Etiology of Liver Disease in Renal-Transplant Patients

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The etiology of 72 episodes of liver disease that developed in 62 of 162 renal-transplant recipients was evaluated. Infection with hepatitis B virus was a minor problem, and none of our patients had evidence of infection with hepatitis A. Cytomegalovirus infection was ubiquitous in the population and probably accounted for many episodes of acute liver disease. This agent's role in causing chronic hepatitis is less secure. Infections with other viruses including Epstein-Barr virus, adenovirus, and the herpes viruses were only rarely associated with hepatic disease. Azathioprine was responsible for some episodes of acute cholestasis but could not be incriminated as a direct cause of chronic disease. A cause could be identified for the majority of episodes of acute hepatic dysfunction, but the cause of most of the chronic hepatitis remains undetermined. It is likely that infection with non-A, non-B hepatitis virus accounts for much of this serious, often fatal, complication of renal transplantation.

THE IMPORTANCE OF LIVER DISEASE and liver failure to the long-term prognosis of patients who are renal-transplant recipients has been recognized with increasing frequency during the past few years (1-3). We have been concerned by both the frequency and the seriousness of the liver disease occurring in our patients who have received renal grafts (4). We have sought, therefore, to define the role played by a number of viruses as well as various drugs and other agents in the development of liver disease during the period after transplantation. Many of these agents have been incriminated as causing liver disease in particular patients with this clinical background, but no comprehensive evaluation of their relative contribution to this serious problem has been presented to date.

Materials and Methods

PATIENTS

From January 1970 through June 1976, 217 patients received 223 renal transplants at Parkland Memorial Hospital. In 61 instances the patient either died or required transplant nephrectomy within 3 months of the procedure. These patients have been excluded from analysis, because their clinical course was either too short or too complicated to allow for adequate assessment of any hepatic disturbance that may have been present. Thus 162 patients were "at risk" for the development of liver disease in the post-transplant period. These patients have been observed until death or for a minimum period of 6 months (mean, 33 months). Some have been followed for as long as 7 years. At every visit to the clinic each patient was assessed for evidence of hepatic dysfunction both clinically and by means of a screening battery of laboratory tests, which included total serum bilirubin, serum albumin, and the serum activities of aspartate aminotransferase (serum glutamic-oxalacetic transaminase [SGOT]) and alkaline phosphatase (SMA-12/60, Technicon Instruments, Inc., Tarrytown, New York). At intervals that varied from 1 to 6 months sera were drawn for the detection of hepatitis B surface antigen (HBsAg) or antibody to cytomegalovirus. These sera were then stored at −20 °C and were used subsequently for the other serologic studies herein reported. Liver biopsies and other laboratory tests were done as dictated by the patients' clinical circumstances.

Liver disease has been defined as the occurrence of two consecutive SGOT measurements drawn at least 1 week apart, which were elevated above the upper limits of the normal range for the method used and which were confirmed, at least once, by a spectrophotometric method. This requirement was adopted to exclude SGOT elevations produced by artefacts of the colorometric method used by the SMA-12 autoanalyser (5). An episode of liver dysfunction was termed "acute" if the results of liver tests returned entirely to normal in less than 6 months or the patient died within 3 months of its onset. The liver disease was considered "chronic" if the patient manifested persistently abnormal liver test results for longer than 6 months or died after at least 3 months of unremitting severe disease.

SEROLOGY

Initial tests were done on sera drawn just prior to transplantation or, when such sera were not available (22 instances), on sera obtained soon after transplantation and before the onset of any liver dysfunction. The various assays were repeated on sera drawn at least 6 months after the onset of liver disease. Patients who remained free of any hepatic disturbance were re-assessed using sera drawn at least 6 months and usually more than 12 months after transplant. When seroconversion was found from the "early" to the "late" sera a more precise point of seroconversion was ascertained by repeating the assay on all of the available interval samples.

Sera from all of the patients with liver disease were not available for all of the assays. The pretransplant sera from some patients was exhausted or lost before all of the tests could be done. This has caused a variation in the number of patients who have been tested in the different assay systems.

