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Endotoxemia and Human Liver Transplantation

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ALTHOUGH orthotopic liver transplantation has become the preferred treatment for many kinds of end-stage liver disease, the operation still has a significant perioperative morbidity and mortality.[1] Problems that can jeopardize or preclude success despite a seemingly perfect donor and recipient operation include unexpected coagulopathy with hemorrhage, cardiovascular collapse, acute renal failure, and respiratory insufficiency. Complications of the same kind have been attributed to endotoxemia in experimental animals or in nontransplant patients with portal vein occlusion,[2] intestinal ischemia,[3] cirrhosis,[4] and several other surgical conditions.[5]

In dogs submitted to liver replacement, a wave of endotoxemia can always be demonstrated during and after the transplantation.[6] Consequently, we have looked systematically for evidence of endotoxemia in a randomly selected group of 90 liver transplantations in adult humans. We found a striking association between perioperative blood endotoxin levels, the difficulty of convalescence, and the ultimate outcome.

MATERIALS AND METHODS

Case Material

Eighty-one adult patients who were 44.0 ± 1.3 (SE) years old (range 18 to 66) had 90 orthotopic liver transplantations between March 15 and August 15, 1988. Males were the recipients of 52 (64.2%) of the grafts. During this 5-month period, investigations were conducted in 50% of all transplantations, the constituency of the studied vs nonstudied groups being determined primarily by availability of the investigators.

Parenchymal disease, of which postnecrotic cirrhosis was the most common example, was the principal indication for liver transplantation (Table 1). Other broad diagnostic categories were cholestatic disease, such as primary biliary cirrhosis, miscellaneous disease including hepatic malignancies, and failure of a previous graft necessitating retransplantation (Table 1). The workup of the recipients included a complete set of standard liver function tests. However, the heterogeneity of native diseases as well as the variability of liver function within specific diseases precluded the use of these results to classify disease severity.[7] Instead, the need for transplantation and, therefore, the degree of illness of each patient was defined prospectively by the criteria for urgency used for the United Network for Organ Sharing (UNOS) distribution system.[8]

1. Working, in school
2. Confined to home, self-care
3. Home, requiring professional care
4. Hospital bound, not in intensive care unit (ICU)
5. ICU bound, not ventilator dependent
6. In ICU, on ventilator, often unconscious

The Transplant Operations

All donor procurements were with previously described in situ techniques.[9,10] The livers were preserved for 14.1 ± 0.7 (SE) hours (range 3 to 32) with University of Wisconsin (UW) solution.[11-13] Donor lymphoid tissue was obtained for standard HLA typing and for the performance of a standard cytotoxic antibody crossmatch with recipient serum. The results of these studies did not influence case selection, since they were not known until after the transplantation.

The transplantations were performed with techniques that could be varied according to the needs of the individual recipients.[14,15] The principles were removal of the diseased native liver and placement of the graft in as anatomically normal a way as possible (Fig 1). During the total hepatectomy and sewing in of the new organ, a motor-driven venovenous bypass without heparin[16-18] was used in all but 3 transplantations to prevent venous hypertension of the occluded inferior vena caval and splanchnic venous beds. Even with this technique, it is necessary to accept 15-30 minutes of portal occlusion time during the performance of the portal venous anastomosis.

The anhepatic phase was counted from the time when the recipient hepatic circulation was interrupted to the time when portal or arterial circulation was restored to the graft. Throughout the operation, particularly during the anhepatic phase, serum lactate, blood glucose, and blood gases were measured at frequent intervals. Coagulation was monitored with thromboelastography and supplementary measurement of platelet counts, prothrombin times, and other parameters.[19]

Biliary tract reconstruction was with choledochocholedochostomy or choledochojejunostomy to a Roux limb (Fig 1). At the conclusion of the operation, the total blood product administration was recorded.

Immunosuppression and Rejection

All patients were treated with CyA and prednisone to which azathioprine (AZA) was added variably if the white blood cell count was above 5000/mm^3. Rejection was treated with 1 g boluses of steroids and an increased maintenance dose of prednisone. OKT3 MAb was given when steroid-resistant rejection was diagnosed, when poor renal function prevented the administration of adequate CyA doses, or when accelerated rejection was suspected to be the cause of primary graft nonfunction. In several patients whose grafts failed to function, one or more treatments with plasmapheresis were given with the hope that antibodies or some other undetected harmful substance could be removed thereby.

