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Recurrent Hepatitis C in Liver Allografts

Prospective Assessment of Diagnostic Accuracy, Identification of Pitfalls, and Observations About Pathogenesis

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Rationale and Design: The accuracy of a prospective histopathologic diagnosis of rejection and recurrent hepatitis C (HCV) was determined in 48 HCV RNA-positive liver allograft recipients enrolled in an "immunosuppression minimization protocol" between July 29, 2001 and January 24, 2003. Prospective entry of all pertinent treatment, laboratory, and histopathology results into an electronic database enabled a retrospective analysis of the accuracy of histopathologic diagnoses and the pathophysiologic relationship between recurrent HCV and rejection.

Results: Time to first onset of acute rejection (AR) (mean, 107 days; median, 83 days; range, 7–329 days) overlapped with the time to first onset of recurrent HCV (mean, 115 days; median, 123 days; range, 22–315 days), making distinction between the two difficult. AR and chronic rejection (CR) with and without co-existent HCV showed overlapping but significantly different liver injury test profiles. One major and two minor errors occurred (positive predictive values for AR = 91%; recurrent HCV = 100%); all involved an overdiagnosis of AR in the context of recurrent HCV. Retrospective analysis of the mistakes showed that major errors can be avoided altogether and the impact of unavoidable minor errors can be minimized by strict adherence to specific histopathologic criteria, close clinicopathologic correlation including examination of HCV RNA levels, and a conservative approach to the use of additional immunosuppression. In addition, histopathologic diagnoses of moderate and severe AR and CR were associated with relatively low HCV RNA levels, whereas relatively high HCV RNA levels were associated with a histopathologic diagnosis of hepatitis alone, particularly the cholestatic variant of HCV.

Conclusions: Liver allograft biopsy interpretation can rapidly and accurately distinguish between recurrent HCV and AR/CR. In addition, the histopathologic observations suggest that the immune mechanism responsible for HCV clearance overlap with those leading to significant rejection.

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Hepatitis C virus (HCV)-induced cirrhosis is the leading indication for liver transplantation throughout the world.[32] Unfortunately, reinfection is nearly universal and recurrent disease occurs in a majority of recipients. HCV replication can be detected in the RNA-positive recipients within days after transplantation.[27] Allograft dysfunction can occur as early as 1 week following transplantation in patients with high viral loads before transplantation,[6] but the majority of HCV-positive recipients usually first show signs of recurrent disease between 2 and 3 months.[11,31]

Distinguishing among recurrent viral hepatitis and AR and CR and other causes of allograft dysfunction is based primarily on liver biopsy evaluation. Guidelines to recognize individual syndromes were proposed more than a decade ago,[13,16] but experience has shown the distinction to be problematic.[1,19,25]

Steatosis is an early nonspecific finding in recurrent HCV[5]; spotty hepatocyte necrosis, lobular disarray, and Kupffer's cell hypertrophy are more reliable features that specifically point toward recurrent HCV as the cause of allograft injury.[31,33] Later in the course of recurrent HCV, predominantly mononuclear portal inflammation with variable interface activity and low-grade bile duct damage signal the transition from acute to chronic hepatitis. Chronic HCV is characterized by mononuclear portal inflammation that is frequently arranged into nodular aggregates and mild bile duct damage that is neither as severe nor as widespread as is seen in acute rejection (AR) or chronic rejection (CR).[31] Interface activity, including a type II ductular reaction, is also more common in hepatitis than in rejection. Retrospective analysis of case material to determine whether specific histopathologic criteria are useful in distinguishing among recurrent HCV and other causes of allograft dysfunction is fraught with pitfalls.

The Electronic Data Interface for Transplantation (EDIT)[15] software simultaneously creates an information portal for patient management and populates a research database. Real-time entry of all pertinent treatment, laboratory, and histopathology data into this software has enabled us to accomplish our primary goal. This objective was to prospectively and rigorously test our ability to distinguish among recurrent HCV, rejection, and other various causes of liver allograft dysfunction in HCV RNA-positive recipients enrolled in a recent "immunosuppression minimization protocol."[36] A secondary goal was to retrospectively analyze errors and determine how they might be avoided.

The treatment protocol used in this patient population is based on the concept that alloantigen migration to the central lymphoid tissues of the recipient early after transplantation is an important event that simultaneously triggers rejection and tolerogenic immune responses.[35–37] Pretransplant immunodepletion with anti-leukocyte antibodies is used to rein in the expected early alloresponse into a manageable range. This is combined with minimal posttransplantation monotherapy immunosuppression in an attempt to facilitate activation-induced apoptosis of donor-reactive lymphocytes.[36] A final goal of this study was to determine whether histopathologic observations in this unique patient population shed any insight into our conceptual understanding of HCV pathogenesis in the allografted liver.

MATERIALS AND METHODS

Patient Population and Pathology Workflow

All primary liver allograft recipients with documented HCV infection (RNA positive by PCR) enrolled in the protocol between July 29, 2001 and January 24, 2003 were initially included in this study (n = 53). Five patients either died within 1 week of transplantation or did not undergo any posttransplantation biopsies and were removed from the study. This left a total of 48 patients for analyses. The remaining patients were followed until March 31, 2003. Rationale for the treatment protocol is reported by Starzl et al.[36]

Briefly, all liver allograft recipients were treated immediately before transplantation with either broadly reactive rabbit anti-thymocyte globulin (Thymoglobulin; Sangstat, Menlo Park, CA; n = 25) or Alemtuzumab (Campath 1H; n = 22) and simultaneously with 1 to 2 g methylprednisolone to concomitantly prevent cytokine reactions.[36] One patient inadvertently missed pretreatment. After transplantation patients were treated with tacrolimus monotherapy with the goal of achieving target trough levels of 10 ng/mL. Any additional immunosuppression, such as steroids or other agents, was added only temporarily to control biopsy-proven rejection. Beginning at 4 months after transplantation, patients that had been on tacrolimus monotherapy for the preceding 60 days were considered for weaning.[36] After obtaining a protocol biopsy that was rejection-free, twice daily tacrolimus doses were consolidated to once daily doses for a few weeks. In the continued absence of rejection, baseline immunosuppressive therapy was further weaned by spacing doses to every other day and subsequently to longer intervals.[36] During immunosuppression weaning, unacceptable elevations of liver injury tests were investigated by liver allograft biopsies and other tests when appropriate. Mostly all biopsies were processed on a "STAT" basis with interpretation occurring on the same day the biopsy was obtained.

Experienced transplant pathologists (A.J.D., M.A.N., P.R., or T.W.) initially reviewed all liver allograft biopsies. Each biopsy was evaluated according to a protocol[15] that listed 31 histologic findings for scoring (http://tpis.upmc.edu/tpis/schema/AlloLiver.html). Consultation among the pathologists in difficult cases was routine at the time of signout. All biopsies were reviewed a second time immediately before a weekly clinicopathologic conference, when the free text diagnosis assigned by the primary pathologist was converted into "coded" diagnosis(es) by a single pathologist (A.J.D.) who re-reviewed the slides.[15] The diagnoses were also ranked in perceived order of importance with the most important listed first and discussed during the conference. Since all difficult cases were also discussed among the pathologists at the time of signout, there was only one instance of a significant disagreement between the signout diagnosis and coded diagnosis, which did not impact the results of this study.