Hepatitis B surface antigen was assayed initially by counterimmunoelectrophoresis (6), but since 1973 these tests have been done routinely by passive hemagglutination (Auscell, Abbott Laboratories, North Chicago, Illinois). All patients have had at least two sera tested by radioimmunoassay (Ausria II, Abbott Laboratories).

Antibody to hepatitis B surface antigen (anti-HBs) was assayed by radioimmunoassay (Ausab, Abbott Laboratories). Antibody to hepatitis B core antigen (anti-HBc) was assayed by a radioimmunoassay technique that has been described in a prior publication (7).

Antibody to hepatitis A virus (anti-HA) was assayed using a radioimmunoassay technique the details of which have been published previously (8, 9).

Antibody to cytomegalovirus was assayed by microtiter complement-fixation (United States Public Health Service [USPHS] Laboratory Branch complement fixation method [10]) using the AD-169 strain (Flow Laboratories, Rockville, Maryland) as antigen. A fourfold ($\log_2$) or greater rise in titer of the antibody was accepted as evidence of recent infection.

An indirect immunofluorescent antibody test was used to detect antibody to the Epstein-Barr virus (11). The P3J cells containing viral capsid antigen (PIR cells without antigen served as controls) were reacted with serial dilutions of the serum sam-

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ples. The cells were then stained with goat antihuman gamma globulin conjugated with fluorescein and examined under an ultraviolet microscope for characteristic fluorescence. Antibody was considered to be present if fluorescence was evoked by serum dilutions of 1:20 or more. Seroconversion was defined as a four-fold or greater rise in antibody titer. The cell preparations were obtained from Associated Biomedic Systems, Inc., Buffalo, New York. The fluoresceinated antihuman gamma globulin conjugate was obtained from Meloy Laboratories, Springfield, Virginia.

A group-specific adenovirus complement-fixation test was done by the microtiter method (10) using antigen obtained from commercial sources (Flow Laboratories).

VIRAL CULTURES

Urine samples (often multiple) were obtained from the majority of these patients during their course and cultured for cytomegalovirus. A sample of all liver-biopsy specimens obtained from those patients with liver disease and many specimens from various organs obtained at autopsy were also cultured for this virus. Cultures were established by inoculating a monolayer of human-embryonic lung cells with the test material and examining the monolayer during the ensuing month for evidence of the characteristic cytopathic effect.

All of the other diagnostic aids used in these patients were done by standard methods in the clinical laboratories of Parkland Memorial Hospital.

ATTRIBUTION OF CAUSE

An episode of liver disease was considered to be the consequence of hepatitis B virus infection if the onset of the liver disease coincided with the appearance of HBsAg in the patient's serum or if the initial manifestations were followed within 2 months by the development of circulating anti-HBc either alone or in company with anti-HBs. Infection with hepatitis A virus, Epstein-Barr virus, or adenovirus was accepted as the cause of the episode if the onset of liver dysfunction was followed within 2 months by seroconversion for the corresponding antibodies. Cytomegalovirus infection was incriminated if seroconversion to cytomegalovirus occurred within 1 month of the onset of the liver disease and if there was no other reasonable etiologic explanation apparent. The diagnosis was strengthened by the occurrence of a typical febrile illness, the appearance, by culture, of cytomegalovirus in urine or especially in the homogenate of a liver biopsy, or the finding of characteristic intranuclear inclusions on microscopic examination of such a biopsy.

The diagnosis of a drug-related disease required the temporal concurrence of the hepatic dysfunction with the initiation of drug therapy (or an increment in dosage) or the resolution of the episode with interruption (or a decrease in the dosage) of the agent under suspicion. Rechallenge trials were not undertaken unless the drug concerned was considered essential for the patients' management. A drug-related cause was only accepted in the absence of any reasonable alternative cause.

STATISTICAL ANALYSIS

Student's *t* test and chi square analysis were used where appropriate.