Because the causes of primary graft nonfunction or early patient death are not easy to determine,[1,20] 18 grafts that were lost within 7 days by death or retransplantation were kept in a separate category

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Table 1. Indications for the Transplantation of 90 Liver Grafts

Category	Diagnosis	Number of Grafts	No. of Grafts Failed Within First Month
Parenchymal liver disease	Cirrhosis, idiopathic	33	(9)
	Cirrhosis, HBsAg+	2	(0)
	Cirrhosis, auto-immune	5	(1)
Cholestatic liver disease	Primary biliary cirrhosis	12	(2)
	Sclerosing cholangitis	4	(0)
	Caroli's disease	1	(0)
Miscellaneous	Fulminant hepatitis	2	(1)
	Malignant tumor	7	(0)
	Budd-Chiari syndrome	2	(0)
Graft failure[a]	1. Rejection 18 days-2 years	7	(2)
	2. Hepatitis 36-142 days	2	(1)
	3. Recurrent tumor 19 months	1	(0)
	4. Undetermined 1-11 days	12	(8)
Total		90	(24)

[a] 1. 221 ± 89.1 (SE) days
2. 36 and 142 days
3. 19 months
4. 4.5 ± 1.0 (SE) days

in which the role of an immune event was considered unknown. With the 72 livers that survived for more than 7 days, rejection was categorized as (1) no clinical or histologic evidence of rejection ($n = 19$), (2) clinical or histologic evidence of rejection that required increased steroids ($n = 28$), (3) evidence of rejection that required increased steroids plus OKT3 treatment within the 1 month period of the study ($n = 25$). In group 3, a histologic diagnosis was made in 16 recipients, but in 9, OKT3 was given blindly, based on the clinical findings of rejection that was so severe that coagulation defects made biopsy unsafe.

Special Infectious Disease Studies

Culture Data. Aerobic and anaerobic bacteriologic, fungal, and viral cultures were obtained preoperatively in all of the patients. Six patients went to the operating room infected. In 3, OL transplant was performed in the presence of a positive blood culture, and in 3 more, either pneumonia or peritonitis was present with a positive sputum or peritoneal fluid culture. Five of these 6 infected patients were undergoing retransplantation for a failed graft; the sixth had fulminant hepatic failure. All 6 patients were treated with appropriate antibiotic therapy preoperatively and intraoperatively.

Endotoxin Measurements. Platelet-poor blood samples of peripheral venous blood were obtained with a sterile technique with venipuncture or through an indwelling catheter and were stored at −80°C after 10 minutes centrifugation at 3000 rpm. The samples were obtained immediately preoperatively (stage 1), at the end of the anhepatic phase just before restoring graft circulation (stage 2), 1 day postoperatively (stage 3), 3 days postoperatively(stage 4), and 7 days postoperatively or the day closest to 7 in the event of graft loss (stage 5). For endotoxin assay,[21,22] 0.32 mol/l perchloric acid, 0.18 N NaOH, and Toxicolor* with pH 8.0 Tris-HCl buffer was used for the chromogenic endotoxin assay with limulus coagulation enzymes. The standard curve was plotted using *Escherichia coli* 0111:B4 endotoxin, Westphal type (Difco Laboratories Inc., Detroit MI), in distilled water.

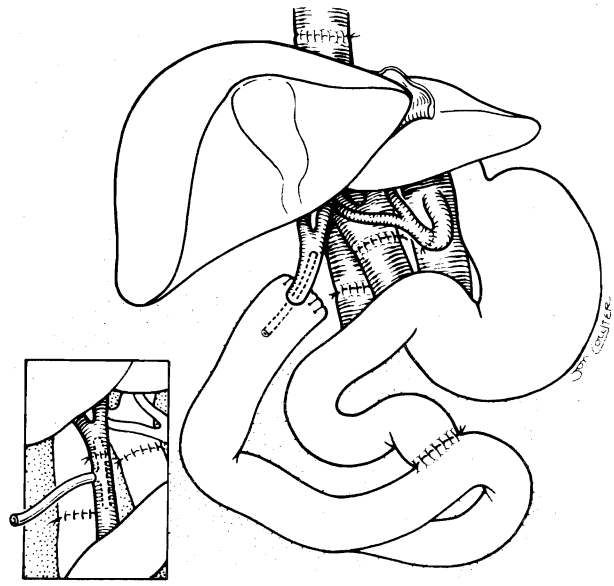

Fig 1. The standard technique of orthotopic liver transplantation, showing the two methods of biliary tract reconstruction.

Statistical Analyses

Survival for any graft was the time from transplantation to patient death or to graft replacement, if this was done. Maximum credit for any graft survival was 31 days.

Cumulative graft survival was obtained with univariate analysis,[23] and the Cox proportional hazards model was used for multivariate analysis in order to examine the influence of various perioperative factors on graft survival. BMDP Statistical Software package was employed (University of California Los Angeles, California).[24] To examine the relationship between endotoxin and total bilirubin, GOT, GPT, albumin, lactate, amount of blood transfusion, duration of cold ischemic time, and duration of anhepatic phase, Pearson correlation coefficients were calculated. *t*-Test was used to determine whether the mean values of endotoxin differed between platelet transfusion, urgency status, preoperative infection, and lymphocytotoxic crossmatch. Statistical values were considered significant when p value was less than 0.05. Analyses were performed with all 90 transplantations initially and then also with only the 68 primary transplantations.

