by 2 to 3 days, apparently in response to earlier periportal hepatocyte damage. A mononuclear portal and perivenular infiltrate became evident at 3 days, and graft failure was attributed to both antibody and cell-mediated rejection (Furuya et al: Preformed lymphocytotoxic antibodies: Hepatology 1992, 16: 1415–1422). The model described resembles observations in crossmatch positive human liver allograft recipients. The mechanisms of hepatic graft resistance to antibody mediated rejection and the possible long term consequences of early damage to the biliary tree are discussed. (Am J Pathol 1993, 142:1383–1391)

We have recently described a characteristic graft syndrome in patients harboring preformed lymphocytotoxic antibodies (LAbs), which included increased intraoperative use of blood products, lower postoperative platelet counts, poorer early posttransplant graft function, and an increased incidence of rejection and graft failure.[1–3] However, hyperacute rejection was rarely seen. The livers showed a variety of pathological changes, including findings similar to preservation injury, cellular rejection, arterial vasospasm, and focal large bile duct necrosis.[3] Although the changes were attributed to antibody (Ab)mediated attack, only focal deposits of IgG and complement could be detected in the failed grafts.[3] It was conceivable, but unlikely, that nonimmunological complications could have led to a similar, if not identical pathology.[3]

We began studies in rats to elucidate the pathophysiological interactions between preformed LAbs and liver allografts. In the first series of experiments, we noted a direct correlation between LAb titer and graft survival and identified IgG Abs as the most destructive.[4] In this study, our goal was to investigate the

Accepted for publication November 2, 1992.

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Absorption Studies

Specificity of the preformed lymphocytotoxic Abs was further investigated by absorption of the immune sera with ACI red blood cells (RBCs), which are known to carry surface class I major histocompatibility complex (MHC, RT1) antigenic determinants. Briefly, 1.0 cc packed ACI RBCs was mixed with 0.5 ml of pooled pretransplant ACI-immune LEW sera at room temperature for 30 minutes and then centrifuged for 10 minutes at 2,000 RPM. LEW RBCs were used as a control. The procedure was repeated twice. The remaining supernatant was collected and tested for lymphocytotoxic activity and binding to normal ACI liver tissue sections by indirect IF (see above).

Electron Microscopy

Tissue samples were immersed in Karnovsky's fixative containing 2% paraformaldehyde and 2.5% glutaraldehyde in 0.2 mol/L cacodylate buffer with 0.01% calcium chloride at pH 7.4. Samples were dehydrated in ethanol and embedded in eponaraldite after propylene oxide/resin infiltration. Specimens were vacuum infiltrated and polymerized at 60 C overnight. Thin sections were cut on a Sorval MP5000 and collected on water. Sections in the pale gold to silver interference spectra were then poststained in alcoholic uranyl acetate and Reynold's lead citrate. Thin sections were viewed on a CM12 Phillips microscope at an accelerating voltage of 60 kv and photographed at a final magnification of ×3,000 to ×24,000.

Results

Characterization of Pretransplant Immune Sera

Pooled immune sera obtained immediately before transplantation from sensitized LEW recipients was cytotoxic to unfractionated ACI lymphocytes at a titer of $1:2^9$. Absorption of the sera with ACI RBCs almost completely depleted the lymphocytotoxic activity ($1:2^1$), in contrast to absorption with LEW RBCs, which only slightly lowered the titer ($1:2^6$), likely because of a dilutional effect.

Indirect IF analysis of nonabsorbed pretransplant immune sera at room temperature revealed diffuse IgG ≫ IgM portal and central veins, sinusoidal, hepatic arterial, hilar capillary endothelial, and bile duct cell reactivity. When the assay was performed at 37 C, IgM binding decreased, whereas IgG binding increased slightly. Immune sera absorbed with ACI RBCs continued to show strong capillary and larger vessel endothelial IgG reactivity, whereas sinusoidal binding decreased substantially, and bile duct cell staining was not seen. The results are summarized in Table 1.