Results

Table 1 outlines the scope of the problem posed by liver disease in this population of renal-transplant recipients. Sixty-two (38%) of the 162 patients "at risk" showed evidence of hepatic dysfunction in the post-transplant period. Twenty-four patients suffered an acute episode, whereas 38 developed a chronic form of liver disease. The distinction between progressive and nonprogressive chronic liver disease was based, in most instances, on the histologic examination of serial liver tissues obtained by percutaneous biopsy or at autopsy. We used the same histologic criteria as those reported by Baggenstoss and associates (12). Fourteen of the 16 patients with progressive disease had from two to four histologic assessments. The other two patients were not subject to biopsy during life but were shown to have cirrhosis at autopsy. Eleven of this group of patients ultimately developed cirrhosis, whereas four showed progression from chronic persistent hepatitis to chronic active hepatitis with or without bridging lesions. One patient died of liver failure after 12 months of liver disease, and at autopsy her liver was massively infiltrated with fat.

Table 1. Liver Disease in Transplant Recipients at Parkland Memorial Hospital 1970-1976

Case Divisions	no.
Patients "at risk" for liver disease*	162
Patients with liver disease	62
Episodes of liver disease	72
Acute episodes	34†
Acute reversible episodes	22
Acute fulminant episodes	2
"Second" acute episodes	10
Chronic disease	38
Chronic progressive disease	16
Chronic nonprogressive disease	16
Chronic disease—indeterminant	6

* Patients "at risk" were patients who survived for 3 months after transplant with an intact graft.

† These episodes occurred in 29 patients. Five patients had two episodes of acute liver disease. Five patients with chronic hepatitis also suffered a "second" separate acute episode of liver dysfunction.

Fourteen of the 16 patients with nonprogressive liver disease were subject to at least one hepatic histologic evaluation (multiple in six patients). The biopsies in this group of patients showed either mild nonspecific abnormalities (three instances), features of chronic persistent hepatitis (eight instances), or chronic active hepatitis without bridges (three instances). In patients with the latter findings a subsequent examination had shown the lesion to be unchanged. Two patients were not biopsied. Elevations of SGOT levels were recorded in these patients for 10 and 12 months respectively. The results of their liver tests then returned entirely to normal. Insufficient data were available to adequately assess progression in six other patients with chronic disease.

Ten patients had more than one attack of liver dysfunction. These 10 acute and reversible "second" episodes either occurred separately from the patients' major illnesses or they developed during the course of, and were superimposed on, episodes of chronic liver disease. Under these latter circumstances the second illness was identifiable as an icteric event associated with a separate and definable cause. With resolution of the second acute lesion the patient was left with the manifestations of the underlying chronic process.

HEPATITIS B VIRUS

At the time of transplantation only one of our patients was known to be a carrier of HBsAg (Table 2). This patient maintained an asymptomatic carrier state but displayed no evidence of liver disease for the 7 months preceding her death from nonhepatic causes. Four patients

Table 2. Role of Hepatitis B Virus in Post-Transplant Liver Disease*

HBV Markers	Number	Liver Disease	Cause of Liver Disease
Present at transplantation			
HBsAg + anti-HBc	1	None	...
Anti-HBs (+ anti-HBc in 18 patients)	21	Various (in nine patients)	Not HBV
Anti-HBc	2	Chronic	Not HBV
? Passive transfer			
Anti-HBc (transient)	4	None	...
Developed after transplantation			
HBsAg	1	Fulminant	HBV
HBsAg + anti-HBc	3	Chronic	HBV
Anti-HBs + anti-HBc	1	Acute	HBV
Anti-HBc	1	Chronic	HBV

* HBV = hepatitis B virus, HBsAG = hepatitis B surface antigen, anti-HBc = antibody to hepatitis B core antigen, anti-HBs = antibody to hepatitis B surface antigen.

developed HBsAg during the post-transplant period. Fulminant acute hepatitis caused the death of one of these people 5 months after the grafting procedure. Another man developed chronic hepatitis with persistent antigenemia and evidence on two liver biopsies of histologic progression to chronic active hepatitis. The other two patients became chronic antigen carriers also: one asymptomatic with normal hepatic histology apart from numerous ground glass cells, the other with mild elevations of SGOT but without biopsy assessment of the histologic consequences.