*Lot No. 310051, a commercially available reagent consisting of lyophilized amebocyte lysate from *Tachypleus tridentatus* and the synthetic chromogenic substrate Boc-Leu-Gly-Arg-p-nitroanilide, Seikagaku Kogyo, Ltd., Tokyo, Japan.

RESULTS

Graft Survival

Of the 90 grafts, 66 (73.3%) survived to the end of the first month. Calculated with a ceiling of 31 days, the mean graft survival was 26.3 ± 1.3 (SE) days. The 1-month patient survival was 86.4% (70/81). Of the 24 graft losses, only 11 were coincident with death of the recipient.

The 24 grafts that failed were designated group A, and the other 66 were called group B. The mean ages of the recipients in group A and group B were 45 and 42 years, respectively ($p > 0.05$), and the male representation was 58.3 and 73.7% ($p > 0.05$).

Conditions Predisposing to Graft Death

There was a 25% incidence of graft death (10 of 40) after primary transplantation in patients with parenchymal hepatic disease. This was higher than with the other native disease categories (Table 1). However, the greatest risk of graft death by far was in patients undergoing retransplantation after failure of a first graft, especially if this was soon after the primary operation. Of the 24 graft failures, 11 were after retransplantation; the failure rate with this indication for operation was 50% (11/22) (Table 1).

One factor contributing to the low success rate with retransplantation was the so-called nonabandonment policy of transplanting a third graft if a second one failed. Thus, the 22 retransplantations in Table 1 were performed in only 18 patients. Of the 4 patients who had consecutive retransplantations and who, therefore, used a total of 8 livers after failure of their primary grafts, only 1 lived for as long as 31 days, and he eventually died after 55 days.

In 12 of the 22 retransplantations in Table 1, there was no obvious explanation for why the previous attempt 4.5 ± 1.0 (SE) days earlier (range 1 to 11) had failed. In 8 of these 12 retransplantations, the effort was unsuccessful, a discouraging record comparable to that reported earlier from our center under similar circumstances.[25] Retransplantation was more successful when the reason for graft failure was known, even if this was rejection (Table 1). One reason probably was the later timing of the retransplantations for rejection, hepatitis, and recurrent tumor. These were 220.8 ± 89.1 (SE) days (range 18 to 730) after the primary grafting (Table 1).

Timing and Findings with Graft Death

Graft death occurred 1-26 days postoperatively after a mean time of 8.5 ± 1.5 (SE) days. Eighteen of the 24 grafts became available for pathologic examination, either at retransplantation or at autopsy. In the other 6 cases in which autopsy was denied, a final graft diagnosis was reached from the clinical events, premortem hepatic biopsies, and infectious disease data.

The majority of the failed grafts had necrosis or severe ischemic injury (Table 2). The clearest examples were in the 9 grafts that had such complete nonfunction that retransplantation or death followed an average of 2.5 days later. Ischemic injury with the supervention of sepsis led more slowly to the same result after an average of 9.5 days in 4 other grafts (Table 2). Sepsis was thought to have caused delayed hepatic failure in 4 additional patients. Humoral or cellular rejection was the primary diagnosis in 3 patients who became septic secondarily (Table 2). Technical causes of death included disruption of a biliary anastomosis and shock, rupture of a splenic artery aneurysm, and portal vein thrombosis (Table 2).

Table 2. Timing and Findings With 24 Graft Deaths

Diagnosis	No. of Grafts	Average Survival (days)
Graft necrosis (primary nonfunction)	9	2.5
Ischemia plus sepsis	4	9.5
Sepsis (plus secondary liver failure)	4	10.8
Rejection (plus sepsis)	2	7
Humoral rejection, A to O (plus sepsis)	1	6
Cytomegalovirus hepatitis	1	25
Bile duct disruption	1	10
Intraperitoneal bleeding	1	7
Portal vein thrombosis	1	1
Total	24	

Six patients underwent transplantation while infected. Three had positive blood cultures, 2 had culture-proven bacterial peritonitis, and 1 had pneumonia. The organisms cultured were *Enterobacter cloacae, Enterobacter aerogenes, Acinetobacter anitratus, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae,* and *Candida albicans.* Multiple organisms were found in 3 patients. Five of 6 grafts transplanted into the infected environment failed after