Graft Survival and Correlation with Antibody Class, Titer, and Specificity

We have previously shown that liver allograft survival after four sensitizing skin grafts in the ACI to LEW model is directly proportional to antibody titer, class, and specificity.[4] In those experiments,[4] it was shown that preformed IgG lymphocytotoxic antibodies accelerated graft failure. If the liver grafts were placed ≤12 weeks after skin sensitization (×4), hyperacute or pure humoral rejection was seen. If however, the liver grafts were transplanted between 12 to 16 weeks after sensitization, graft survival was less than unsensitized controls, and graft failure was attributable to a combination of antibody and cell-mediated rejection.[4]

The experiments described below were conducted to determine the sequence of rejection in sensitized recipients, who do not hyperacutely reject but experience accelerated graft injury and failure. The model closely mimics the clinical situation in patients with a positive crossmatch.[3]

Table 1. *The Effect of Absorption and Temperature on Intrahepatic Binding of Pretransplant Immune Sera*

Sera	Crossmatch titer		Immunofluorescent staining (20 C/37 C)		
			Vascular endothelium*	Biliary epithelium	Sinusoids
Pretransplant	$1:2^9$	IgG	+++/+++	+++/+++	++/++
		IgM	+/−	+/−	++/+
After absorption with LEW RBCs	$1:2^6$	IgG	++/++	++/++	+/++
		IgM	+/+	−/−	++/+
After absorption with ACI RBCs	$1:2^1$	IgG	++/++	−/−	−/−
		IgM	+/+	−/−	−/−

* Hepatic artery, portal and central vein, and hilar capillary endothelium.

Table 2. *Pathological Course of Sensitized Liver Allograft after Revascularization*

	Postrevascularization periods					
	1–3 minutes	10–30 minutes	1–3 hours	6–24 hours	36–48 hours	72–96 hours
Platelet plugging	+	++	++	+	+	+
Endothelial hypertrophy/vacuolization	–	+	++	++	++	++
Hepatocyte necrosis	–	–	+	++	++	++
Sinusoidal neutrophilia	–	–	+	++	+	+
Neutrophilic portal venulitis	–	–	+	++	+	+
Portal artery thickening	–	–	+	++	++	++
Arterial myocyte vacuolization	–	–	–	–	+	++
Cholangiolar proliferation (focal)	–	–	–	–	+	++
Mononuclear inflammatory infiltrate	–	–	–	–	+	++
Biliary sludge	–	–	–	–	–	+
IgG						
Sinusoids	+	++	++	+	+	+
Portal and central veins	+	++	+	+	–	–

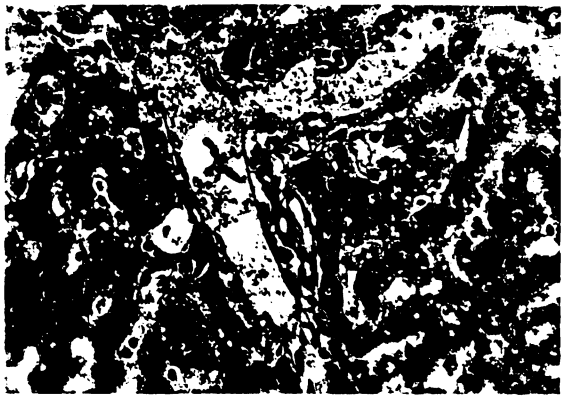

Figure 2. *Sludging of platelets could be detected in the portal veins and periportal sinusoids within 1 to 3 minutes after reperfusion (hematoxylin and eosin [H&E], × 768).*

denudation, mild focal periportal sinusoidal, and intravenular sludging of neutrophils and fibrin could be seen. However, the neutrophil sludging was not particularly striking, as is seen with humoral rejection of kidney or heart allografts.

Between 6 to 12 hours, muscular arteries in the hilum showed medial thickening and more pronounced wrinkling of the internal elastic lamina, suggestive of arterial spasm (Figure 4). The congestion in the peribiliary vascular plexus increased, and collarettes of partially degranulated mast cells rimmed the arteries. Mast cells also appeared increased in the connective tissue around large hilar bile ducts (Figure 4).

