PostScript

LETTERS

Autoimmune hepatitis triggered by hepatitis A

We describe a case of autoimmune hepatitis triggered by an acute hepatitis A infection. A young woman presented with a 10 day history of nausea and dark urine having returned from Tunisia a month previously. She had marked transaminitis. Viral serology demonstrated positive hepatitis A immunoglobulin M. Other viral serology was negative. Autoantibody screening revealed positive antismooth muscle antibodies (titre 1 in 80) but negative antinuclear (ANA), antimitochondrial, and antiliver, kidney and pancreas (LKM) antibodies. Immunoglobulin levels revealed normal IgA and IgG levels with a raised IgM at 5.53 g/l (range 0.60–2.50). Abdominal ultrasound scan was normal. She was diagnosed with acute hepatitis A.

Liver function tests normalised within two months. One month later her alanine aminotransferase levels began to increase. Autoantibody screen was positive for ANA (titre 1 in 40) and SMA was negative. However, immunoglobulin G was raised at 18.6 g/l (range 6–16) and serum electrophoresis showed a polyclonal increase in gamma globulins. Liver histology showed interface portal inflammation with plasma cell (fig 1) associated interface hepatitis with involvement of lobular and perivenular hepatocytes, consistent with autoimmune hepatitis (AIH). She was successfully treated with immunosuppression.

Studies suggesting triggering factors for the development of AIH have been reported in the past and include Epstein-Barr virus,[1] and hepatitis B and hepatitis A infection.[2] Although SMA was initially positive in our patient, this was probably because of the viral infection.

Using the diagnostic scoring system of the American Association for the Study of Liver Diseases (AASLD) (see table 1) for the diagnosis of AIH, our patient scored 16 before steroid treatment (definite diagnosis is >15) and 18 after steroid treatment (definite diagnosis is >17). The sensitivity of this scoring system for the diagnosis of AIH is quoted by the AASLD as 97–100%.

Acute hepatitis secondary to hepatitis A infection is a common cause of viral hepatitis in humans and is usually self limiting and resolves within weeks. However, it may lead to fulminant liver failure in a small percentage of cases. Hepatitis A does not lead to chronic viral hepatitis. We have documented a patient with acute hepatitis A who subsequently went on to develop AIH. Hence diligent follow up of patients with acute hepatitis A needs to be carried out. Hepatitis A may not be, as has long been thought, an acute infection with no long term sequelae.

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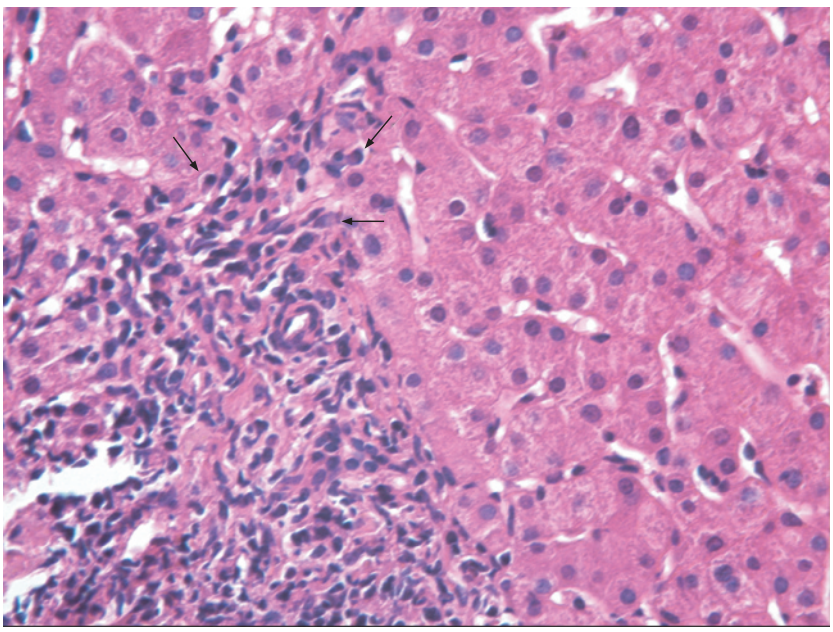

Figure 1 Liver histology revealing multiple plasma cells (arrowed).