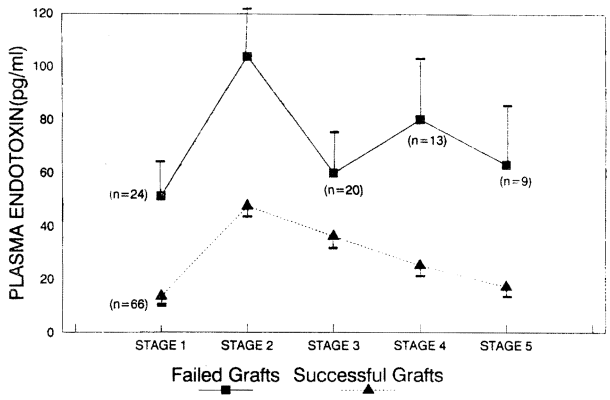

Fig 2. Plasma endotoxin levels in transplantations that failed vs those that succeeded. Data were from 90 transplantations (68 primary and 22 retransplantations). Stage 1, preoperative. Stage 2, end of anhepatic phase. Stage 3, 1 day postoperative. Stage 4, 3 days postoperative. Stage 5, 7 days postoperative or day of sampling closest to 7 days. Mean ± SE.

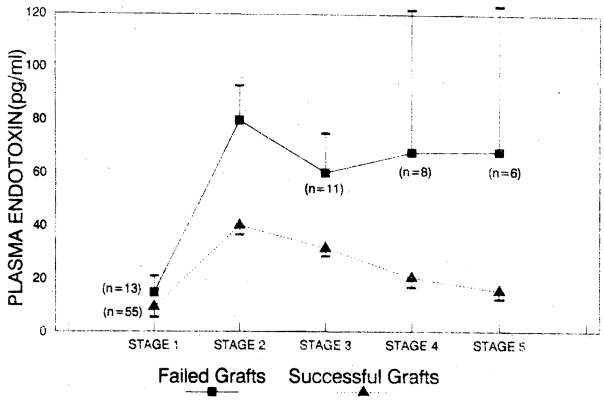

Fig 3. Same study as in Fig 2 but for only 68 primary transplantations. Stages as in Fig 2. Mean ± SE.

2-20 days, and the sixth failed 2.5 months later. All patients died. Five of these 6 patients were undergoing emergency retransplantation, and the other had fulminant hepatic failure.

Endotoxemia

Controls. With the method used in this study, endotoxin was not detectable in the plasma of 24 normal volunteers (<10 pg/ml). Plasma samples from 6 cadaveric liver donors were analyzed. The samples contained 16.7, 1.4, 1.7, 1.4, 0.1, and 19.5 pg/ml endotoxin. Thus, 2 of the donors had abnormally high endotoxin levels.

Failed (group A) vs successful (group B) grafts. Endotoxin concentrations were abnormally elevated in both groups A and B. However, the 24 patients in group A had considerably higher preoperative endotoxin levels than the patients in group B, and this differential was maintained throughout all stages of sampling. The highest values in both groups were at the end of the anhepatic phase (Fig 2). When the retransplantations were removed from both groups and only primary transplantations were considered (Fig 3), the relative endotoxemia in group A was still evident.

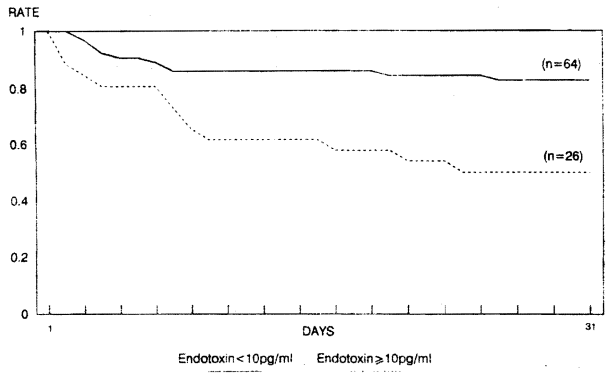

Fig 4. Effect of preoperative endotoxin on outcome in 90 transplantations (68 primary and 22 retransplantation).

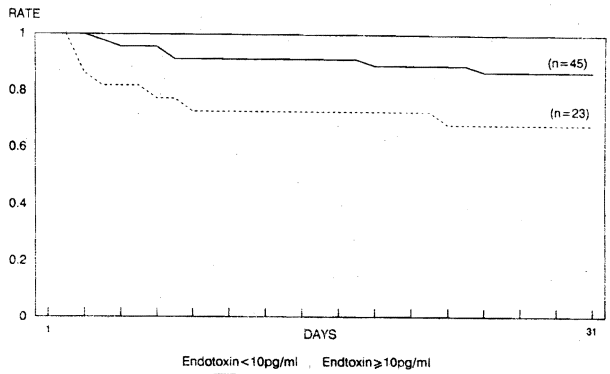

Fig 5. Same study as in Fig 4 but for only 68 primary transplantations.

Endotoxemia vs mortality. When the preoperative plasma endotoxin concentrations were ≥10 pg/ml, graft survival was significantly reduced (Fig 4). Because the inclusion of 22 retransplantations could have distorted the results, a separate analysis was conducted of the 68 primary transplantations (Fig 5). The influence of preexisting endotoxemia was equally evident in this culled group.