Figure 3. *Marked periportal necrosis at 6 hours after revascularization (H&E, × 480).*

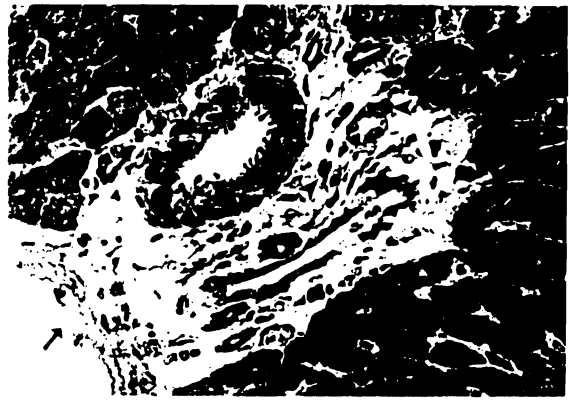

Figure 4. *Changes suggestive of arterial spasm, such as medial thickening, wrinkling of the internal elastica lamina, medial myocyte vacuolization, and endothelial hypertrophy occurred in the peribilar hepatic arteries between 6 and 12 hours posttransplant (plastic embedded thick section for electron microscopy, toluidine blue, × 768). Note also the early infiltration of mast cells (arrowheads) and platelet margination along the portal vein (arrow).*

Small infarcts developed in the hepatic parenchyma and periductal connective tissue and focally involved the walls of hilar excretory bile ducts by 12 hours (Figure 5). Although neutrophils were present, they were not conspicuous, except in the case of infarction.

Small intralobular and larger septal bile duct cells showed an increased nuclear:cytoplasmic ratio between 24 to 36 hours, and cholangioles appeared focally at the edge of the limiting plate by 72 hours.