Histopathologic Criteria for the Distinction Between Recurrent HCV and AR and CR and Assessment of Follow-up

The criteria used to distinguish between AR and recurrent HCV were based on those originally developed for HBV.[13,16] Specifically, mild AR was diagnosed when either of the following conditions was met: 1) portal inflammation with inflammatory bile duct damage involving ≥50% of the bile ducts; or 2) mononuclear perivenular inflammation involving ≥50% of the terminal hepatic venules, associated with hepatocyte necrosis and/or dropout. These criteria for mild AR require more extensive tissue injury than listed for the Banff criteria[3] in allografts not otherwise affected by a coexistent disease. Slightly more extensive tissue injury than usually seen in allografts without coexistent disease was also required for a diagnosis of moderate and severe AR, but the Banff criteria did not have to be adjusted.

In general, a biopsy was considered adequate when it contained six or more portal tracts and four or more terminal hepatic venules. Early and late CR was diagnosed using the Banff criteria.[12] Recurrent HCV was diagnosed when lobular or interface necro-inflammatory activity was more prevalent and prominent than bile duct inflammation and damage.

Patient outcome was used to gauge the accuracy of the prospectively entered histopathologic diagnoses. AR treat-

ment consisted of bolus steroid therapy. If unsuccessful, daily tacrolimus therapy was re-instituted and other agents were added. For the purpose of this study, the diagnosis of AR was considered correct if peak liver injury test abnormalities at the time of diagnosis showed a sustained improvement of at least 50% during the first week after additional immunosuppressive therapy. Liver injury tests eventually normalized with increased immunosuppression in all of the patients with AR alone.

Return to daily tacrolimus therapy until whole blood levels registered at least 10 ng/mL was used to treat CR; this was supplemented in some cases by simultaneous administration of other agents. A diagnosis of early CR was considered correct if there was a sustained decrease of 50% or more in total serum bilirubin therapy during the 2 months following treatment. Liver injury test eventually returned to normal or near-normal levels in all of the patients with early CR alone.

Recurrent hepatitis C was treated either by no change in immunosuppression therapy or weaning of immunosuppression in patients more than 4 months after transplantation. This was supplemented by interferon-α (INTRON A or PEG-INTRON; Schering, Kenilworth, NJ) and/or ribavirin (REBETROL; Schering) in patients that agreed to treatment and were able to tolerate the side effects. A diagnosis of recurrent HCV was considered correct if there was either no worsening of liver injury tests for at least 2 weeks following the decision not to augment immunosuppression and/or introduce anti-viral therapy. However, most patients specifically treated for HCV with decreased immunosuppression and anti-viral therapy showed noticeable improvement.

EDIT

The EDIT software used to collect data for this study was designed and developed specifically for the Thomas E. Starzl Transplantation Institute at UPMC and described earlier in greater detail.[15]

Data Handling and Statistical Analysis

Pertinent data from EDIT were first rendered anonymous by stripping it of unique patient identifiers, according to the exempt institutional review board-approved protocol (IRB#020177). The cohort was described using estimates of central tendency (means, medians) and spread (standard deviation, range) for continuous data and frequencies and percentages for categorical data. Groups were compared using the χ^2 test for differences in proportions (categorical data) and the Wilcoxon Rank Sum test (continuous data). To identify potential predictors of AR and CR, Cox regression models were constructed. Time-dependent covariates were used when appropriate. For comparison of liver injury tests, only those laboratory values that were obtained within −2 to 0 days prior to biopsy were eligible. However, the time range for eligible laboratory results was increased from −14 to 0 days for biopsies showing CR because of the slower changes in liver injury tests associated with this diagnosis. All analyses were performed using Statistical Analysis System (version 8.2).

RESULTS

Patient Characteristics and Graft and Patient Survival

Donor and recipient age, sex, race, and viral genotype (if available) are shown in Table 1. Coexistent diseases complicating HCV-induced cirrhosis are shown in Table 2. The mean and median follow-up for this group of patients was 292 and

TABLE 1. Donor and Recipient Characteristics, Follow-up Period, and HCV Genotypes in Study Population

	Study Group (N = 48)	
Variable	**N**	**[Column %]**
Gender		
Male	35	72.9%
Female	13	27.1%
Donor gender		
Male	29	60.4%
Female	19	39.6%
Race		
White	45	93.8%
Black	0	0.0%
Other	3	6.3%
Donor race		
White	42	87.5%
Black	5	10.4%
Other	1	2.1%
Age (yr)		
Mean (SD)		52.3 (8.3)
Median		50.8
Range		36.1–70.6
Donor age (yr)		
Mean (SD)		47.6 (14.6)
Median		50.7
Range		13.7–78.4
Follow-up (days)		
Mean (SD)		292.0 (172.7)
Median		240.0
Range		10–650
Genotype		
1a	16	33.3%
1b	8	16.7%
3a	2	4.2%
Other	7	14.6%
Missing	15	31.3%

TABLE 2. Coexistent Diseases in Patients Who Underwent Liver Transplantation Primarily for HCV-Induced Cirrhosis

Coexistent Disease 1	Coexistent Disease 2	No. of Recipients
None		28
Hepatocellular carcinoma		11
Alcoholic liver disease		5
Alcoholic liver disease	Hepatocellular carcinoma	1
Chronic HBV		1
Metabolic disease		2
Total		48

240 days, respectively, with a range of 10 to 650 days. Patient survivals at 1, 3, and 6 months and 1 year after transplantation were 98%, 94%, 85%, and 80%, respectively; graft survivals for the same intervals were 94%, 90%, 83%, and 78%. There were eight deaths and nine graft failures. The causes of death included liver allograft failure from primary dysfunction (n = 3) complicated by myocardial infarction (n = 1), cerebral anoxia (n = 1), or multiorgan failure (n = 1). Three patients died with functioning allografts from an intracranial bleed (n = 1), sepsis (n = 1), and a motor vehicle accident (n = 1). Two other patients died because of liver allograft failure secondary to recurrent cholestatic HCV (n = 1) and a combination of hepatic artery thrombosis and recurrent HCV (n = 1). The causes of graft failure included patient death (with functioning graft; n = 3), primary dysfunction without (n = 1) or with patient death (n = 3), and liver allograft failure from cholestatic hepatitis (n = 1) or a combination of hepatic artery thrombosis and recurrent HCV (n = 1). None of the allografts failed primarily from either AR or CR.