Antibody to hepatitis B surface antigen was detected in the serum of 21 patients at the time of transplantation. Antibody to hepatitis B core antigen was detected in the sera from 18 of these patients. Nine of them (all with anti-HBc) subsequently developed some form of liver disease, which varied in its clinical expression and was not attributed to hepatitis B virus infection. Only one of the 141 patients who were initially anti-HBs-negative subsequently developed this antibody. This seroconversion was accompanied by the appearance of anti-HBc (HBsAg was not detected), and these antibody responses followed an asymptomatic episode of mildly abnormal liver tests that lasted only 2 weeks and was caused, presumably, by infection with hepatitis B virus.

Antibody to hepatitis B core antigen (anti-HBc) without coexistent HBsAg or anti-HBs was present in the sera of two patients at the time of transplantation. Neither patient had any evidence of liver disease during hemodialysis, and their liver test results were quite normal for some months after transplantation. Both patients subsequently developed a progressive form of chronic liver disease, and although the anti-HBc persisted in their sera the titer remained low throughout. The subsequent liver lesions in these patients probably were not caused by hepatitis B virus infection. Four other patients were noted to have low titers of anti-HBc in the first sera drawn after transplantation. These titers diminished during the subsequent few months, and then the antibody disappeared. In only one of these patients was pretransplant serum available, and anti-HBc was not present in this sample. In none of these patients was HBsAg or anti-HBs ever detected, and the presence of anti-HBc was not associated with any evidence of liver dysfunction. These findings probably these represent the passive transfer of anti-HBc from blood transfusions received at transplantation. Seroconversion for anti-HBc (in the absence of HBsAg and anti-HBs) was documented in one patient. This occurred coincidently with the onset of a chronic liver disease that has not progressed histologically and is probably the consequence of hepatitis B virus infection. Thus hepatitis B virus could be incriminated as the cause of only six episodes of liver disease in 141 susceptible patients and accounted for only 10% of the liver disease that was observed.

Table 3. Role of Hepatitis A Virus in Transplant Recipients*

	Anti-HA Positive at Transplant		Anti-HA Negative at Transplant	Seroconversion
	no.	(%)	*no.*	
Acute liver disease (n = 17)	9	(53)	8	0
Chronic liver disease (n = 37)	23	(62)	14	0
No liver disease (n = 30)	17	(57)	13	0
Total (n = 84)	49	(58)	35	0

* Anti-HA = antibody to hepatitis A.

HEPATITIS A VIRUS

The results of the assays for anti-HA are shown in Table 3. Approximately 60% of the population has preformed anti-HA at the time of the transplant. No instance of seroconversion was observed through the period of follow-up in those patients whose initial serum did not contain antibody. No episode of liver disease could be attributed to hepatitis A virus infection.

CYTOMEGALOVIRUS

Adequate serologic data were available in 150 of the 162 patients and are summarized in Table 4. Approximately 80% of patients whose pretransplant serum did not contain complement-fixing antibody to cytomegalovirus subsequently showed seroconversion for this antibody. Approximately 60% of patients whose pre-

Table 4. Seroconversion to Cytomegalovirus in Transplant Recipients

Liver Disease	CF Ab* Titers at Transplantation					
	<1:8			>1:8		
	Number	Seroconversion		Number	≥ Fourfold ↑ Titer	
		no.	%		no.	%
Acute disease	21	18	86	3	0	...
Chronic disease	28	22	79	8	6	75
No disease	53	44	83	37	22	59
Total	102	84	82	48	28	58

* Complement-fixing antibody.