The adverse implications on graft survival of elevated plasma endotoxin at the end of the anhepatic period (Figs 6, 7) or on day 1 (Figs 8, 9) also were apparent with the total 90 transplantations (Figs 6, 8) or with the subgroup of 68 primary grafts (Figs 7, 9).

Endotoxemia vs rejection. There were 72 transplantations in which graft survival for at least a week allowed rejection to be assessed. Although there was a trend toward greater endotoxemia in patients who ultimately developed severe enough rejection to require OKT3, this was significant ($p < 0.05$) compared to patients with mild or no rejection only on postoperative day 3 (Fig 10).

Endotoxemia and primary graft nonfunction. The 9 grafts with immediate hemorrhagic necrosis (Table 2) were classified as having primary nonfunction. Positive cytotoxic crossmatches with their donors were demonstrated with the sera of only 2 of the 9 recipients (Table 3). Patient 4 died

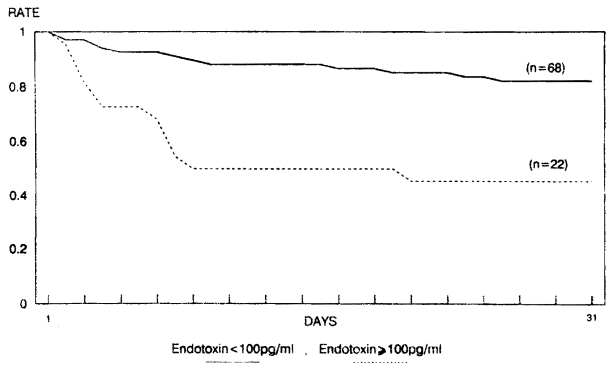

Fig 6. Correlation of endotoxin level at the end of the anhepatic phase with graft survival for all 90 transplantations.

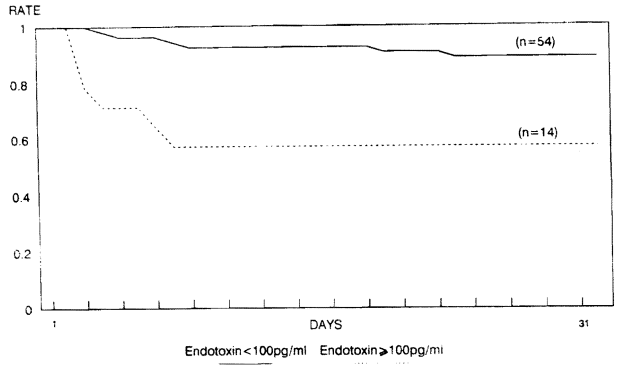

Fig 7. Same study as in Fig 6 but for only 68 primary transplantations.

before another liver could be found. Five other patients died within a month in spite of retransplantation, usually with similar nonfunction of the next liver. Only 3 of the 9 patients survived (Cases 1, 2, and 8).

Although none of these 9 patients was thought to be infected preoperatively, the plasma endotoxin rose significantly between the beginning of the case and the end of the anhepatic phase. By this time (Table 3), the plasma endotoxin was between 101 and 295 pg/ml in 7 of the 9 recipients (Table 3).

Other associations with graft death and endotoxemia. Eight other factors associated with graft loss by Kaplan-Meier univariate analysis are listed in Table 4. In Table 5 are summarized correlation analysis or *t*-test, showing that total bilirubin, GOT, GPT, creatinine, lactate, amount of blood transfusion, urgency status, cold ischemic time, and preoperative infection were associated with endotoxemia before, during, or after the transplantation.

With the Cox proportional hazards model, the most powerful independent factors associated with graft death were endotoxemia ≥100 pg/ml at the end of the anhepatic period, lactate level greater than 10 mmol/l at the same time, and SGPT ≥200 IU/l preoperatively (Table 6).

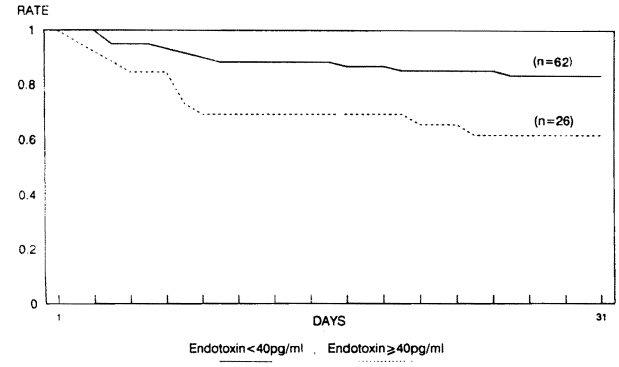

Fig 8. Correlation of endotoxin level 1 day postoperatively on graft survival for all 90 transplantations. (Two of 90 grafts failed before reaching this stage.)