A randomised controlled trial of total immunosuppression withdrawal in stable liver transplant recipients

Total withdrawal of immunosuppression (TIW) without causing rejection has been reported in some stable liver recipients.[1–3] Patient characteristics which predict this clinical tolerance have not been determined. Ursodeoxycholic acid (UDCA) has been reported to reduce the risk of early graft rejection following hepatic and cardiac transplantation.[4] We conducted a double blind controlled trial of UDCA therapy followed by TIW in 26 liver recipients to (a) determine if UDCA would facilitate TIW, (b) assess the safety of attempting TIW and (c) determine predictors of success of TIW.

Patients and methods

Records were reviewed and all patients who had been free of rejection for a minimum of 2 years, and on single or double drug immunosuppression, with transaminase levels <1.5 times the upper limit of normal, were invited to participate. Twenty six (13 male) patients gave informed consent and entered the study. Baseline liver biopsies were obtained, and a priori data related to pre-transplant and post-transplant patient variables were recorded. UDCA (15 mg/kg) or identical placebo capsule was administered, followed by sequential withdrawal of azathioprine (AzA) or prednisone and then graded reduction in ciclosporin (CyA) dose. Endpoints were defined as graft dysfunction (alanine aminotransferase >2× normal) with biopsy confirmation of abnormalities, or 6 months of no immunosuppression and no rejection on repeated biopsy. Rescue therapy for rejection was reinstitution of previous treatment, bolus steroid treatment with tapering or conversion to Tacrolimus based therapy.

Results

The UDCA and placebo groups had similar baseline characteristics (table 1). Rejection episodes occurred in 6/14 (43%) patients in the UDCA group and in 9/12 (75%) of those on placebo ($p = 0.09$) (fig 1). Time to rejection, degree of rejection (blind biopsy review) and immunosupression at the time rejection developed were similar in the two groups.

All responded to rescue therapy; none developed chronic rejection. All rejection episodes developed during CyA tapering, with a mean daily dose of 105 mg with whole blood levels <50 ng/ml in all patients. Only 1/6 patients (17%) with alcoholic liver developed rejection. Nine of the remaining 11 patients developed graft dysfunction without evidence of rejection. Three of four patients (75%) with autoimmune hepatitis (AIH) had recurrence of the disease with immunosuppression withdrawal.

Table 1 Diagnostic scoring system of the American Association for the Study of Liver Diseases

Category	Factor	Score
Sex	Female	+2
ALP:AST (or ALT) ratio	>3	−2
	<1.5	+2
γ-globulin or IgG (× above upper limit of normal)	>2.0	+3
	1.5–2.0	+2
	1.0–1.5	+1
	<1.0	0
ANA, SMA or anti-LKM1 titres	>1:80	+3
	1:80	+2
	1:40	+1
	<1:40	0
AMA	Positive	−4
Viral markers of active infection	Positive	−3
	Negative	+3
Hepatotoxic drugs	Yes	−4
	No	+1
Alcohol	<25 g/day	+2
	>60 g/day	−2
Concurrent immune disease	Any non-hepatic disease of an immune nature	+2
Other autoantibodies	Anti-SLA/LP, actin, LC1, pANCA	+2
Histological features	Interface hepatitis	+3
	Plasma cells	+1
	Rosettes	+1
	None of the above	−5
	Biliary changes	−3
	Atypical features	−3
HLA	DR3 or DR4	+1
Treatment response	Remission alone	+2
	Remission with relapse	+3
Pretreatment score		
Definite diagnosis		>15
Probable diagnosis		10–15
Post-treatment score		
Definite diagnosis		>17
Probable diagnosis		12–17

ALP, alkaline phosphatase; ALT, alanine aminotransferase antinuclear; AMA, antimitochondrial antibodies; ANA, antinuclear antibodies; anti-SLA/LP, antibodies to soluble liver antigen/liver pancreas; anti-LC1, antibodies to liver cytosol type 1; AST, aspartate aminotransferase; LKM, antiliver, kidney and pancreas antibodies; pANCA, perinuclear antineutrophil cytoplasmic antibodies.

