1454

Reprinted from HEPATOLOGY, St. Louis
Vol. 16, No. 6, pp. 1415-1422, December, 1992 (Printed in the U.S.A.)

Preformed Lymphocytotoxic Antibodies: The Effects of Class, Titer and Specificity on Liver vs. Heart Allografts

TOMOKI FURUYA,[1] NORIKO MURASE,[1] KENJIRO NAKAMURA,[2] JACKY WOO,[1] SATORU TODO,[1] ANTHONY J. DEMETRIS[2] AND THOMAS E. STARZL[1]

Pittsburgh Transplant Institute, Departments of [1]Surgery and [2]Pathology, University of Pittsburgh, Pittsburgh, Pennsylvania 15261

The effect on liver and heart allograft survival (ACI rats to Lewis rats) was studied after three methods of recipient presensitization and after different intervals between sensitization and transplantation. With comparable lymphocytotoxic antibody titers, liver allografts always survived longer than heart grafts. The titer, class and specificity of the antibodies varied with the method of sensitization. Four skin grafts produced IgG and IgM lymphocytotoxic antibody titers of 1:2,000 to 4,000. The IgG fraction was shown to have hepatic vascular endothelial specificity by indirect immunofluorescence. These primed recipients hyperacutely rejected both heart and liver allografts, which showed vascular deposition of IgG antibodies. Survival of either kind of graft was inversely proportional to the lymphocytotoxic antibody titer and length of time after placement of the last skin graft. Presensitization with a single heterotopic heart graft produced an even higher mixed IgG and IgM lymphocytotoxic antibody titer of 1:8,000 but with less IgG vascular endothelial specificity. These animals also hyperacutely rejected heart or liver grafts with tissue deposition of IgG but less consistently and with a weaker correlation with lymphocytotoxic antibody titers and time after sensitization. Sensitization with two pretransplant blood transfusions produced the lowest titer (1:500 to 1,000) and the least IgG vascular endothelial specificity. Liver allograft survival was routinely enhanced in these animals, and little effect was seen on heart grafts. Collectively, the experiments showed that the liver is not only resistant to antibody-mediated rejection relative to the heart but is more easily enhanced. A more precise characterization of preformed antibodies may increase the ability to predict the outcome of liver transplantation in sensitized recipients or guide pretransplant strategies to foster enhancing antibodies. (HEPATOLOGY 1992;16:1415-1422.)

The relationship between preformed complement-fixing lymphocytotoxic antibodies (LAbs) and rapid kidney allograft rejection is well known (1, 2). However, liver allografts are relatively resistant to preformed LAbs; hyperacute rejection is rarely observed in clinical practice (3, 4) and is difficult to produce in experimental animal models (5, 6). The liver's resistance is thought to be caused by many factors, but recent clinical evidence and studies of highly sensitized animal models have shown that this privileged state is only relative (4-10). Because of conflicting results in clinical practice with sensitized liver allograft recipients (3-4, 7-10), the practical significance of LAbs in an individual patient and whether they should interdict candidacy is difficult to judge.

In an attempt to learn more about the interactions between preformed LAbs and liver allografts, we sensitized rats with heart, skin or whole blood and varied the time between the last priming and placement of the test heart or liver allograft.

MATERIALS AND METHODS

Animals

Male inbred Lewis (LEW, $RT1^l$) rats weighing 180 to 250 gm and ACI ($RT1^a$) rats weighing 180 to 300 gm (Harlan Sprague Dawley Inc., Indianapolis, IN) were used as recipients and donors, respectively. The animals were housed in conventional facilities with water and commercial rat chow provided *ad libitum*.

Sensitization Protocols

Skin Grafting. LEW recipients were sensitized with four successive skin grafts using a modification of the method described by Guttmann (11). Full thickness ACI tail skin grafts (2 cm in diameter) were sutured to the flank of LEW recipients with 4-0 Novafil (Davis and Geck, Inc. Manati, PR), and a plaster bandage was applied for 5 days. The procedure was repeated three more times at 14-day intervals.

Heart Grafting. ACI heterotopic heart grafts were anastomosed to the infrarenal abdominal aorta and vena cava of LEW recipients as described by Ono and Lindsey (12). The grafts were palpated daily until the heartbeat ceased, which uniformly occurred on the sixth or seventh day.

Received February 3, 1992; accepted July 6, 1992.
Aided by research grants from the Department of Veterans Affairs and Project Grants DK 29961 and CA 44602 from the National Institutes of Health, Bethesda, Maryland.
Address reprint requests to: Thomas E. Starzl, M.D., Ph.D., Department of Surgery, 3601 Fifth Avenue, 5C Falk Clinic, University of Pittsburgh, Pittsburgh, PA 15213.
31/1/41322

Whole-blood Transfusions. One milliliter of heparinized ACI whole blood was given intravenously to LEW recipients on two different occasions at a 7-day interval.

Experimental Design

Operative Methods. Heart grafts were placed in the neck of LEW recipients using the method of Heron (13). The graft aorta and pulmonary artery were connected to the carotid artery and jugular vein of the recipient, respectively. The heart grafts were directly monitored for the first 30 min, palpated through the skin each hour for the first 6 hr and every 12 hr thereafter. Graft rejection was diagnosed when no palpable contractions were observed; it was confirmed by direct inspection and histological examination.