transplant serum did contain antibody also underwent a four-fold or greater rise in titer. Neither the fact of liver disease nor the specific form of such liver disease was influenced by these rates of seroconversion. The association of cytomegalovirus seroconversion with an acute febrile illness or with a temporally related episode of acute rejection was not confined to any one group of patients, although these events tended to be more frequent in patients who developed liver disease (Table 5). The application of the liberal criteria we used to incriminate cytomegalovirus as the cause of liver disease in this study resulted in the identification of 21 such episodes. Fourteen of these were acute: one being fatal, whereas in seven patients the liver disease became chronic and in three there was histologic evidence of a progressive lesion. Forty-eight fragments of liver tissue from 28 patients were placed in viral culture. Seven specimens from five patients induced in the monolayer cytopathic changes characteristic of cytomegalovirus infection. Five of these positive cultures were derived from three patients with chronic liver disease. Two of these patients had positive cultures from two liver specimens obtained more than 12 months apart. A liver biopsy from a patient with acute liver disease and an autopsy specimen from a patient without any hepatic dysfunction also gave positive results on culture. Typical cytomegalovirus intranuclear inclusions were seen in parenchymal cells in only four of the liver specimens examined by light microscopy.

EPSTEIN-BARR VIRUS

Table 6 summarizes the serologic data for Epstein-Barr virus obtained in 55 patients with liver disease. The sera from approximately 70% of the patients who subsequently developed liver disease contained antibodies to this virus at the time of transplantation. Seroconversion was documented in 11 patients whose sera was initially negative for antibody. In 10 patients this was not associated with the development of liver dysfunction. Seroconversion in one patient occurred, however, during the course of an asymptomatic but histologically progressive form of chronic liver disease. It was associated with a striking icteric illness, which was self-limited and resolved within 3 months leaving the patient with just the manifestations of his underlying chronic disease. This second acute illness was considered to result from Epstein-Barr virus infection.

In only one other patient was seroconversion for Epstein-Barr viral antibody associated with clinical manifestations. This patient developed a febrile illness, but there were no associated hepatic abnormalities.

ADENOVIRUS

Complement-fixing antibodies to adenovirus were found in the pretransplant sera in approximately 40% of patients (Table 7). Two patients who subsequently developed liver disease underwent seroconversion for adenovirus antibody. The onset of the liver disease and the seroconversion were not temporally related, however, and it was concluded that adenovirus infections were not responsible for any of the observed liver disease.

DRUGS

Two patients developed acute liver disease within a few

Table 5. Accompaniments of Cytomegalovirus in Transplant Recipients

	No Liver Disease (N = 90)	Acute Liver Disease (N = 24)	Chronic Liver Disease (N = 36)	Total (N = 150)
	no.			
Serological Evidence of Recent Infection	66	18	28	112
Accompanied by				
Febrile illness*	16	9	10	35
Acute rejection†	10	6	9	25
Positive urine culture	21/42	10/13	17/29	48/84
Onset of liver disease	...	14	7	21
Positive liver culture	1/1	1/1	3/25	5/28

* Defined as the development of an otherwise unexplained temperature >37.8 °C within 1 month prior to seroconversion.
† Defined by the receipt of intravenous bolus steroid therapy within 1 month prior to seroconversion.

Table 6. Epstein-Barr Antibody in Transplant Recipients

	Acute Liver Disease (N = 19)		Chronic Liver Disease (N = 36)	
	no.	(%)	no.	(%)
Positive titer at transplant	12	(63)	25	(69)
≥ Four-fold rise in titer	3		1	
Associated liver disease	0		0	
Negative titer at transplant	7		11	
Seroconversion	4		7	
Associated liver disease	0		1*	

* Associated with a "second" acute episode in patient with chronic disease.

weeks of starting therapy with isoniazid and sulfamethoxazole-trimethoprim (Bactrim), respectively. Both drugs are known to produce idiosyncratic liver injury (12, 13), and in both instances the liver disease resolved with cessation of the agents. Rechallenge trials were not conducted in these patients, but the drugs were accepted, although not proved, to be the cause of the episodes of liver dysfunction.

The initial mean daily dose of azathioprine and the distribution of its dosage ranges were the same in patients who developed liver disease and those who did not (Table 8). Thirty-two patients displayed an inordinate sensitivity to the effects of azathioprine, which was reflected by repeated falls in their peripheral leukocyte counts and necessitated a marked reduction in their maintenance dosage. This sensitivity was seen more frequently in patients with liver disease than in those without ($P < 0.05$).