Biopsy Timing and Diagnoses

There were a total of 179 biopsies included in this study. The timing of the biopsies and the number of biopsies per patient are shown in Table 3. In total, grade mild AR or greater was diagnosed on 45 of 179 (25%) biopsies from 23 of 48 (48%) patients (Table 4). The mean and median time to first onset of AR was 107 and 83 days, respectively, with a range of 7 to 329 days. Early CR was diagnosed on 17 of 179 (9.5%) biopsies from 6 of 48 (12.5%) patients. The mean and median times until the first onset of early CR were 302 and 300 days, respectively, with a range of 170 to 413 days. None of the patients developed late CR.[8,12] Acute and/or chronic hepatitis was diagnosed on 86 of 179 (48%) biopsies from 31 of 48 (65%) patients. The mean and median times until the first onset of recurrent HCV were 115 and 123 days, respectively, with a range of 22 to 315 days.

TABLE 3. Number and Timing of Liver Allograft Biopsies Obtained to Determine the Cause of Allograft Dysfunction

	No.	%
Biopsies/person		
1	11	22.9
2	8	16.7
3	10	20.8
4	6	12.5
5–12	13	27.1
Biopsies/time period		
0–7 days	12	6.7
8–30 days	40	22.3
31–60 days	16	8.9
61–90 days	11	6.1
90–180 days	50	27.9
181–365 days	37	20.7
>365 days	13	7.3

Correlation of Histopathologic Diagnoses With Liver Injury Test Profile

Correlation of the histopathologic diagnosis with the liver injury test profile is shown in Table 5. Patients with a primary diagnosis of AR alone showed significantly lower serum aspartate aminotransferase (AST) and γ-glutamyl transpeptidase (GGTP) levels than patients with a primary diagnosis of AR and a secondary diagnosis of recurrent HCV. This is likely attributable to the more restrictive criteria used for a primary diagnosis of AR in the context of recurrent HCV. Conversely, however, there was no significant difference in the liver injury test profile in patients with a primary diagnosis of recurrent HCV alone versus those with a primary diagnosis of recurrent HCV and a secondary diagnosis of AR (Table 5).

TABLE 4. Timing of First Onset of AR and CR and Recurrent HCV in the Study Population

Time to first AR (days)	
Mean (SD)	106.6 (109.8)
Median	83.0
Range	7–329
Time to first CR (days)	
Mean (SD)	302.3 (80.8)
Median	300
Range	170–413
Time to recurrent HCV (days)	
Mean (SD)	114.6 (58.5)
Median	123.0
Range	22–315

AR, acute rejection; CR, chronic rejection; SD, standard deviation.

TABLE 5. Correlation of Liver Injury Test With Histopathologic Diagnoses

Primary Diagnosis	Other Diagnoses		AST	GGTP	TB
AR (n = 11)	HCV −	Mean	222.1	232.5	7.0
		SD	203.0	137.9	6.0
		Median	110.0	181.5	4.7
AR (n = 19)	HCV +	Mean	497.7	738.1	5.0
		SD	464.2	520.0	4.3
		Median	270.0	589.0	5.0
		P value	0.049	0.001	0.39
HCV (n = 42)	AR −	Mean	184.1	519.4	4.5
		SD	143.5	578.6	5.4
		Median	163.0	395.0	2.0
HCV (n = 7)	AR +	Mean	178.2	632.0	2.6
		SD	116.8	374.2	2.4
		Median	149.0	774.5	1.0
		P value	0.9	0.4	0.4
HCV (n = 39)	AR−/CR−	Mean	186.2	416.7	4.2
		SD	145.7	453.1	5.3
		Median	154.5	287.0	1.7
CR (any position) (n = 17)	HCV +/−	Mean	335.4	558.4	11.2
		SD	324.5	253.6	5.0
		Median	154.0	513.5	12.8
		P value	0.4	0.04	0.0004

AR, acute rejection; CR, chronic rejection; HCV, hepatitis C virus hepatitis; TB, total bilirubin; +, present; −, absent.

A diagnosis of CR, regardless of ranking, was associated with significantly higher GGTP and total bilirubin levels compared with patients with HCV alone. This difference is attributable to the more diffuse bile duct damage and senescence seen in CR,[26] which is not part of the histopathologic spectrum of HCV alone.

Examples of Correct Identification and Treatment of Rejection and Recurrent Hepatitis

Two predominant histopathologic patterns comprised an "AR profile" in the context of recurrent HCV: 1) portal inflammation with bile duct damage involving a majority of the portal tracts; and/or 2) perivenular mononuclear inflammation involving a majority of central veins. The latter finding was associated with perivenular hepatocyte necrosis and dropout with or without portal inflammation. Early CR was recognized by senescence changes involving a majority of the bile ducts.[26] A representative example of each AR profile and one for recurrent HCV are described below.

The clinical course of the first patient, a 54-year-old man, is represented graphically in Figure 1. A protocol preweaning biopsy obtained 109 days after transplantation showed recurrent HCV alone, characterized by mild portal inflammation that was focally arranged into nodular aggregates, mild focal interface activity, and mild steatosis. No bile duct damage or perivenular inflammation was seen and liver injury tests were minimally abnormal, so weaning from immunosuppression began (Fig. 2A). Fifty days later (postoperative day 206), elevated liver injury tests prompted a repeat biopsy that showed moderate to severe AR (Figs. 2B, C), characterized by moderate portal inflammation with prominent bile duct damage involving virtually all of the bile ducts. The patient was initially treated with a pulse of corticosteroids. A follow-up biopsy obtained 11 days later (day 217) showed partial resolution of the portal inflammation, but senescence changes appeared in a majority of the bile ducts signaling the onset of

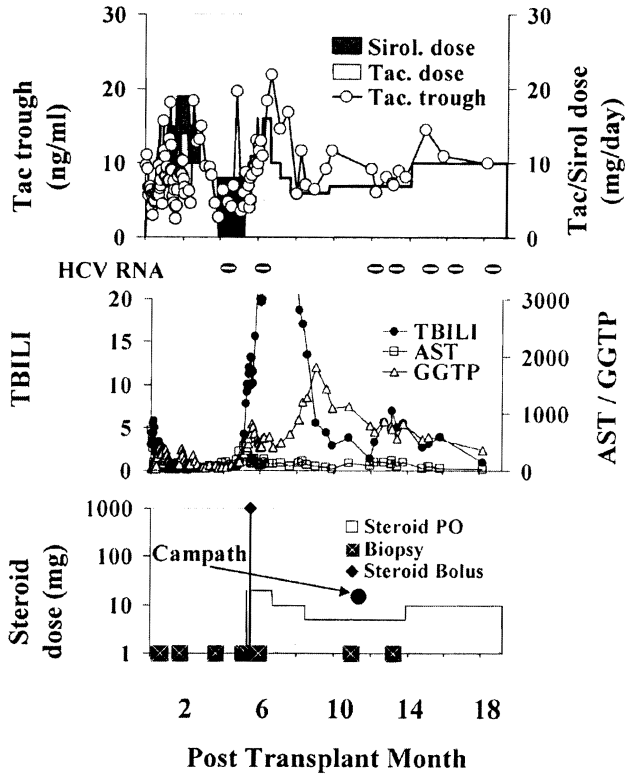

FIGURE 1. Dose and blood levels of baseline immunosuppression (top panel), HCV RNA levels and anti-viral therapy (second panel from top), liver injury test (third panel from top), and timing of biopsies and augmentation of immunosuppression (bottom panel) in a patient correctly diagnosed as developing AR after weaning of immunosuppression. Note the dramatic increase in total bilirubin (Tbili), aspartate aminotransferase (AST), and γ-glutamyl transpeptidase (GGTP) after weaning of immunosuppression. A correct histopathologic diagnosis (see Fig. 2) of rejection prompted return to daily tacrolimus therapy and treatment with corticosteroids, which eventually resulted in resolution of liver injury tests abnormalities. Despite the low levels of HCV RNA, the PCR for HCV was positive.