The orthotopic liver transplant procedure used the cuff technique for the portal and infrahepatic vena cava anastomoses, according to the method of Kamada and Calne (14). Arterial reconstruction was omitted because in pilot experiments many grafts were destroyed or gravely damaged by accelerated rejection between the time of portal revascularization and completion of the arterial anastomosis. Graft rejection was diagnosed by the death of the animals and confirmed by histological examination.

Timing of Test Grafting. ACI heart or liver grafts were placed into LEW recipients at 2, 4, 6, 9, 12 and 15 wk after completion of the skin and heart sensitization protocols. Heart or liver transplantation was completed 1 wk after the second blood transfusion. Six or seven experiments were performed at each time point.

Lymphocytotoxic Activity and Flow Cytometric Analysis

Serum samples for antibody analysis were obtained immediately before transplantation and at the time of graft rejection. Samples were stored at −70° C until the cytotoxic crossmatch, flow cytometric analysis or indirect immunofluorescent assays were performed.

A complement-fixing LAb assay was performed with unfractionated donor ACI lymphocytes and immune LEW sera according to the method of Terasaki et al.(15). Serum samples were serially diluted from 1:1 to 1:2 (16) with RPMI 1640 (Gibco, Grand Island, NY) supplemented with 25 mmol/L HEPES buffer (Gibco), 1.6 mmol/L L-glutamine (Gibco), 50 μg/ml gentamicin (Gibco) and 10% heat-inactivated normal rat serum. Cell lysis was scored as follows: 0 = undetectable, 1 = 0% to 10%; 2 = 11% to 20%; 4 = 21% to 50%; 6 = 51% to 80%; 8 = 81% to 100%. All samples were run in duplicate. The LAb titer was defined as the most dilute serum sample with a score greater than 6 (greater than 50% cell lysis).

ACI peripheral lymph node cells (5×10^5) were used as targets for flow cytometric analysis; the phenotypic composition was determined using OX19 (T cells; Accurate Chemical and Scientific Corp., Westbury, NY), OX33 (B cells, Accurate Chemical and Scientific Corp.) and goat antirat IgG or IgM (B cells; Accurate Chemical and Scientific Corp.). Target lymphocyte phenotype was determined by incubation of the target lymphocytes with the primary antibody (OX19 or OX33) for 45 min, followed by two washes and labeling with FITC-conjugated goat antimouse immunoglobulin (Accurate Chemical and Scientific Corp.). The percentage of B cells (OX33+ or IgG and IgM+) varied between 30% to 38%, with T cells (OX19+) comprising most (65% to 75%) of the remaining population.

For ACI lymphocyte binding activity of the immune sera and determination of titers, diluted immune LEW serum samples (1:10, 1:100, 1:500 and 1:1,000 in HBSS with 1% BSA and 0.1% NaN_3) were incubated with the targets for 45 min at 4° C. Nonimmune LEW sera served as a control. After incubation, cells were washed and FITC-conjugated goat antirat IgG (Accurate Chemical and Scientific Corp.) or IgM antibody (1:75 dilution) was added and incubated for another 30 min at 4° C. Cells were then washed twice and fixed in 1% paraformaldehyde.

Samples were analyzed by FASTAR (Becton Dickinson & Co., Franklin Lakes, NJ). A total of 5,000 cells were analyzed, and the fluorescent profiles of the ACI cells after labeling with immune serum samples were compared with the profiles obtained after labeling with normal LEW serum and after OX19, OX33, IgM or IgG labeling. The percentage of positive cells and the phenotype of the targets was determined by subtraction from the normal control background.

Routine and Immunopathological Studies

Failed allograft tissues were processed for routine pathological analysis, and a portion was frozen for direct immunofluorescent studies. A diagnosis of pure humoral rejection of the heart or liver was made when histological examination of the grafts revealed hemorrhagic necrosis, with platelet/fibrin thrombi and neutrophils in the microvasculature but without a significant mononuclear inflammatory infiltrate. Mixed humoral and cellular rejection was diagnosed when the above findings were found in conjunction with a predominantly mononuclear graft infiltrate combined with tissue deposition of IgG.

Direct immunofluorescence studies on failed allografts were performed in representative animals from each group, using goat antirat IgG and IgM (Accurate Chemical and Scientific Corp.) to detect immunoglobulin deposits. Indirect immunofluorescent studies were used to detect the target antigen specificity, class and intensity of binding of the LEW immune sera in the ACI liver. Normal frozen ACI liver sections (2 μm) were blocked with protein-blocking agent for 30 min, blotted and then overlaid with undiluted immune LEW serum for 45 min. The slides were then washed two times and incubated with goat antirat IgG or IgM for 30 min, washed two times and evaluated on a fluorescent microscope. Nonimmune LEW sera served as a control. All of the pathological analyses were performed without knowledge of the type of sensitization or primary immunoreactant (blinded review).

Statistical Analysis

All results were analyzed for statistical significance by the Mann-Whitney U test, except for the results of changes in antibody titer before and after transplantation, which were analyzed by the Wilcoxon signed rank test. The differences were considered statistically significant if $p < 0.01$.