Four discrete episodes of acute cholestasis characterized by marked pruritus and varying degrees of jaundice were observed. Withdrawal of azathioprine resulted in resolution of the cholestasis in three instances. A reduction in azathioprine dosage was followed by marked amelioration of the episode in the fourth patient. Restitution of the dosage to its original level in this patient was followed by a recurrence of the disorder, which resolved completely when the drug was discontinued. These four episodes were considered to have been caused by azathioprine in a "dose-related" rather than an "idiosyncratic" fashion.

All but one of the patients who were identified as having chronic progressive liver disease and eight of the 16 with nonprogressive chronic disease were treated by interrupting azathioprine therapy. In some instances cyclophosphamide was substituted to maintain adequate immunosuppression. In each case azathioprine was withheld for at least 1 month and in most instances for more than 6 months. An apparent amelioration of the chronic liver disease was seen in only two instances. One patient was a carrier of HBsAg, and the other patient subsequently relapsed (while still off azathioprine) and died of liver failure. Azathioprine was considered unlikely to have been directly responsible for any of the chronic liver disease observed in these patients.

MISCELLANEOUS CAUSES

There was one example each of hepatic infection with *Cryptococcus neoformans* and with lethal varicella zoster virus infection. Although 10 of our patients are known to have had cholelithiasis, there were no instances where bile-duct obstruction or pancreatitis accounted for the observed liver disease. In no instance could alcohol abuse be incriminated as the cause, and there were no immunologic markers present consistently in these patients to suggest either primary biliary cirrhosis or "lupoid" hepatitis.

Table 9 summarizes the causes that could be applied to the various forms of liver disease. Although most of the acute episodes had a definable cause, the bulk of the chronic liver disease could not be explained by any of the agents evaluated.

Discussion

The potential causes of liver disease in the immunosuppressed host are legion (3). Chief consideration must be given, however, to viral infections and drug reactions. Hepatitis B virus infection has been the major culprit in many of the previously reported experiences with liver disease occurring in patients after renal transplantation (1, 2, 15). In our unit this virus has been of only minor significance. Five of the six events shown to be related to hepatitis B virus occurred before 1972 when the routine testing of donated blood by sensitive assays for HBsAg became available. This factor together with the small reservoir of carriers in our unit probably accounts for our

Table 7. Adenovirus in Transplant Recipients

	CF Ab* Positive at Transplant	CF Ab* Negative at Transplant	Seroconversion	
			Number	With Onset Liver Disease
	← no. →			
Acute liver disease (N = 19)	8	11	1	0
Chronic liver disease (N = 37)	14	23	1	0
No liver disease (N = 8)	4	4	1	0
Total (N = 64)	26	38	3	0

* Complement-fixing antibody.

Table 8. Azathioprine in Transplant Recipients

	No Liver Disease (N = 100)	Acute Liver Disease (N = 24)	Chronic Liver Disease (N = 38)	Total (N = 162)
Daily dose, *mg/kg body weight*				
<2.0	39%	42%	24%	35%
2.0 to 2.5	48%	38%	55%	48%
2.6 to 3.0	12%	13%	16%	13%
>3.0	1%	8%	5%	3%
Mean daily dose, *mg/kg body weight*	2.1	2.2	2.2	2.2
Leukocyte sensitivity, *no.*	10	8	14	32
Cholestasis, *no.*	...	2	2	4

patients' relative freedom from infection with a virus to which only 21 of the 162 were protected by preformed anti-HBs. We have accepted the presence of anti-HBs in serum together with normal liver test results to represent evidence of previous infection with hepatitis B virus and have presumed that any subsequent liver disease occurring in the absence of re-emergence of HBsAg was caused by agents other than hepatitis B virus (16). The basis for this presumption rests on the lack of good evidence that hepatitis B virus, in contrast to the herpes viruses, is capable of latency. Four of the six hepatitis B virus infections were identified by the presence of HBsAg, but two required the demonstration of seroconversion to anti-HBc with or without the appearance of anti-HBs. This underlines the limitations of using HBsAg as the sole means of detecting hepatitis B virus infections. The avoidance of hepatitis B virus infections, moreover, has not eliminated the serious problem of chronic liver disease in our transplant unit.