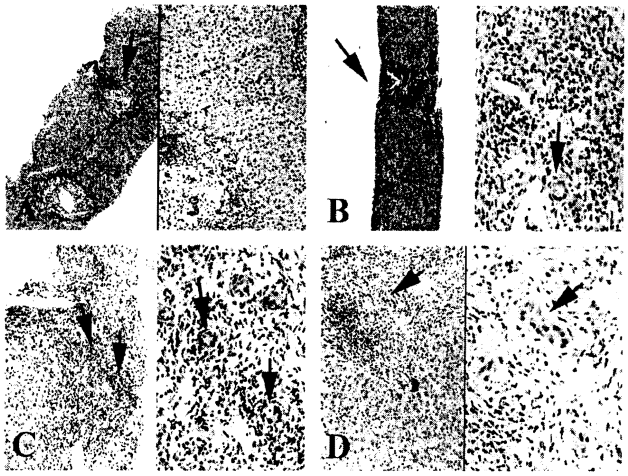

FIGURE 2. Appearance of liver allograft biopsies from the patient whose clinical course is shown in Figure 1. A: A protocol preweaning liver biopsy obtained on day 109 showed mild chronic portal and spotty hepatocyte necrosis but without bile duct damage or portal or central venulitis. These findings prompted a diagnosis of recurrent HCV alone. B and C: Another biopsy was obtained after weaning of immunosuppression on day 206 because of markedly increased liver injury test (see Fig. 1). Note the prominent mononuclear portal inflammation and prominent bile duct damage (arrows), which involved the majority of the ducts. Attention is drawn to the bile ducts (C; arrows), which are shown at higher magnification on the right side of (C). D: A follow-up biopsy obtained 11 days later (day 217) after treatment with increased immunosuppression showed partial resolution of the portal inflammation, but a majority of the bile duct showed senescence-related changes, prompting a diagnosis of early CR (arrows). An example of an affected bile duct (arrow) is shown at higher magnification in the right panel of D. A return to daily tacrolimus and maintenance corticosteroids eventually resulted in near normalization of the liver injury tests.

early CR (Fig. 2D). Re-institution of daily tacrolimus and maintenance corticosteroid therapy eventually lowered the markedly elevated liver injury test (total serum bilirubin peaked >50 mg/dL) to near-normal levels without a concomitant increase in HCV RNA levels.

The clinical course of the second patient, a 52-year-old woman, is represented graphically in Figure 3. A preweaning biopsy obtained about 3.5 months after transplantation showed low-grade perivenular inflammation, which was not treated with increased immunosuppression because of normal liver injury tests. There was minimal histopathologic evidence of recurrent HCV at this time. Approximately 3.5 months after the start of weaning, a sharp rise of AST and GGTP prompted the liver biopsy shown in Figure 4. Mild portal inflammation with minimal interface activity and mild focal bile duct damage combined with prominent perivenular inflammation and hepatocyte dropout resulted in focal central-to-central bridging necrosis. A primary histopathologic diagnosis of mild acute cellular rejection prompted treatment with 2 pulses of corticosteroids, followed by a single injection of Alemtuzumab. This led to a prompt return of liver injury tests to normal levels, without a significant rise in the HCV RNA levels.

The clinical course of a 45-year-old man successfully recognized and treated as suffering from recurrent HCV alone after withdrawal from immunosuppression is shown in Figure 5. This patient first had evidence of recurrent HCV in a biopsy obtained on day 150 after transplantation, manifest as primarily as spotty acidophilic necrosis of hepatocytes with minimal portal inflammation. This was followed by biopsies on days 232, 296, and 388, all of which showed changes characteristic of recurrent chronic HCV including variable mononuclear portal inflammation, interface activity, lobular disarray, and spotty hepatocyte necrosis (Fig. 6). No significant bile duct

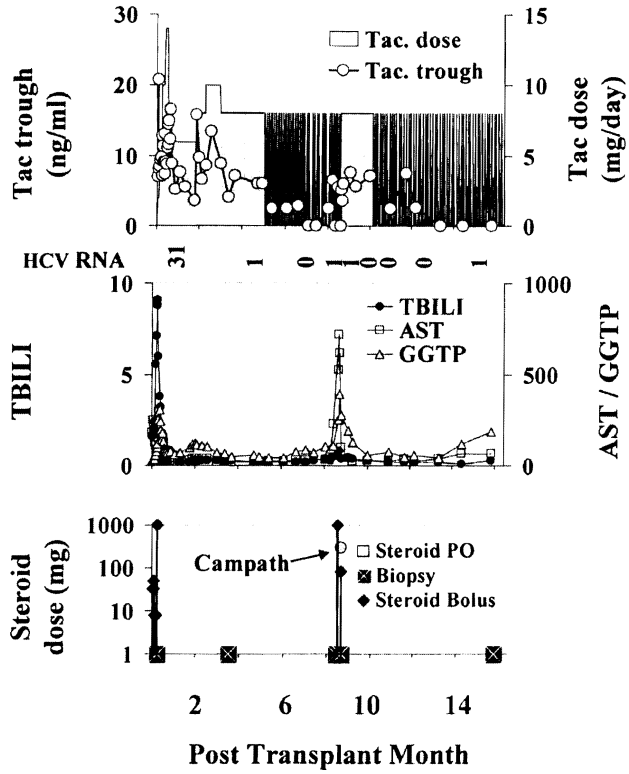

FIGURE 3. Clinical course of a patient who developed AR after being weaned from immunosuppression. Approximately 5 months after transplantation (top panel) and several weeks after a protocol biopsy that showed minimal focal perivenular inflammation, the immunosuppression was lowered (top panel). Approximately 3 months after spaced dosing of tacrolimus and low whole blood levels of tacrolimus <5 ng/mL (top panel), the patient developed elevations of the AST and GGTP to levels >600 IU/L (third panel from top). This prompted a repeat liver biopsy shown in Figure 4. Note the low levels of HCV RNA during the course.