RESULTS

Lymphocytotoxic Antibody Titer, Class and Specificity After Sensitization

Both routine cytotoxic and flow cytometric crossmatches were negative using nonimmune LEW sera. However, all three sensitization protocols produced antibodies cytotoxic to ACI lymphocytes. The highest titers were observed during the first 6 wk after heart (1:8,000) and the final skin (1:2,000 to 4,000) sensitization (Fig. 1). Antibody titers after whole-blood sensitization were only measured 1 and 2 wk after the last

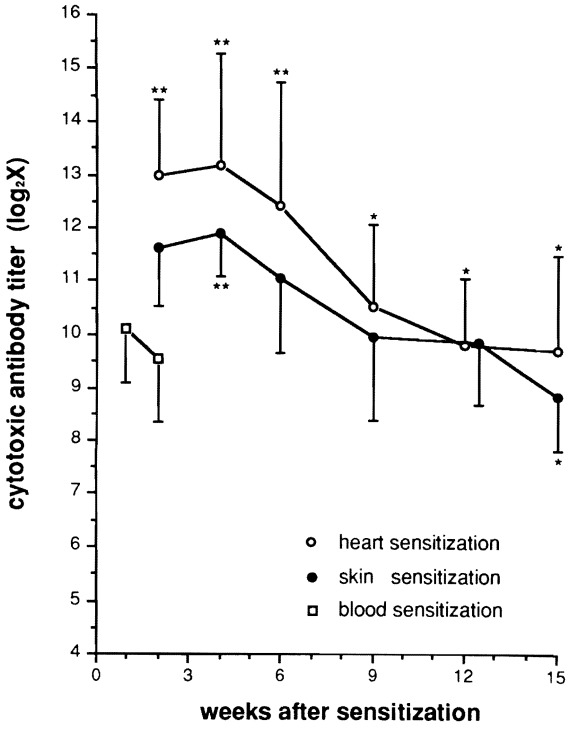

FIG. 1. The changes in LAb titer after sensitization (mean ± S.D.). Titers more than 9 wk after heart sensitization and more than 15 wk after skin sensitization were significantly different than initial titers at 2 wk (*$p < 0.01$). In addition, titers less than 6 wk after heart sensitization and 4 wk after skin sensitization were significantly higher than after blood sensitization (**$p < 0.01$).

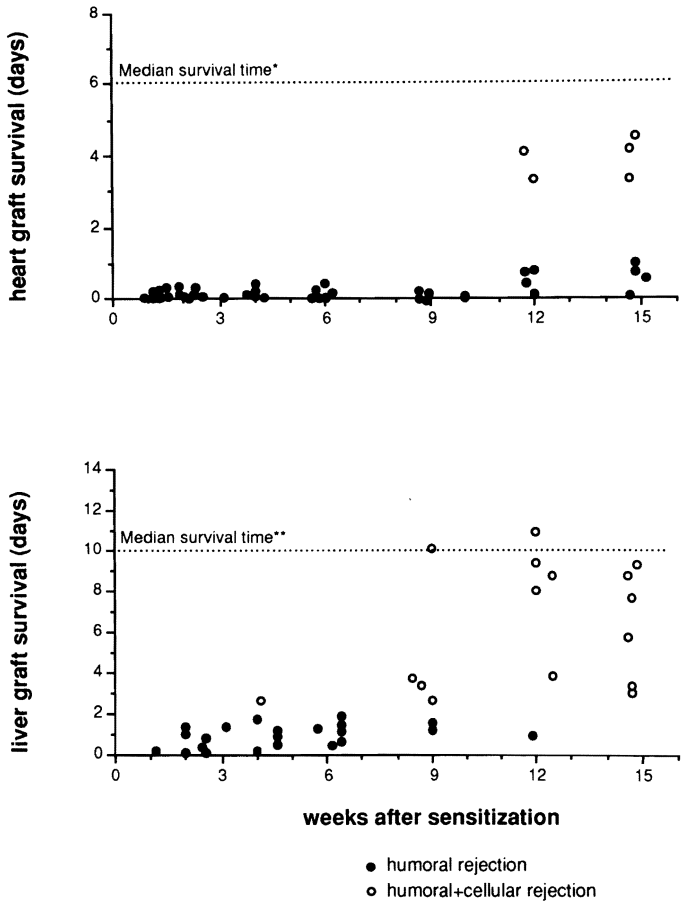

FIG. 2. Graft survival time after skin sensitization. Heart or liver grafts surviving more than 3 days showed a mixed humoral and cellular rejection *(open circles),* whereas those failing before 3 days showed more humoral rejection *(closed circles).* *Median survival time of ACI heart graft (6 days, N = 7). **Median survival time of ACI liver graft (10 days, N = 7) in unsensitized LEW recipients.

transfusion and were lower (1:500-1,000). The specificity of the cytotoxic antibodies as defined by flow cytometry and indirect immunofluorescence is given below.

Skin Sensitization. Two weeks after concluding the sensitization, flow cytometry revealed that skin priming produced immune sera containing both IgG and IgM antibodies, both of which reacted with more than 90% of ACI cells, which contained both T and B cells. Dilutional analysis revealed that the IgG fraction was present in higher concentration than IgM (> 1:1,000 vs. < 1:500).

Indirect immunofluorescent studies using 2-wk immune LEW sera revealed intense linear IgG > > IgM reactivity with all endothelia, bile duct epithelia and weaker hepatocyte staining (Table 1).

Heart Sensitization. After heart sensitization, flow cytometric studies revealed IgG and IgM antibodies (both > 1:1,000) and binding with more than 90% of ACI lymphocytes, which contained both T and B cells. However, despite the higher titers, less intense hepatic endothelial IgG reactivity by indirect immunofluorescence was seen (Table 1). IgM localization revealed a distinctly granular pattern in sinusoidal cells and on hepatocytes.