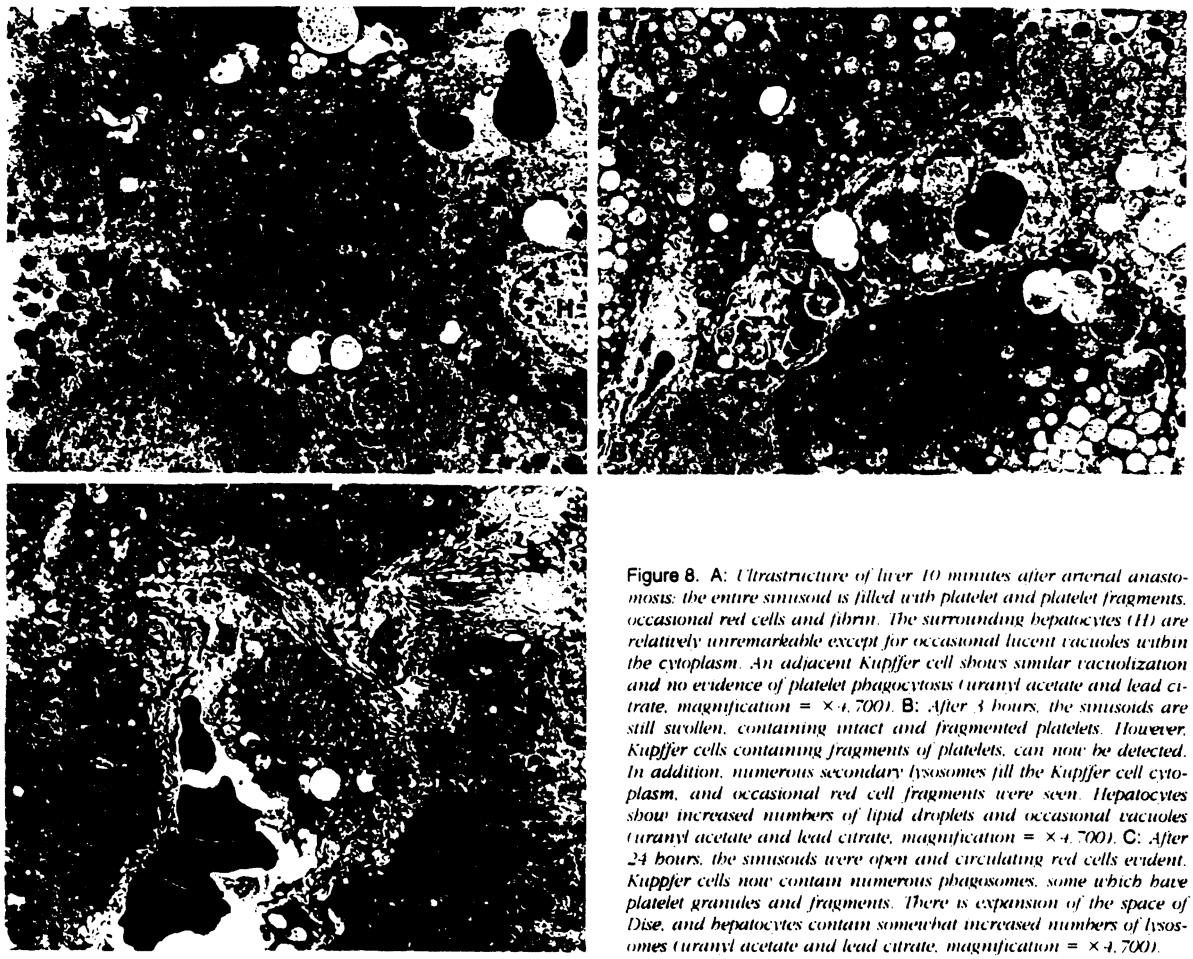

Figure 8. A: *Ultrastructure of liver 10 minutes after arterial anastomosis: the entire sinusoid is filled with platelet and platelet fragments, occasional red cells and fibrin. The surrounding hepatocytes (H) are relatively unremarkable except for occasional lucent vacuoles within the cytoplasm. An adjacent Kupffer cell shows similar vacuolization and no evidence of platelet phagocytosis (uranyl acetate and lead citrate, magnification = ×4,700).* B: *After 3 hours, the sinusoids are still swollen, containing intact and fragmented platelets. However, Kupffer cells containing fragments of platelets, can now be detected. In addition, numerous secondary lysosomes fill the Kupffer cell cytoplasm, and occasional red cell fragments were seen. Hepatocytes show increased numbers of lipid droplets and occasional vacuoles (uranyl acetate and lead citrate, magnification = ×4,700).* C: *After 24 hours, the sinusoids were open and circulating red cells evident. Kupffer cells now contain numerous phagosomes, some which have platelet granules and fragments. There is expansion of the space of Disse, and hepatocytes contain somewhat increased numbers of lysosomes (uranyl acetate and lead citrate, magnification = ×4,700).*

marked by the appearance of focal infarcts. Because of these similarities, we feel this represents a reasonable model to explain some of the histopathological findings in humans and to study the role of other donor or recipient co-factors, which may influence graft survival in crossmatch-positive patients.

The hypersensitized system reported by Knechtle et al[11] was important to show that liver allografts were susceptible to damage from preformed lymphocytotoxic Abs but lack of arterial reconstruction, and the rapidity and mode of graft failure was largely inconsistent with clinical observations.[1,3] Studies by Gubernatis et al[12] in primates were more akin to clinical observations, but limitations are imposed by the use of large animals. Other models of liver rejection in sensitized small animal recipients have emphasized hepatic resistance to preformed Abs,[13-15] although a deleterious effect on graft survival was observed.[13-15]

An allograft liver placed into a sensitized recipient may begin to hyperacutely reject, like kidney or heart allografts. If the preformed allo-Abs are of high titer, IgG class and show endothelial binding, rapid graft failure may ensue.[4] However, if the allo-Abs are of lower titer or IgM class or show less endothelial specificity,[4] we have shown that the liver is often able to withstand the initial damage, adapting and responding to the injury. In humans, lymphocytotoxic Abs are usually of relatively low titer and are only occasionally detected in titers >1:512, which may at least partially account for the rarity of hyperacute rejection in clinical liver transplantation.

The immune sera produced by the skin sensitization protocol contained a mix of IgG and IgM antibodies[4] with a predominant specificity for anti-class I MHC (RT1) antigens and a lesser component, apparently specific for endothelial cells. This conclusion is based on absorption of lymphocytotoxic activity by ACI RBCs but continued presence of anti-endothelial IgG Abs by indirect IF despite absorption. Further studies are currently underway to characterize more precisely the specificities and to determine which Ab is the more destructive in passive administration assays. Not unexpectedly, increased temperature decreased the intrahepatic

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