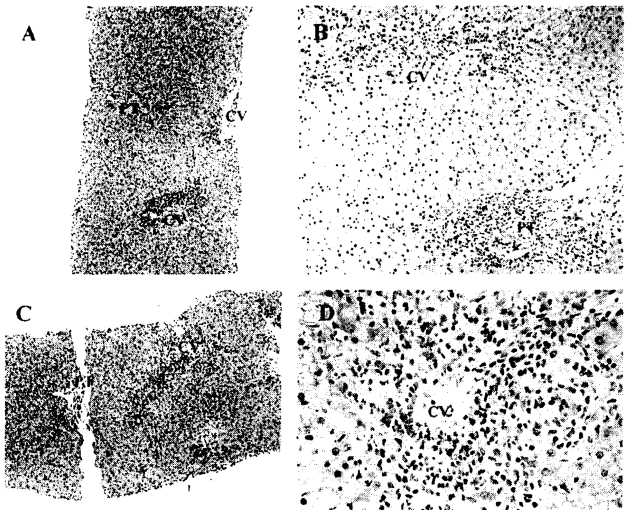

FIGURE 4. Liver biopsy from the patient whose clinical course is shown in Figure 3. It was obtained 8.5 months after transplantation and 3 months after weaning of immunosuppression. The most striking changes were perivenular inflammation, centrilobular hepatocyte dropout, and early perivenular fibrosis, which prompted a diagnosis of mild AR. A: Note that the inflammation is concentrated around the central veins (CV). B and C: Note the mild portal tract (PT) inflammation with focal mild bile duct damage but prominent perivenular inflammation. D: Higher magnification of an involved central vein showing the prominent perivenular inflammation consisting of lymphocytes and plasma cells. There is also red blood cell congestion, hepatocyte dropout, and early perivenular fibrosis.

damage or perivenular inflammation was seen in any of the biopsies. While being maintained on low levels of baseline immunosuppression (Fig. 5), the patient experienced fluctuating liver injury tests and HCV RNA levels over 6.5 months until treatment with a combination of interferon and ribavirin caused a dramatic lowering of the liver injury tests.

Identification and Explanation of Errors

We identified one major error and two minor errors in the pathology diagnoses. The major error occurred in a 36-year-old man who was subjected to an initial biopsy on day 39 because of elevated AST levels. The biopsy showed changes consistent with recurrent HCV alone, manifest as very mild chronic portal inflammation and spotty acidophilic necrosis of hepatocytes without bile duct damage or venulitis. Weaning of immunosuppression resulted in an increase in liver injury tests (Fig. 7), which in turn, prompted a repeat biopsy on day 82. It showed markedly increased portal inflammation. Bile duct damage was present but involved a minority of the portal tracts. There was also prominent lobular disarray, a type II ductular reaction, and hepatocyte necrosis. A mistaken primary diagnosis of mild AR with a secondary diagnosis of recurrent HCV (Fig. 8) prompted re-institution of daily tacrolimus therapy and a short cycle of steroids.

Treatment with more immunosuppression caused an immediate worsening of liver injury tests. Another follow-up biopsy obtained almost a week later (day 88) showed noticeably less portal inflammation but centrilobular hepatocyte swelling and hepatocanalicular cholestasis appeared, clear indicators of the development of cholestatic hepatitis (Fig. 9). Recognition of the mistake in this follow-up biopsy prompted a lowering of immunosuppression; the patient was also started on pegylated interferon and ribavirin. The anti-viral therapy resulted in dramatic lowering of the liver injury tests and lower viral loads (Fig. 7). An additional follow-up biopsy obtained on day 100 showed changes of recurrent HCV alone.

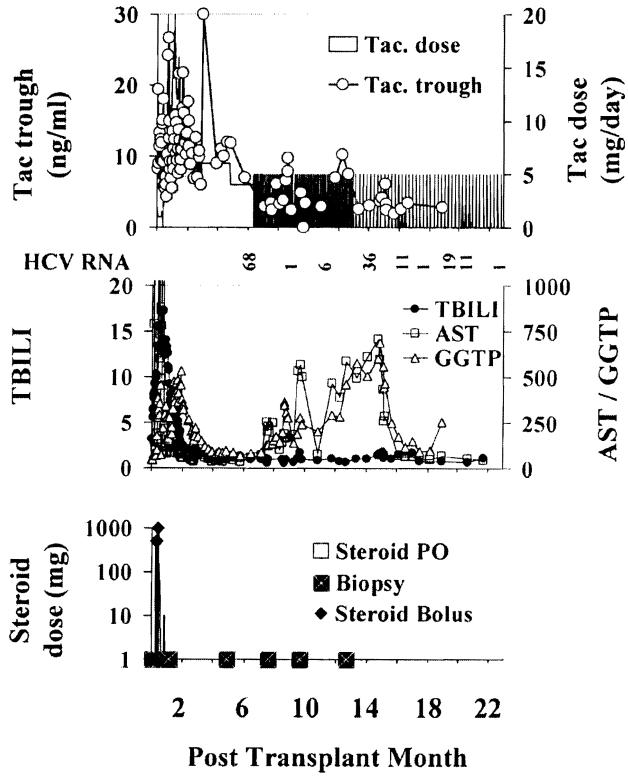

FIGURE 5. Clinical course of a patient correctly recognized and treated as recurrent HCV alone. Note that weaning from immunosuppression began approximately 4 months after transplantation (top panel) after a protocol biopsy (bottom panel) showed changes of recurrent HCV alone. Marked fluctuation of liver injury tests after weaning from immunosuppression prompted several liver allograft biopsies, all of which showed changes of recurrent HCV alone (see Fig. 6). Consequently, the patient was treated with a combination of interferon and ribavirin (panel second from top), which eventually resulted in a normalization of liver injury test (middle panel).