This is the first published assessment of the experience with hepatitis A virus obtaining in a group of renal-transplant recipients. The prevalence of 60% of patients showing preformed antibody at the time of transplantation is not very different from that found in a general adult population (17-20). The failure to identify a single instance of seroconversion for this antibody exonerates hepatitis A virus as a cause of liver disease in our patients and is in accord with other evidence that suggests hepatitis A virus is associated with neither a carrier state (21) nor the development of chronic liver disease (21).

Serologic and cultural evidence of infection with cytomegalovirus is almost universal in renal-transplant recipients (22-24). Our own results confirm this well-described observation. The attribution of a cause-and-effect relation to cytomegalovirus and *any* clinical event in such patients is difficult to support because of the likelihood that a documented temporal coincidence has occurred by chance. At the onset of their liver disease some of our patients developed a characteristic febrile illness, which was accompanied by positive urine cultures for cytomegalovirus and was followed by the appearance in serum of antibodies to the virus. Such episodes could be attributed to cytomegalovirus infection with some confidence. Proving, however, that cytomegalovirus is the cause of a chronic disease or one that pursues a fulminant course is much more difficult. Even the demonstration that the virus was still present in a diseased liver months or even years after infection was first acquired does not prove that the virus was causing the disease. One of our patients who manifested no evidence of liver disease during life was found to have characteristic nuclear inclusions in his hepatic nuclei on light microscopy and a positive cytomegalovirus culture from liver tissue obtained at autopsy 5 months after seroconversion to cytomegalovirus occurred. Although we have attributed seven instances of chronic hepatitis and one fulminant episode to possible infection with this virus, we acknowledge that proof of this relation is weak and that we may well have overestimated the contribution this virus has made to these categories of liver disease.

Table 9. Cause of Liver Disease in Transplant Recipients (72 Episodes in 62 Patients)

Agent	Acute Episodes		Chronic Disease			Total
	Primary	"Second"	Not Progressive	Progressive	Indeterminant	
	no.					
Possible cytomegalovirus	12	2	4	3	...	21
Hepatitis B virus	2	...	2	1	1	6
Hepatitis A virus	...	...	...	...	...	0
Adenovirus	...	...	...	...	...	0
Epstein-Barr virus	...	1	...	...	...	1
Varicella zoster virus	1	...	...	...	...	1
azathioprine (Imuran)	1	3	...	...	...	4
Other drugs	1	1	...	...	...	2
Cryptococcus	1	...	...	...	...	1
Undetermined	6	3	10	12	5	36
Total	24	10	16	16	6	72

The prevalence of antibodies to Epstein-Barr virus in our patients at the time of transplantation was quite high. Moreover, the majority of patients who were initially seronegative developed such antibodies during the period of follow-up. This evidence of widespread exposure to Epstein-Barr virus is in accord with the report of an 87% rate of positive throat cultures for this virus in renal-transplant recipients (25). Only two of our patients suffered a clinical illness temporally associated with Epstein-Barr virus seroconversion, however, and in only one of these was their evidence of hepatic involvement.

Varicella zoster virus and the adenoviruses contributed little or nothing to the liver disease we have observed. Although we did not conduct a systematic serologic evaluation for evidence of infection with herpes simplex viruses in these patients, we have no reason to believe that these viruses contributed to the problem. Previous serologic studies from this unit showed no association between the acquisition of HSV_1 antibodies and the development of hepatic dysfunction in renal-transplant recipients (24). None of the 48 liver specimens examined by fibroblast culture produced cytopathic lesions characteristic for herpes simplex; nor did any of the 14 patients who were documented to have clinically apparent herpes simplex infections manifest any associated hepatic disturbance. The occurrence of herpes simplex hepatitis is well described in immunosuppressed hosts (26) but apparently is an uncommon event.