Blood Sensitization. Cytotoxic antibody titers after blood sensitization were significantly lower (1:500 to 1,000; $p < 0.01$) than either heart or skin. By flow cytometry the concentration of IgG equaled IgM (< 1:1,000), and both T-cell and B-cell binding were observed. However, by indirect immunofluorescence the 1-wk antiserum reacted only weakly with hematolymphoid cells amid the portal connective tissue for both IgG and IgM (Table 1). The larger vessel endothelium was only weakly or equivocally positive. Sera obtained after 2 wk showed slight increases for both IgG and IgM, but the binding did not approach that seen with 2-wk heart or skin sera.

Evolution of Antibody Titer, Class and Specificity

Sequential analysis of the cytotoxic activity of the immune sera revealed a gradual decline in antibody titer between 6 and 15 wk after both heart and skin sensitization (Fig. 1). Compared with levels at 2 wk, the decline became statistically significant by 9 wk for heart priming and by 15 wk for skin immunization.

The decrease in IgG and IgM titers was also noted by flow cytometry dilutional analysis and by a shift to a lower channel for both IgG and IgM. For the skin-

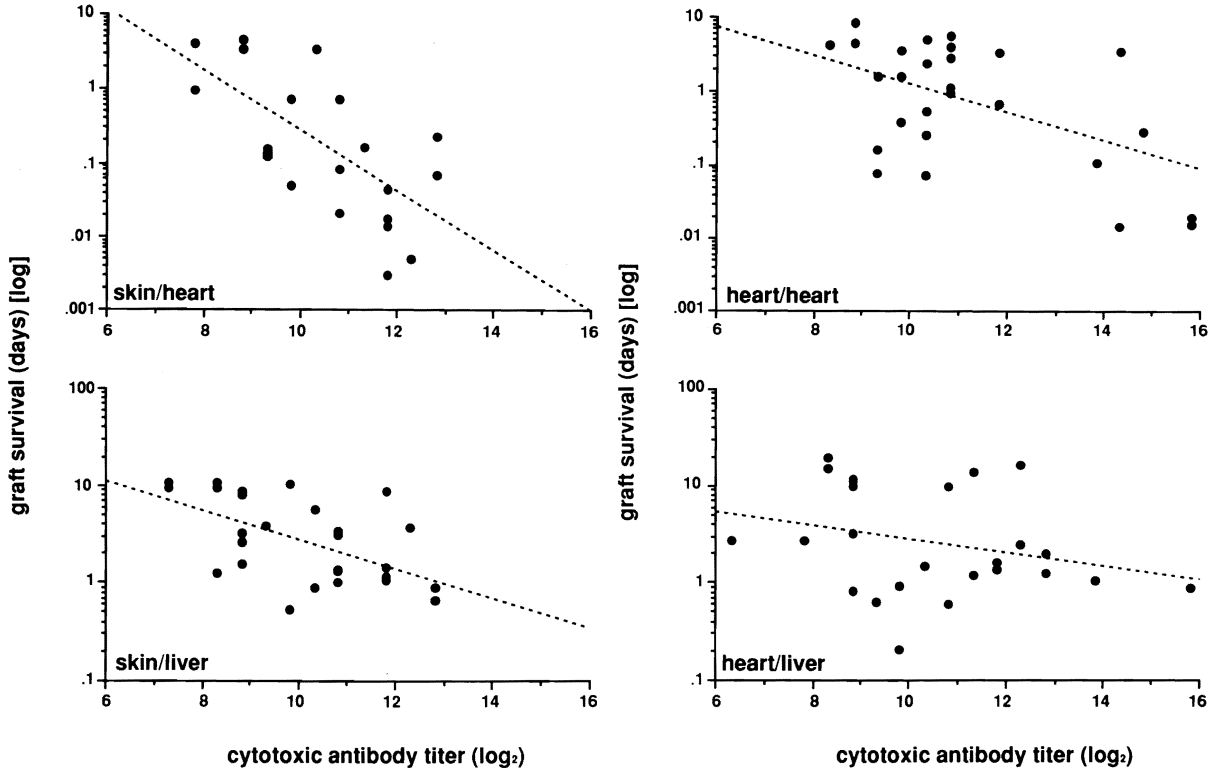

FIG. 3. Correlation between heart or liver graft survival and LAb titer after skin or heart sensitization. Skin/heart = heart graft survival and cytotoxic antibody titer after skin sensitization. Regression equation: log10 (Y) = 3.5713 − 0.41796X, r = 0.6766, p < 0.01. Skin/liver = liver graft survival and cytotoxic antibody titer after skin sensitization. Regression equation: log10 (Y) = 1.9549 − 0.15054X, r = 0.5690, p < 0.01. Heart/heart = heart graft survival and cytotoxic antibody titer after heart sensitization. Regression equation: log10 (Y) = 2.3070 − 0.18928X, r = 0.5042, p < 0.01. Heart/liver = liver graft survival and cytotoxic antibody titer after heart sensitization. Regression equation: log10 (Y) = 1.1692 − 0.071396X, r = 0.2811; NS.