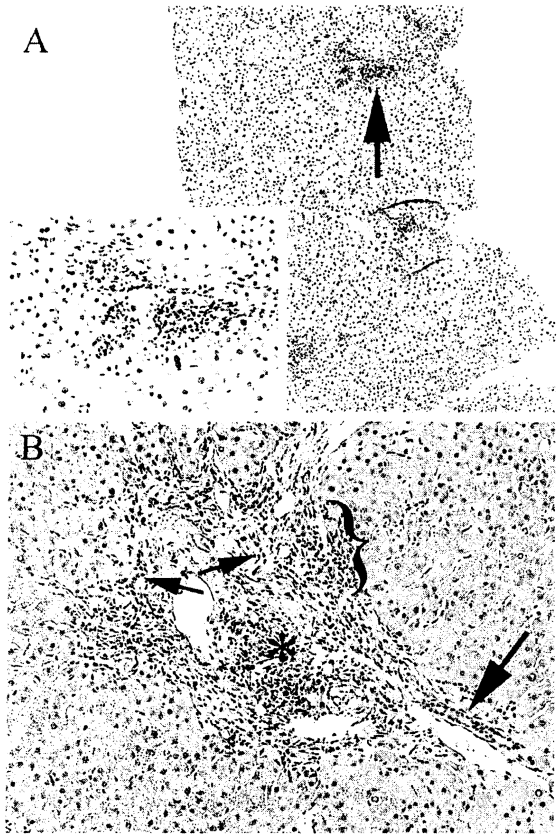

FIGURE 6. Liver allograft biopsies obtained from the patient whose clinical course is shown in Figure 5. A: The protocol preweaning biopsy obtained approximately 4 months after transplantation showed mild mononuclear portal inflammation, mild interface activity, and spotty acidophilic necrosis of hepatocytes. There was no evidence of bile duct damage or perivenular inflammation. The inset in the lower left corner shows the portal tract marked by the arrow at higher magnification. B: A repeat biopsy obtained about 6 months after weaning, during the peak of liver injury test abnormalities also showed changes of recurrent HCV alone. Note the mild mononuclear portal inflammation arranged into a small nodular aggregates (*), intact bile ducts (arrows), and type II ductular reaction at the interface zone (brace). None of the biopsies from the patient showed any changes of AR or CR.

A minor diagnostic error occurred approximately 10 months after transplantation and 6 months after weaning of immunosuppression. A secondary diagnosis of mild AR resulted in treatment with a single bolus of steroids, which in turn resulted in a slight worsening of liver injury tests. Follow-up biopsies 1, 4, and 6 months later showed predominantly or only recurrent HCV. Rejection activity, if present at all in any of these biopsies, was of indeterminate severity.

The final minor error occurred during the interpretation of a protocol biopsy obtained 4 months after transplantation, just before beginning weaning of immunosuppression. A secondary diagnosis of mild AR was ignored by the clinicians and weaning proceeded without any worsening of the near-normal liver injury tests.

A total of 105 of the 179 (59%) biopsies were obtained more than 30 days after transplantation and therefore were considered "at risk" for confusing AR with recurrent HCV. All errors were similar: AR was overdiagnosed in the context of recurrent HCV. The positive predictive values of rejection and hepatitis diagnoses were 91% and 100%, respectively.

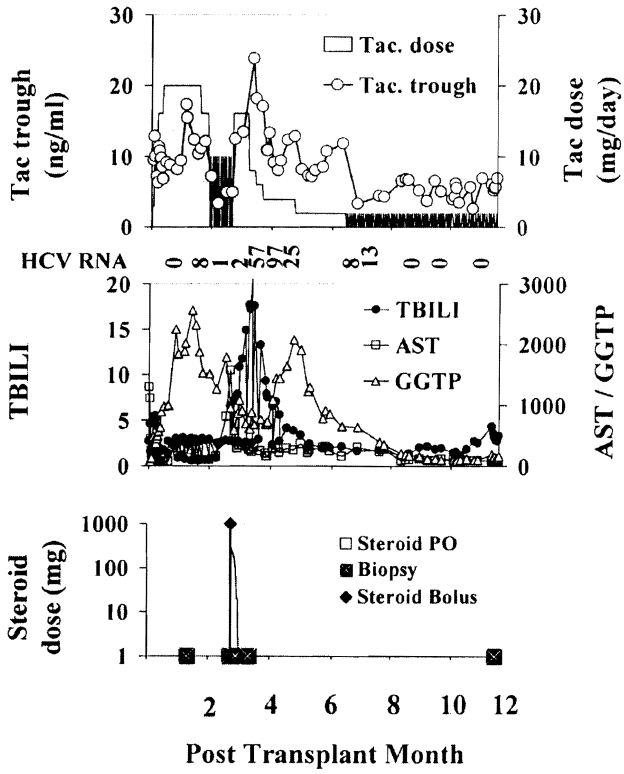

FIGURE 7. Clinical course of the patient whose liver allograft biopsy was misinterpreted as primarily AR, when in retrospect, the changes represented aggressive recurrent HCV. Weaning from immunosuppression (top panel) began several months after transplantation because a preweaning biopsy obtained on day 39 showed changes of recurrent HCV alone. By day 82, the liver injury tests as well as total serum bilirubin increased dramatically (third panel from top). This prompted a repeat liver biopsy, shown in Figure 8, which was misinterpreted as showing primarily AR with a secondary diagnosis of recurrent HCV. Return to daily tacrolimus therapy (top panel) and treatment with a pulse of corticosteroids (bottom panel) resulted in a further worsening of liver injury tests (third panel from top). A repeat biopsy 6 days later (Fig. 9; day 88) showed features of cholestatic hepatitis, a diagnosis that led to a decrease in immunosuppression and treatment with interferon and ribavirin. Eventually the anti-viral therapy led to a marked improvement in liver injury tests and a dramatic fall in HCV RNA levels (second panel from top).

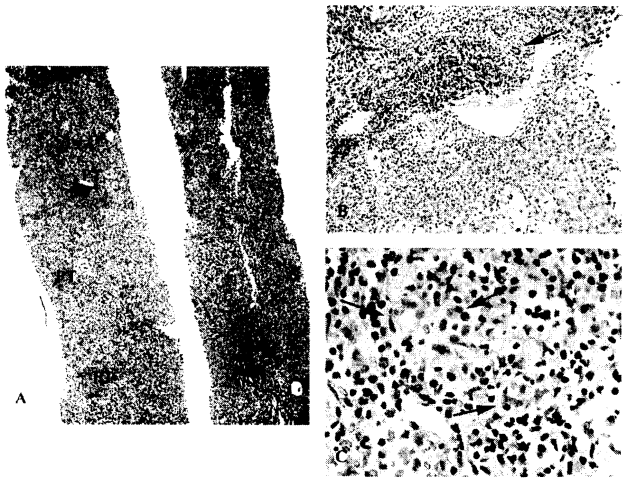

FIGURE 8. Liver allograft biopsy misinterpreted as showing primarily AR with a secondary diagnosis of recurrent HCV. The clinical course of this patient is shown in Figure 7. A: Note the prominent portal tract (PT) inflammation. B: In this portal tract, there is mild to moderate mononuclear portal inflammation and a ductular reaction at the interface zone, but minimal inflammatory bile duct damage (arrow). C: In contrast, other portal tracts from the same biopsy showed easily identifiable lymphocytic infiltration and damage of the small bile ducts (arrows). The prevalence of inflammatory bile duct damage was greater than is usually seen with HCV alone, but in retrospect, did not involve a majority of the bile ducts.

Retrospective Analysis of Errors and How They Might Be Avoided

In our opinion, the most serious error occurred because of anxiety over the uncertainty about the impact of conflicting influences of the treatment protocol on the biologic and histopathologic manifestations of recurrent HCV and rejection. It was reasoned that depleting antibody pretreatment might raise viral levels[11] early after transplantation. Subsequent weaning of immunosuppression had the potential to "re-arm" the immune system that could cause either severe hepatitis or severe rejection.[13,16] In retrospect, the major mistake would have probably not occurred if we had strictly adhered to our original histopathologic criteria and ignored the low blood levels of immunosuppression. In addition, retrospective analysis of the clinical course of this patient showed that HCV RNA levels were >50 million IU/mL at the time of the misinterpreted biopsy. Thereafter, an attempt was made to include routine monitoring of HCV RNA levels in patient management, but values were routinely not available until recently during preparation of this manuscript.