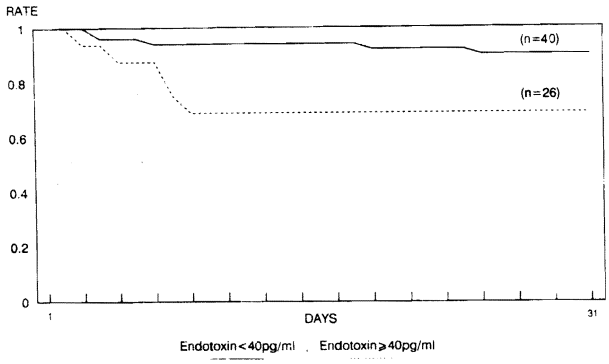

Fig 9. Same study as in Fig 8 but only for the 68 primary transplantations. (Two of 68 grafts failed before reaching this stage.)

DISCUSSION

Endotoxin is a macromolecule of which the most specific and active component is lipid A.[26] However, it has been recognized increasingly that protein and polysaccharide components of the molecule can influence its potency and specificity.[27,28] Because endotoxin is found in the wall of gram-negative bacteria that are indigenous to the gastrointestinal tract, an enteric problem must be suspected when symptomatic endotoxemia is diagnosed.[29]

Almost no information exists about endotoxemia in hepatic transplantation despite the fact that changes might be predicted. Intravenous endotoxin is removed mainly by the Kuppfer cells of the liver.[30,31] Not only is this detoxification system absent during the anhepatic phase of transplantation, but there is a subsequent transformation in the graft whereby donor Kuppfer cells are replaced with macrophages of recipient origin.[32,33] Finally, the operation exposes the liver to intestinal bacteria that reach the liver in splanchnic blood

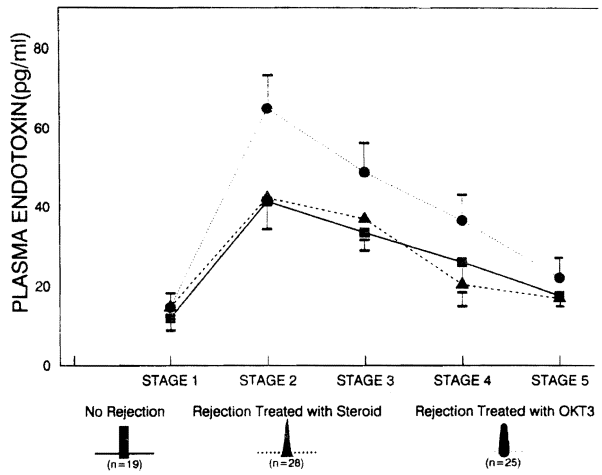

Fig 10. Endotoxin levels in 72 patients in whom rejection could be assessed. Group A, no rejection. Group B, mild or moderate rejection. Group C, severe rejection requiring OKT3 therapy. Stages are the same as in Fig 2. Mean ± SE.

Table 3. Primary Nonfunction of 9 Grafts

Case	No of Previous Grafts	Endotoxin Concentration (pg/ml) Preop	Anhepatic	PO[a] day 1	PO day 3	PO day 7	Preop Status	Crossmatch	Graft Survival (Days)
1	0	17.7	111.2	26.0	—	—	4	–	3
2	0	0	15.7	42.5	7.8	—	3	–	3
3	0	8.9	199.3	74.4	73.3	—	4	–	3
4	0	13.3	101.1	—	—	—	4	+	2
5	1	18.7	153.3	34.7	—	—	5	–	3
6	1	40.1	295.0	60.0	—	—	6	+	2
7	0	41.8	137.2	67.6	—	—	5	–	2
8	0	47.6	13.4	60.6	12.1	12.5	4	–	7
9	0	10.8	135.2	—	—	—	5	–	1

[a]PO, postoperative.

and through the biliary tract and then leak through to the system circulation.[34,35] Thus, it was not surprising in healthy dogs to always find rises in the plasma endotoxin during and after liver replacement.[6]

What this wave of endotoxemia means is an unresolved matter. Although there is evidence that small quantities of endotoxin can cause serious or lethal syndromes in animals and humans,[5,36] a cause and effect relationship may be difficult to establish in specific situations.[37] One reason is that the presence of endotoxin, even in large amounts, may not necessarily be associated with symptoms.[38] Another reason is that the responses elicited by endotoxin are not specific or