TABLE 1. Hepatic specificity analysis of preformed antibodies by indirect immunofluorescence[a]

Method of sensitization	Time (wk)	PV	HA	BD	HLC	SIN	CV
Skin	2	3/2	3/2	3/1	3/1	3/2[b]	3/2
	15	2/0	2/0	1/0	1/0	2/2[b]	2/0
Heart	2	2/1	2/0	1/1	2/2	2/2[b]	2/1
	15	1/±	2[c]/0	1/1	2/2	1/1[b]	1/1
Blood	1	±/1	0/0	±/1	1/1	±[b]/1[b]	0/0
	2	1/1	1/1	2/1	1/1	2[b]/2[b]	0/1

PV = portal vein; HA = hepatic artery; BD = bile duct; HLC = portal tract hematolymphoid cells; SIN = sinusoids; CV = central vein. The indirect immunofluorescence was scored subjectively on a scale from 0 to 3, with 0 = negative and 3 = strongly positive.

[a]The fluorescent intensity of IgG deposits/IgM deposits.

[b]Sinusoidal binding was distinctly granular.

[c]Reactivity with arterial smooth muscle cells.

sensitized rats, overall titers were lower but the ratio of IgG/IgM did not change. However, immune sera produced by heart priming at 15 wk showed a shift to an IgG-predominant response (> 1:1,000) compared with IgM (< 1:500).

Indirect immunofluorescence of immune sera 15 wk after heart or skin sensitization revealed a decreased binding, which was greater for IgM than IgG compared with 2-wk sera. No appreciable change in tissue specificity was detected for skin-primed rats. However, heart-sensitized immune sera demonstrated a greater predilection for portal tract structures, including vascular smooth muscle cells, than did similar sera obtained after 2 wk (Table 1).

Graft Survival as a Function of Postsensitization Time and Antibody Titers

Skin Sensitization. Survival of heart and liver grafts as a function of the time after sensitization is shown in Figure 2. If the heart grafts were placed less than 10 wk after the last skin transplant, they always were hyperacutely rejected. Typically, these heart grafts became

cyanotic, edematous and hemorrhagic within a few minutes after adequate revascularization. Microscopic examination revealed classic hyperacute rejection with vascular deposition of IgG.

If heart placement was delayed until 12 to 15 wk, an accelerated mixed humoral and cellular rejection was nearly as common as pure humoral or hyperacute rejection. Heart grafts survived significantly longer when placed after 12 wk (mean survival = 43.7 hr; median = 18.8 hr) compared with heart transplants completed before 10 wk (mean survival = 1.7 hr; median = 0.9 hr; $p < 0.01$). In addition, a strong inverse correlation was seen between LAb titers and heart graft survival (Fig. 3).

Liver allografts always survived longer than heart grafts placed after skin sensitization (Fig. 2). During the first 6 wk after sensitization, all livers were rejected within 3 days. Characteristically, the hepatic grafts became mottled and pale soon after portal revascularization. This was accompanied by severe portal hypertension and mesenteric congestion. During the next 24 hr, the portal hypertension waned, the livers became small and pale and the recipient became anemic and had copious thin bloody ascites develop. Pathologically, characteristic findings included marked platelet plugging of the vasculature, congestion, maplike areas of coagulative necrosis and diffuse vascular deposition of IgG alone.

If liver placement was delayed for more than 9 wk, a range of graft survivals was observed, some of which approached unsensitized controls (Fig. 2). Pathologically, the failed grafts showed a mixed humoral and cellular rejection characterized by a mixed cellular portal infiltrate, platelet plugs, infarcts and focal sinusoidal IgG deposits. An inverse relationship between antibody titers and liver graft survival was seen (Fig. 3), but this was less strong than when the heart was the test graft.

Heart Sensitization. Heart and liver graft survival in LEW recipients presensitized with ACI heart grafts was more variable than after skin priming (Fig. 4), although the trends after heart priming were the same. In addition, a less strong correlation was seen with LAb titers (Fig. 3), which were actually higher than after skin sensitization.

Most heart grafts placed during the first 6 wk were hyperacutely rejected, but with a delay of more than 9 wk median survival was between 50 and 60 hr. Most of the latter hearts were rejected by a mixture of humoral and cellular mechanisms.

Liver allograft survival in heart-sensitized LEW recipients was even more variable. Although many of the liver grafts were rejected before 4 days during the entire 15 wk after sensitization, slightly enhanced graft survival was not uncommon in grafts placed after 6 wk (Fig. 4). Grafts surviving between 3 and 7 days often showed a mixed humoral and cellular rejection at the time of failure.

Blood Sensitization. Prior blood transfusions had little effect on subsequent heart allograft survival, although a slight but statistically insignificant prolongation was seen. In contrast, liver graft survival was