Correlation of Histopathology Diagnosis With HCV RNA Levels

Serial plasma HCV RNA levels according to the histopathologic diagnosis were plotted versus time after transplantation (Fig. 10). In general, HCV RNA levels were greatest during the first 6 months after transplantation, although the results varied among patients and values fluctuated significantly over time in individual patients.

Quantitative HCV RNA levels near the time of biopsy were available for 10 of 14 patients with histopathologic diagnoses of moderate or severe AR or early CR (Fig. 10). All of the episodes in these 10 recipients occurred more than 100 days after transplantation, and all but one of these patients, who had titers of 11.8 million IU/mL, showed HCV RNA levels of <10 million IU/mL at the time of the rejection diagnosis. HCV RNA levels were not available near the time of the biopsies in the 4 remaining patients, all of whom experienced moderate or severe AR or early CR less than 30 days after transplantation. The two patients who developed cholestatic HCV showed HCV RNA levels of >50 million IU/mL at the time of diagnosis. There was a wide range of HCV levels (0–30 million IU/mL) in recipients with a primary histopathologic diagnosis of recurrent HCV (Fig. 10).

Analysis of Risk Factors for the Development of Acute and Chronic Rejection

Six of the 11 HCV-positive recipients simultaneously maintained on low immunosuppression and treated with a combination of interferon and ribavirin developed significant rejection, defined as moderate or severe AR or CR. The remaining 5 of 11 patients treated with a combination of interferon and ribavirin, 2 other recipients treated with interferon alone, and a third patient treated with ribavirin alone did not develop significant rejection.

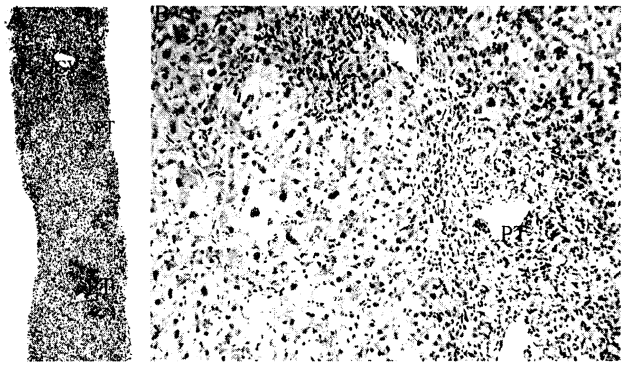

FIGURE 9. Follow-up biopsy from the patient whose clinical course and previous liver allograft biopsy are shown in Figures 7 and 8, respectively. A and B: Treatment with increased immunosuppression caused a dramatic decrease of the portal inflammation compared with the biopsy shown in Figure 8; it also caused marked hepatocyte swelling, hepatocanalicular cholestasis (left side of B), and a prominent ductular reaction at the interface zone (right side of B), all of which are characteristic features of cholestatic hepatitis. HCV RNA levels measured retrospectively from a sample obtained near the time of this biopsy were >50 million IU/mL (PT, portal tract).

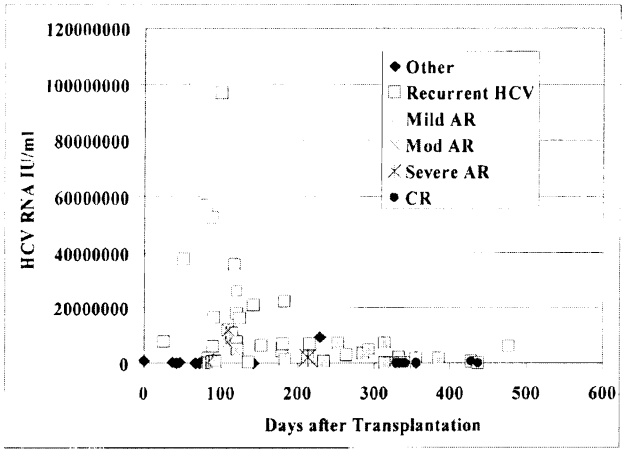

FIGURE 10. Correlation between quantitative HCV RNA levels and primary histopathologic diagnosis plotted against time after transplantation. The HCV RNA levels were obtained within a window from 14 days before until 3 days after the corresponding biopsy. Note that the highest levels of HCV RNA are detected during the first 6 months after transplantation, and thereafter levels generally decrease. In addition, HCV RNA levels >10 million IU/mL are almost invariably associated with a histopathologic diagnosis of recurrent HCV alone, and very high levels are seen in association with cholestatic hepatitis. Conversely, moderate and severe AR, and especially early CR, almost invariably shows relatively low HCV RNA levels. The point corresponding to the major mistake was not included in this graph.

Univariate models were first examined for predictors of AR and/or CR. Those parameters that reached a significance level of 0.10 were then used in multivariable models. Parameters that were considered for these models included basic donor and recipient characteristics such as age, race, and gender. Other variables included were cold ischemia time, pretransplantation crossmatch, antibody pretreatment, and posttransplantation anti-viral therapy. Only moderate or severe AR was used in the analysis for AR.

Predictors of AR in this patient population include female recipients ($P = 0.04$; hazard ratio [HR] = 4.0) and pretreatment with Thymoglobulin versus CamPath pretreatment ($P = 0.05$ HR = 8.0). Since older donor livers tended to develop CR sooner than younger donors, the CR model and the AR or CR model was adjusted for donor age. Treatment with interferon and ribavirin appeared to be a predictor of CR ($P = 0.03$; hazard ratio = 12.6); AR was weakly predictive of CR ($P = 0.11$; HR = 2.8), probably because of the small number of patients that experienced CR. Predictors of either AR or CR included female recipients ($P = 0.02$; HR = 4.7), pretreatment with Thymoglobulin ($P = 0.08$; HR =6.6), and treatment with interferon and ribavirin ($P = 0.09$; HR 4.0). Indeed, 13 of 14 patients who developed moderate or severe AR or early CR received Thymoglobulin pretreatment and of these, 4 of 6 who went on to develop early CR were also treated with anti-viral therapy for recurrent HCV.

DISCUSSION

In conventionally treated liver allograft recipients, AR usually occurs during the first month after transplantation; recurrent HCV usually first appears more than 1 month after transplantation. Thus, timing alone can be used to determine the cause of new onset liver allograft dysfunction. For patients enrolled in this protocol, the mean time to first onset of AR was 107 days, which significantly overlapped with first onset of recurrent HCV at 115 days. Consequently, distinguishing between rejection and recurrent HCV could not be based solely on time since transplantation and was especially troubling and particularly reliant on biopsy evaluation. The unusually long interval until the first onset of AR[15,40] is likely attributable to the protocol: pretransplantation immunodepletion followed by weaning of immunosuppression. Regardless, this study prospectively documents that interpretation of liver allograft biopsies can be used to quickly (6–7 hours) and accurately distinguish recurrent HCV from AR and CR, even under challenging conditions.