Table 4. Various Factors Affecting Graft Survival

Factor	Category	Graft No. (Graft Death)	Mean Survival Time (Days) (Mean ± SE)	Statistics
Total bilirubin (mg/dl)	<5	38 (7)	28.1 ± 1.9	NS[a]
	≥5, <10	15 (3)	26.9 ± 3.2	
	≥10	37 (14)	23.2 ± 2.1	
GOT	<80	36 (4)	30.1 ± 1.6	$p < 0.001$
(IU/l)	≥80, <250	31 (7)	26.8 ± 2.0	
	≥250	23 (13)	18.1 ± 2.9	
GPT	<70	38 (4)	29.5 ± 1.7	$p < 0.001$
(IU/l)	≥70, <200	31 (7)	26.9 ± 2.0	
	≥200	21 (13)	17.9 ± 3.1	
Albumin (g/dl)	<3.0	48 (10)	27.2 ± 1.5	NS
	≥3.0	42 (14)	24.3 ± 2.1	
Lactate (preoperative)	<3	71 (13)	28.3 ± 1.3	$p < 0.001$
(mmol/l)	≥3, <6	9 (4)	23.4 ± 4.7	
	≥6	10 (7)	13.4 ± 4.2	
Lactate (intraoperative)	<5	38 (4)	30.3 ± 1.5	$p < 0.0001$
(mmol/l)	≥5, <10	30 (7)	26.9 ± 2.0	
	≥10	22 (13)	16.9 ± 3.0	
Blood transfusions	<10	44 (8)	28.2 ± 1.4	$p < 0.05$
(units)	≥10	46 (16)	23.7 ± 2.0	
Platelet transfusion	none	57 (18)	25.3 ± 1.7	NS
	yes	33 (6)	27.2 ± 2.0	
Urgency status	2-5	68 (15)	28.5 ± 1.4	$p < 0.05$
(preoperative)	6	22 (9)	20.9 ± 2.9	
Cold ischemic time	<12	51 (9)	28.1 ± 1.3	$p < 0.05$
(hours)	≥12	39 (15)	22.8 ± 2.2	
Anhepatic duration	<2	62 (18)	27.2 ± 2.0	NS
(hours)	≥2	28 (6)	25.5 ± 1.6	
Lymphocytotoxic	Positive	16 (5)	24.1 ± 3.4	NS
crossmatch	Negative	74 (19)	26.6 ± 1.4	
Infection	No	84 (19)	27.2 ± 1.3	$p < 0.01$
	Yes	6 (5)	13.5 ± 4.8	

[a]NS, not significant.

Table 5. Correlation Between Various Clinical Parameters and Endotoxin Level at Different Stages

Clinical Parameters	Endotoxin Stage 1 (preop)	Endotoxin Stage 2 (anhepatic)	Endotoxin Stage 3 (postop Day 1)
Total bilirubin	+[a]	+	
GOT	+	−	
GPT	+	−	
Albumin	−	−	
Creatinine	+	+	
Preoperative lactate	+	−	
Intraoperative lactate		+	+
Amount of blood transfusion		+	−
Amount of platelet transfusion		−	−
Urgency status	+	−	
Duration of cold ischemic time			−
Duration of anhepatic phase		−	+
Preoperative infection	+	−	
Lymphocytotoxic crossmatch			−

[a] +, statistically significant $p < 0.05$; −, not significant.

unique.[28,39] Endotoxin can induce the release of a complete spectrum of biologically active substances, including soluble mediators of the inflammatory response and cytokines (Table 7). Activation of the individual mediators, including the cytokines, is induced by a direct effect of the endotoxin on complement, macrophages, monocytes, and other formed blood elements, including lymphocytes and endothelial cells (Table 7).

The soluble mediators that can be released into the circulation or locally as a consequence theoretically could have devastating physiologic effects (Table 7), including fever, shock, vasodilatation, vasoconstriction, coagulation disorders, smooth muscle contraction, endothelial injury, chemotaxis, tissue necrosis, and even neuropsychiatric changes. In addition, the majority of the mediators have immunoregulatory functions, predominantly augmentating either cellular or humoral immunoreactivity or both (Table 7). This latter feature of the soluble mediators may be particularly important in the context of transplantation. However, what results from exposure to endotoxin could be a combination of the effects of many or even all of the mediators. The difficulty of interpretation is compounded by the fact that many factors other than endotoxin can activate the mediators and by the variable functional interactions between the mediators themselves.[28,40]

In our patients, high levels of endotoxin at any time, but particularly before operation and at the end of the anhepatic phase, had more serious prognostic implications than any other factor except lactate accumulation. However, it was difficult to distinguish cause from effect. Were the high levels of endotoxin merely a reflection of the terminally ill state of the patient or the transplantation of a suboptimally performing graft? Or was the preexisting or secondarily appearing endotoxin responsible for the failure of multiple organ systems, including the new liver?

Table 6. Cox Proportional Hazards Model Analysis Showing Factors That Correlate Most Strongly with Postoperative Graft Death

Factor	*p* Value	Relative Risk
Endotoxin ≥ 100 pg/ml at stage 2	0.0001	2.3
GPT ≥ 200 IU/l at stage 1	0.0056	2.9
Lactate ≥ 10 mmol/l at stage 2	0.0002	2.5

The observations most clearly suggesting a cause and effect relationship were in the 9 patients who had primary nonfunction of their grafts. In these patients, most of the endotoxin levels were moderately elevated preoperatively. However, large further increases occurred in the plasma by the time the new livers were revascularized in 7 of the 9 patients. The livers acted as if they had been revascularized in a hostile environment. Only 2 of the 9 patients had positive cytotoxic crossmatches with their donors, but all 9 of the livers behaved as if hyperacute rejection had occurred.