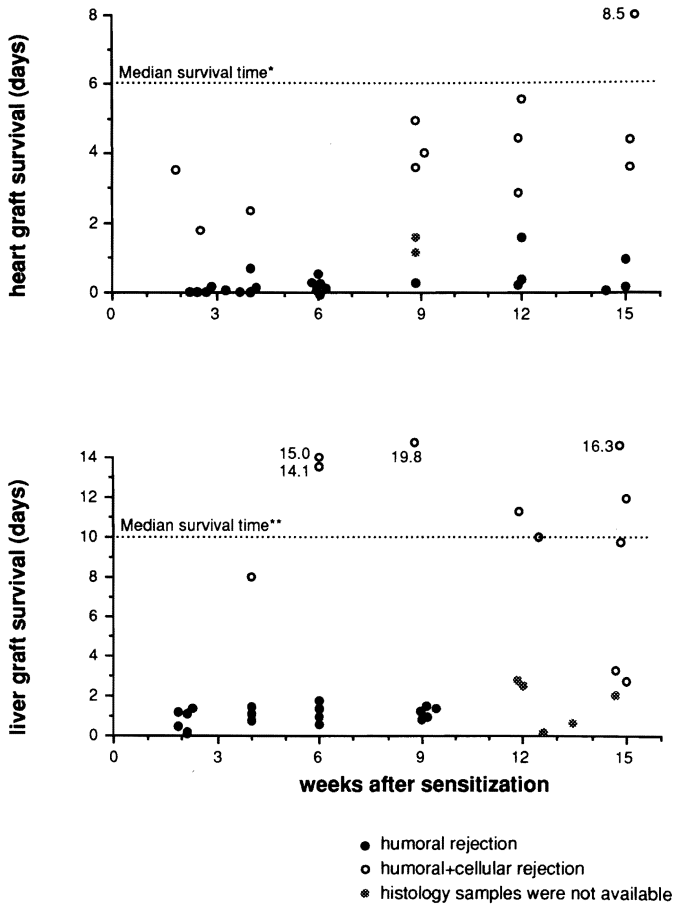

FIG. 4. Graft survival after heart sensitization. *Median survival of ACI heart grafts (6 days, N = 7). **Median survival of ACI liver graft (10 days, N = 7) in unsensitized LEW recipient.

significantly enhanced in all animals ($p < 0.001$) (Table 2). Only one of the failed liver allografts showed classic cellular rejection. The others showed marked sinusoidal Kupffer cell hypertrophy and sinusoidal lymphohistiocytosis with a modest portal infiltrate.

Cytotoxic Antibody Titers Before and After Graft Rejection

When the liver grafts were rejected within 4 days of transplantation, a significant decrease in LAb titer was seen in both skin-sensitized and heart-sensitized animals (Fig. 5). Antibody titers fell less dramatically after heart transplantation.

When the liver and heart grafts were rejected after 4 days, significant falls in antibody titers were not observed, and in fact an increase in titer was observed in some.

DISCUSSION

Our results suggest that at least three variables influence the results obtained with transplantation in sensitized recipients: the type of organ allograft, the organ used for sensitization and the time elapsed after priming. The repetitiveness of priming is likely a fourth factor. Consistent with prior observations (3-10, 16, 17)

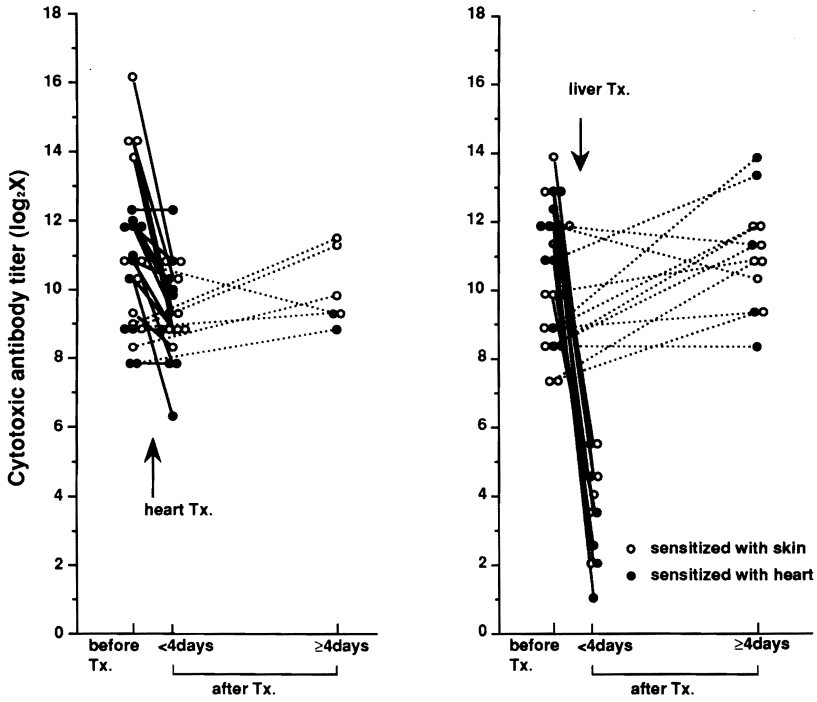

FIG. 5. Cytotoxic antibody titer before and after heart or liver transplantation. Heart Tx = heart transplantation; liver Tx = liver transplantation; before Tx = titers before heart or liver transplantation; after Tx = titers after heart or liver transplantation; < 4 days = grafts rejected within 4 days; ≥ 4 days = grafts survived for 4 or more days.

TABLE 2. Graft survival after whole-blood sensitization compared with unsensitized LEW controls

Sensitization	N	Survival time (days)	MST	p Value
Heart graft survival				
Control	7	6, 6, 6, 6, 6, 7, 7	6.0	–
Whole blood	6	6, 6, 7, 7, 8, 9	7.0	NS
Liver graft survival				
Control	10	9, 9, 9, 9, 10, 10, 10, 11, 12, 13	10.0	–
Whole blood	6	18, 19, 21, 22, 78, 79	21.5	< 0.001

MST = median survival time (days).
p values vs. control (Mann-Whitney U test).

liver grafts were more resistant to antibody-mediated damage than cardiac grafts. The dual hepatic vasculature, secretion of soluble major histocompatibility complex antigens and the protective activity of Kupffer cells, which clear immune complexes, platelet aggregates and activated coagulation proteins, may contribute to hepatic resistance (16-19).