We found that the most problematic biopsies are a subset of those showing changes primarily of recurrent HCV. The troubling subset also shows bile duct damage or perivenular inflammation that is more prevalent than usually encountered with HCV alone, but still involving ≤50% of bile ducts or central veins, respectively. In such cases, it is our opinion that AR should be considered mild at most, and as a secondary diagnosis. These patients should not be treated with additional immunosuppression. Instead, they should be closely followed and subjected to re-biopsy if liver injury tests continue to rise.

Increased immunosuppression should be considered as a treatment option only when rejection is judged to be the primary insult. This condition is met when obvious bile duct damage or perivenular inflammation and hepatocyte dropout clearly involves most of the bile ducts or central veins, respectively. In our experience, AR-related findings should be obvious for biopsies in which AR is the primary insult. Such biopsies are usually graded as moderate or severe AR according to the Banff schema (1997) and usually associated with higher liver tests than HCV alone or mild rejection alone,[15] as confirmed in this study.

The above algorithm is recommended because liver allografts are very "forgiving" compared with other allografts: they can recover from most rejection-related insults and repair without fibrosis.[15] Conversely, unnecessary additional immunosuppression can significantly worsen hepatitis or even trigger fibrosing cholestatic hepatitis, and such patients usually suffer significant and often permanent liver damage from severe recurrent HCV. Furthermore, all of the mistakes in this series and in most other reports[1,19,25] were in the same direc-

tion; too great an emphasis was placed on mild AR findings in the context of recurrent HCV.

Although this study was carried out under a specific immunosuppressive protocol, it is our opinion that histopathologic findings and recommendations are generically applicable to other liver allograft recipients subjected to different approaches to immunosuppression. This contention is supported by the following observations: 1) the algorithm used in this study to distinguish between viral hepatitis and rejection was developed long ago in patients under a different immunosuppression protocol[13,16,31]; 2) even with low immunosuppression and the increased risk of rejection, we still overestimated the risk of AR; and 3) the same tendency to overdiagnose AR in the context of recurrent HCV occurs in liver allograft recipients treated with different approaches to immunosuppression. This statement is particularly true if the histopathologic findings are not clear-cut.[1,19,25]

Correlation of the biopsy findings with the clinical course, including an examination of serum HCV titers, serial liver injury tests in relation to immunosuppression, weekly meetings to discuss and collate all of this information, and importantly, insistence by the clinicians that there be unequivocal histopathologic evidence of significant rejection before giving any additional immunosuppression offered the best approach to optimal management. Real-time availability of graphical representations of the clinical course (ie, pertinent clinical, biochemical, and treatment data) made possible by the EDIT software greatly facilitated the entire process and reporting of the results.

Serial HCV RNA levels provided information useful for the histopathologic interpretation, but caution is urged against placing too much emphasis on HCV levels alone. There is a wide variation of HCV levels among patients, the values fluctuate significantly over time in individual patients, and there is considerable overlap in patients with different histopathologic diagnoses, particularly early after transplantation. Nevertheless, similar to other studies, the highest HCV RNA values were observed early after transplantation and during episodes of cholestatic HCV.[6,28,42] In contrast, all but one of the patients with late onset (>60 days) moderate or severe AR or CR showed HCV RNA levels <10 million IU/mL and most showed levels <2 million IU/mL. Thus, a diagnosis of moderate or severe AR or CR occurring more than 60 days after transplantation should be made with extreme caution in a patient with HCV RNA levels of >10 million IU/mL. Similar results were obtained by Gottschlich et al[19] who showed that higher HCV RNA levels were more frequently associated with an unequivocal diagnosis of recurrent HCV.

Relatively high HCV RNA levels during the first 6 months followed by a return to relatively low levels thereafter is most probably related to antibody pretreatment, disruption by transplantation of the previously established equilibrium between the host and virus, and later weaning of immunosuppression.[21,22,24,41] The unusual responsiveness of HCV levels and liver injury tests to anti-viral therapy in the weaning patients (unpublished observation) is likely attributable to lower overall immunosuppression, and in particular, the infrequent and sparing use of pulse or recycle corticosteroid therapy.[6,9,18,30,42] Our hope is that HCV might be more easily controlled or eliminated after transplantation, but using this protocol in HCV-positive recipients requires very close patient monitoring. We have already observed an increased incidence of early CR,[15] but there have been no graft failures from either AR or CR. In addition, most of the cases of early CR occurred early in the protocol and have recovered to near-normal liver injury tests with appropriate treatment. We are also aware that assessment of efficacy for both rejection and HCV will require longer-term follow-up because "re-arming" the immune system after immunodepletion has the potential to accelerate liver damage from recurrent HCV.[7,32]

The relatively low (<10 million IU/mL) HCV RNA levels and a paucity of hepatitis histopathologic findings during moderate or severe AR and CR versus high HCV RNA levels in cholestatic hepatitis and a complete absence of rejection-related histopathologic findings are interesting observations. Significant AR and CR in liver allografts have been associated with a strong T_H1-type hepatic microenvironment and cytotoxic T-lymphocyte response.[20,34,38,42] These effector mechanisms also are crucial determinants of HCV clearance and HCV-induced liver damage.[10,21,22,41] In contrast, cholestatic HCV has been associated with high viral levels and a strong T_H2-type intrahepatic microenvironment[42] and hepatic tolerogenesis.[29] Consequently, rejection and HCV clearance appear to be closely linked because all of the risk factors for significant rejection in this study (pretreatment with Thymoglobulin vs. CamPath, low-immunosuppression and anti-viral therapy including interferon) simultaneously enhanced viral clearance.

It is tempting to speculate about the role that hepatic dendritic cells might play in the outcome because of their ability to initiate and perpetuate immune responses. HCV interacts with DC-SIGN,[17,39] a receptor on dendritic cells that has the capacity to regulate their maturation and promote T_H2-type microenvironment.[2,39] Since dendritic cells are classically associated with rejection[14] and HCV appears to diminish their allostimulatory capacity,[4,23] HCV may be particularly suited for co-survival of the virus as well as the liver recipient. Early immunosuppression needed to prevent rejection enhances HCV replication, which in turn helps to subvert the alloresponse. Thus, the virus protects itself from clearance and the allograft from rejection. Conversely, while weaning of immunosuppression with addition of interferon may re-arm the immune system and promote HCV clearance, it also could increase the risk of rejection, and both rejection and the immune response leading to HCV clearance can significantly damage the liver. It seems, therefore, that the optimal approach for the treatment of HCV-positive liver allograft recipients would be

similar to hepatitis B. Agents that directly interfere with viral replication are needed.

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