The possibility was discussed two decades ago that endotoxin might be able to destroy kidney grafts in a way analogous to the hyperacute rejection caused by cytotoxic antigraft antibodies.[41] At that time, little was known about soluble mediators and cytokines. Now, it is easy to conceive that these substances, of which many are immunoregulatory (Table 7), could participate in an endotoxin-initiated injury, a humoral immune reaction, or a combination of these. The patient with high endotoxin potential during the anhepatic phase could be the liver eater familiar to liver transplant surgeons, who destroys successive grafts even though crossmatches with the donors are negative.[42]

If endotoxemia can be shown to be a negative factor in the transplantation of the liver or other organs, therapeutic strategies might be devised to prevent this complication. Possibilities could include the use of anti-endotoxin MAbs[43] or, less specifically, the control of the gram-negative intestinal flora with antibiotics, as described by Weisner et al.[44] Polymyxin B is an antibiotic with a strong anti-endotoxin activity.[45]

This study was concerned primarily with recipient endotoxin. However, endotoxin also could adversely affect the liver and other organs of brain dead donors, particularly if these are victims of severe trauma.[5] It was of interest that 2 of 6 cadaveric donors of livers not used in this study had plasma endotoxin levels in the 10-20 pg/ml range. More investigations on this matter are under way.

SUMMARY

Ninety liver transplantations were performed in 81 patients. Plasma endotoxin was measured preoperatively, at the end of the anhepatic phase, and on postoperative days 1, 3, and 7. The presence of high endotoxin levels preoperatively and at the end of the anhepatic period was associated with graft failure and a high mortality. Patients with primary nonfunction of their transplants typically had severe endotoxemia. Endotoxemia could be a cause rather than an effect of perioperative complications and graft loss.

Table 7. Soluble Mediators (Including Cytokines) That Are Activated by Endotoxin

	Description of Mediator	How Endotoxin Initiates Mediator Production	Physiologic Consequences
Anaphylatoxins C3a and C5a	Cleavage products of C3 and C5 complement	Activates serum complement (classic and alternative pathways)	Vasodilatation, smooth muscle contraction, mononuclear cell and neutrophil chemotaxis, immunomodulation of humoral response
Prostaglandins	Cyclooxygenase pathway from arachidonic acid	Activates macrophages and monocytes	Vasodilatation, activate or collaborate with other mediators, modulate macrophage effect on function
Leukotrienes	Lipoxygenase pathway from arachidonic acid	Activates macrophages and monocytes	Vasoconstriction, activate or collaborate with other mediators, modulate macrophage effect on function
Platelet activating factor (PAF)	Lipid mediators derived from platelets, neutrophils, basophils, mononuclear phagocytes, endothelial cells	Binds to platelets, neutrophils, etc with mediator release	Platelet aggregation, neutrophil degranulation, smooth muscle contraction, increased vascular permeability, hypotension, tissue necrosis, modulate endothelial cell function
Tissue factor (TF)	Glycoprotein from monocyte or macrophage cell surfaces	Activates factor XII (intrinsic coagulation pathway), stimulates mononuclear cells (extrinsic coagulation pathway)	Microvascular thrombosis
Interleukin I (IL-1)	Family of immunoregulator cytokines	Stimulates mononuclear phagocytes and other cells	Fever, lymphocyte activation, coagulation, increases endothelial cell adhesiveness, enhancement of T and B cell immunity, secondarily activate PAF, arachidonic acid products, etc
Tumor necrosis factor (cachectin)	Product of activated macrophages	Activates macrophages production	Fever, induces IL-1 from mononuclear and endothelial cells, cytotoxic to tumor cells, amplifies microvascular coagulation
Colony-stimulating factor	Heterogeneous glycoproteins from macrophages and B lymphocytes	Induces production by macrophages and B lymphocytes	Stimulates proliferation and differentiation from marrow-derived precursor cells, activates mature macrophages to produce other mediators
Gamma-interferon	Lymphokine from activated T lymphocytes	Complex pathway[a] (see ref 40)	Increases antibacterial and antitumor activity of macrophages, increases expression of Fc receptors, augments other immune responses, amplifies endotoxin effects (?vicious cycle)
Endorphins	Endogenous opioids	Unknown, could stimulate mononuclear cells	Hypotension, analgesia, behavior changes, immune regulation (enhancing and suppressing)

[a]Alpha and beta interferon are induced by endotoxin directly from B lymphocytes and macrophages.
From Morrison, Ryan.[28]

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