However, our studies and earlier reports show that the liver's immunological privilege is only relative (4-8). In earlier reports of experimental liver transplantation in rats (6) and primates (5), no association was seen between the antibody titers and the outcome. Our data provide this sought-after association that others have claimed for cardiac grafts (20, 21). The clinical implication for liver transplantation is that determination of cytotoxic antibody titers may be equal in importance or more so than the presence of a positive crossmatch. In clinical practice it is uncommon to have titers greater than 1:512, whereas this was the usual condition in our sensitized animals. Therefore determination of antibody titer pretransplantation may increase the predictive value of the crossmatch test.

An additional reservation about conventional clinical crossmatch procedures is that the recipient antibody state is dynamic rather than fixed. The LAb titer may be affected by antibody class, specificity and antiidiotype antibodies (22), which change with time. The quality of these changes apart from quantitative reduction of antibody levels is under investigation. Such studies are needed because it is clear that the LAb titer alone does not sufficiently explain the results obtained with different methods of sensitization. Similar high titers of preformed LAb were observed in some animals who subsequently hyperacutely rejected their grafts and in others who experienced enhancement.

Until now, the best clue to explain such disparate results has come from indirect immunofluorescent analysis, which showed differences in target antigen

specificity in the liver, depending on the method of sensitization. Sensitization with skin protocol produced a subfraction of IgG antibodies with specificity for the hepatic vascular endothelium, and this appeared to be associated with accelerated graft failure. Such an observation is in accord with most clinical studies in which IgG antibodies (in contrast to IgM) with endothelial cell specificity have seemed to be the most dangerous (4, 7, 23-27).

Blood transfusions also produced LAb but with appreciably less hepatic endothelial specificity and with the consequence of frequent enhancement of hepatic graft survival. Thus the lymphocytotoxic crossmatch is only a surrogate test for graft-reactive antibodies. Further or different characterization of preformed antibody may be needed to predict the outcome in sensitized liver allograft recipients.

To our knowledge, this is the first demonstration that liver grafts are enhanced with blood transfusions. Enhancement was first observed in kidney transplantation after blood transfusion (28, 29). Numerous experimental and clinical studies have been performed in an attempt to unravel underlying mechanisms (30-38). Our experiments do not add to the earlier hypothesis beyond the demonstration of the ease of hepatic graft enhancement relative to that of heart grafts.

Acknowledgements: We thank Makoto Kobayashi, Shiro Fujita, Isao Hamamoto and Tomoya Matsuzaki for their technical assistance and Atsushi Yagihashi and Yuichi Iwaki for helpful suggestions.