Azathioprine (or its parent compound, 6-mercaptopurine) is known to produce a dose-related cholestatic syndrome in dogs (27) and man (28, 29). The dose required to produce this phenomenon is less in patients with chronic liver disease than in those with normal livers (30). Cholestatic episodes attributable to azathioprine occurred in four of our patients. One episode was clearly related to an increase in the drug dose. In one other patient the dose was stable, but the patient had underlying and unrelated chronic progressive liver disease. We suggest that a previously tolerable dose of azathioprine became cholestatogenic when his chronic liver disease had eroded a critical mass of his hepatic reserve. We have observed this same sequence in two other transplant recipients with chronic progressive liver disease, but these episodes fell outside the temporal confines of this study. The phenomenon is reminiscent of the early experience with 6-mercaptopurine (the major metabolite of azathioprine) in patients with chronic active hepatitis. A marked reduction in dosage was necessitated in such patients by the frequency of hepatotoxicity induced by therapeutic regimens standard for diseases not involving the liver (30). The two other episodes of azathioprine-associated cholestasis occurred in two women during the second trimester of their pregnancies. It is tempting to speculate that the well-described interference with organic anion transport induced by pregnancy (31) was sufficient to render a previously tolerable dose of azathioprine capable of inducing cholestasis in these women.

The immunosuppressive effect of azathioprine probably constitutes a very important determinant of the altered response to viral infections exhibited by renal-transplant recipients. We could provide no evidence, however, to support the view that azathioprine is directly responsible for any of the chronic liver disease we have observed. Therapy with this agent was interrupted for periods of months in all but one of the patients with a progressive liver lesion and in half of those whose disease appeared to be benign. Amelioration resulted only twice. In one patient chronic hepatitis was clearly caused by hepatitis B virus and in the other situation, benefit was only temporary before progressive hepatic deterioration led ultimately to the patient's death despite the continued avoidance of azathioprine.

Despite the plethora of medications to which transplant recipients are exposed, we were able to explain very few episodes of liver disease by an idiosyncratic response to medication. The potentially devastating nature of the liver disease has precluded rechallenge trials in these patients and we have not proved a cause-and-effect relation in the few instances in which drug reactions have been incriminated. Nonetheless, presumptive reversibility of such drug-induced lesions has led us to adopt a policy of withdrawing all drugs not considered essential therapy in patients who develop abnormal liver-test values in the post-transplant period.

Table 9 shows that although we can offer a likely cause for most of the acute and reversible hepatic lesions no etiologic agent was defined in 27 of the 38 patients with chronic hepatitis. None of these patients gave a history of excessive alcohol ingestion, and alcoholic liver disease was not suggested by the findings on liver biopsy. Serologic markers suggestive of primary biliary cirrhosis or "lupoid" hepatitis such as antimitochondrial, antinuclear, and anti-smooth-muscle antibodies were detected intermittently and in low titer in only three patients. It is still possible that this liver disease is principally the expression of an immunologic abnormality associated with organ transplantation. No evidence is available to support or refute such a hypothesis. We believe, however, that some, if not most, of these patients have chronic hepatitis secondary to infection with the non-A, non-B hepatitis virus (viral hepatitis C). This agent is responsible for approximately 90% of post-transfusion viral hepatitis (32). It shares many of the clinical and epidemiologic characteristics of hepatitis B virus, although its mean incubation period is shorter (33). Hepatitis caused by this virus may have a prolonged course and may be associated with the development of histologic evidence of chronic active hepatitis and cirrhosis (33-35). Almost without exception, our patients received blood transfusions at the time of transplantation. The majority of the episodes of liver disease began within 6 months of the transplant procedure (4). Although these data are suggestive, definitive evaluation of the role played in our patients by non-A, non-B hepatitis virus must await the availability of techniques for its specific serological identification. It is to be hoped that, in the future, the capacity to recognize the presence of this virus in blood will essentially eliminate chronic liver disease from the list of serious complications associated with renal transplantation.

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