REFERENCES

1. Terasaki PI, Marchioro TL, Starzl TE. Sero-typing of human lymphocyte antigens: preliminary trials on long-term kidney homograft survivors. In: Conference and workshop on histocompatibility testing. Washington DC: National Academy of Sciences, National Research Council, 1965:83-96.
2. Kissmeyer-Neilsen F, Olsen S, Peterson VP, Fjeldborg O. Hyperacute rejection of kidney allografts, associated with pre-existing humoral antibodies against donor cells. Lancet 1966;2:662-665.
3. Iwatsuki S, Iwaki Y, Kano T, Klintmalm G, Koep LJ, Weil R, Starzl TE. Successful liver transplantation from crossmatch positive donors. Transplant Proc 1981;13:286-288.
4. Takaya S, Duquesnoy R, Iwaki Y, Demetris AJ, Yagihasi A, Bronsther O, Iwatsuki S, et al. Positive crossmatch in primary human liver allografts under cyclosporine or FK506 therapy. Transplant Proc 1991;23:396-399.
5. Gubernatis G, Lauchart W, Jonker M, Steinhoff G, Bornscheuer A, Neuhaus P, Van Es AA, et al. Signs of hyperacute rejection of liver grafts in rhesus monkeys after donor-specific presensitization. Transplant Proc 1987;19:1082-1083.
6. Knechtle SJ, Kolbeck PC, Tsuchimoto S, Coundouriotis A, Sanfilippo F, Bollinger RR. Hepatic transplantation into sensitized recipients. Transplantation 1987;43:8-12.
7. Demetris AJ, Nakamura K, Yagihashi A, Iwaki Y, Takaya S, Hartman G, Murase N, et al. A clinicopathological study of human allograft recipients harboring preformed IgG lymphocytotoxic antibodies. HEPATOLOGY 1992;16:671-681.
8. Starzl TE, Demetris AJ, Todo S, Kang Y, Tzakis A, Duquesnoy R, Makowka L, et al. Evidence for hyperacute rejection of human liver grafts: the case of the canary kidneys. Clin Transplant 1989;3:37-45.
9. Starzl TE, Tzakis A, Makowka L, Banner B, Demetris A, Ramsey G, Duquesnoy R, et al. The definition of ABO factors in transplantation: relation to other humoral antibody states. Transplant Proc 1987;19:4492-4497.
10. Sanchez-Urdazpal L, Sterioff S, Janes C, Schwerman L, Rosen C, Krom RAF. Increased bile duct complications in ABO incompatible liver transplant recipients. Transplant Proc 1991;23:1440-1441.
11. Guttmann RD. Genetics of acute rejection of rat cardiac allografts and a model of hyperacute rejection. Transplantation 1974;17: 383-386.
12. Ono K, Lindsey E. Improved technique of heart transplantation in rats. J Thorac Cardiovasc Surg 1969;57:225-229.
13. Heron I. A technique for accessory cervical heart transplantation in rabbits and rats. Acta Pathol Microbiol Scand (A) 1971;79: 366-372.
14. Kamada N, Calne RY. Orthotopic liver transplantation in rats: technique using cuff for portal vein anastomosis and biliary drainage. Transplantation 1979;28:47-50.
15. Terasaki PI, Bernoco D, Park MS, Dztark GO, Iwaki Y. Microdroplet testing for HLA-A, -B, -C, and -D antigens. Am J Clin Pathol 1978;69:103-120.
16. Houssin D, Bellon B, Brunaud MD, Gunenheim J, Settaf A, Meriggi F, Emond J. Interactions between liver allografts and lymphocytotoxic alloantibodies in inbred rats. HEPATOLOGY 1986; 6:994-998.
17. Houssin D, Gugenheim J, Bellon B, Brunaud MD, Gigou M, Charra M, Crougneau S, et al. Absence of hyperacute rejection of liver allografts in hypersensitized rats. Transplant Proc 1985;17: 293-295.
18. Davies HFFS, Pollard SG, Calne RY. Soluble HLA antigens in the circulation of liver graft recipients. Transplantation 1989;47: 524-527.
19. Demetris AJ, Markus BH. Immunopathology of liver transplantation. Crit Rev Immunol 1989;9:67-92.
20. Dohi K, Tabe Y, Ezaki H. An experimental model for hyperacute rejection in inbred rat cardiac transplantation: correlation cardiac graft survival time and anti T-cell warm cytotoxic antibody titer. Hiroshima J Med Sci 1984;33:251-257.
21. Dohi K, Tabe Y, Ono E, Ezaki H. Relationship between the decrease of cytotoxic antibody with the elapse of time and hyperacute rejection in hyperimmunized rats. Hiroshima J Med Sci 1985;34:131-136.
22. Feldman M, Male D. Cell cooperation in the antibody response. In: Riott IM, Brostoff J, Male DK. Immunology, 2nd ed. London: Gower Medical Publishing Ltd., 1989:1-12.
23. Ahern AT, Artruc SB, Della Pelle P, Cosimi AB, Russell DS, Colvin RB, Fuller TC. Hyperacute rejection of HLA-A, B-identical renal allografts associated with B lymphocyte and endothelial reactive antibodies. Transplantation 1982;33:103.
24. Mohanakumar T, Rhodes C, Mendez-Picon G, Goldman M, Moncure C, Lee H. Renal allograft rejection associated with presensitization to HLA-DR antigens. Transplantation 1981;31: 93-95.
25. Iwaki Y, Lau M, Terasaki PI. Successful transplants across T-warm positive crossmatches due to IgM antibodies. Clin Transplant 1988;2:81-84.
26. Karuppan SS, Lindholm A, Moller E. Characterization and significance of donor reactive B cell antibodies in current sera of kidney transplant patients. Transplantation 1990;49:510-515.
27. Taylor CJ, Chapman JR, Ting A, Morris PJ. Characterization of lymphocytotoxic antibodies causing a positive crossmatch in renal transplantation: relationship to primary and regraft outcome. Transplantation 1989;48:953-958.
28. Opelz G, Terasaki PI. Poor kidney transplant survival in recipients with frozen blood transfusions or no transfusions. Lancet 1974;1:696-698.
29. Opelz G, Graver B, Mickey MR, Terasaki PI. Lymphocytotoxic antibody responses to transfusion in potential kidney transplant recipients. Transplantation 1981;32:177-183.
30. Wood KJ, Evins J, Morris PJ. Suppression of renal allograft rejection in the rat by class I antigens on purified erythrocytes. Transplantation 1985;39:56-62.
31. Fabre JW, Morris PJ. The effect of donor strain blood pre-

treatment on renal allograft rejection in rats. Transplantation 1972;14:608-617.
32. Maki T, Okazaki H, Wood ML, Monaco AP. Suppressor cells in mice bearing intact skin allografts after blood transfusions. Transplantation 1981;32:463-466.
33. Hutchinson IV. Suppressor T cells in allogenic models. Transplantation 1986;41:547-555.
34. Terasaki PI. The beneficial transfusion effect on kidney graft survival attributed to clonal deletion. Transplantation 1984;37: 119-125.
35. Downey WE III, Baldwin WM III, Sanfilippo F. Association of donor specific blood transfusion enhancement of rat renal allografts with accelerated development of antidiotypic antibodies and reduced alloantibody responses. Transplantation 1990;49: 160-166.
36. Baldwin WM, Rhoton K, Sanfilippo F. IgM and IgG alloantibody production by splenocytes and deposition in rat renal allografts are decreased by donor specific blood transfusion. Transplantation 1991;51:481-485.
37. MacLeod AM, Power DA, Mason RJ, Stewart KN, Shewan WG, Edward N, Catto GRD. Possible mechanism of action of transfusion effect in renal transplantation. Lancet 1982;2:468-470.
38. Davies DAL, Staines NA. A cardinal role for I-region antigens (Ia) in immunological enhancement and the clinical implications. Transplant Rev 1976;30:18-39.