One year after withdrawal only two patients were completely free of immunosupression usage but 4/5 previous users of prednisone were using no steroids, and the mean dose of CyA was <50% of that at entry. Age greater than 60 years, underlying primary alcoholic liver disease (ALD) and no (CyA+AzA) immunosuppression regimen were the only three favourable variables that could predict successful total immunosuppression withdrawal in 82% of cases.

Discussion

UDCA did not appear to decrease the frequency of acute rejection although the number of patients is insufficient to exclude an effect. UDCA may prevent rejection by decreasing HLA class 1 antigen and interleukin 6 expression, both important mediators in the process of transplant rejection.[5–7] Although the rate and intensity of rejection were similar to that of previous reports,[1–3] all but one patient responded to rescue therapy and no cases of chronic rejection or mortality were seen. The mechanism of recurrence of AIH early after withdrawal of immunosuppressions is unknown. However, HLA-DR3 or HLA-DR4 positive recipients are at risk of recurrence regardless of donor HLA status.[8]

The reasons why liver grafts develop tolerance in ALD patients after immunosuppression withdrawal in unclear. One explanation is that these patients may have continued to drink alcohol after liver transplantation which maintains some degree of immunosuppression[9]; however, liver biopsy at 6 months did not show any evidence of alcoholic injury. Whether these patients had more liver endothelial cell chimerism or more diminished dendritic cell numbers than those experiencing rejection is under investigation.

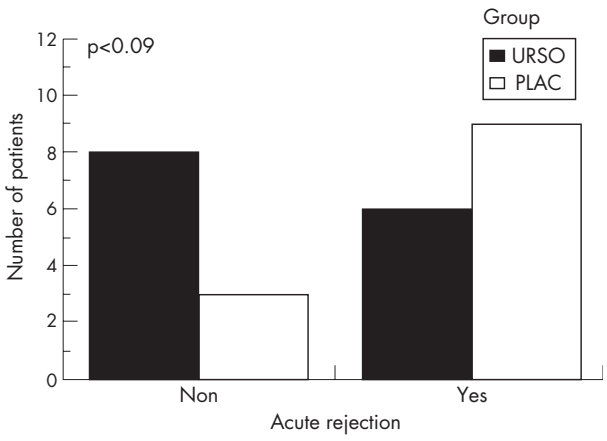

Figure 1 Number of patients with acute rejection in the ursodeoxycholic acid (UDCA) and placebo groups after complete immunosuppression withdrawal.

Conclusion

We have shown that late total immunosuppression withdrawal in stable liver transplant recipients is safe but seldom successful and most useful for patients transplanted for ALD and not for patients transplanted for autoimmune liver disease. We suggest that the search for an accurate means of identifying allograft tolerance among immunosuppressed recipients should become a priority in liver transplantation.

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Table 1 Baseline characteristics of liver transplant recipients with ursodeoxycholic acid (UDCA) therapy and controls

Characteristic	UDCA group (n = 14)	Placebo group (n = 12)	p Value*
Age (y)	56.3 (12.6)	50.7 (15.7)	0.3
Sex (M:F)	7:7	6:6	NS
OLT indications (%)			
Cryptogenic	7	25	
Cholestatic	36	8	
Alcoholic	36	8	
Autoimmune	14	17	
Miscellaneous	7	42	
No of previous rejections	0 (1)	0 (1)	NS
Time since OLT (months)	51.8 (12.8)	60.2 (29.2)	0.3
Biochemistry			
ALT (U/l)	18.8 (8.7)	29.9 (29.5)	0.1 (4–26)
AST (U/l)	23.8 (9.5)	32.8 (20.3)	0.1 (5–27)
ALK PH (U/l)	93.1 (25.2)	123 (50.6)	0.08 (16–98)
Bilirubin (μmol/l)	12.4 (4.5)	15.2 (6.6)	0.2 (3.4–17.1)
Creatinine (μmol/l)	144.1 (55.7)	163.6 (60.3)	0.3 (71–168)
Immunosuppression			
CyA dose (mg twice daily)	128 (42.6)	139.58)	0.6
CyA level (ng/ml)	130.3 (68)	172.5 (50)	0.1
Regimens			NS
CyA+AzA	n = 8	n = 6	
CyA+prednisone	n = 2	n = 3	
CyA alone	n = 4	n = 3	

Data are expressed as mean (SD).
ALK PH, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AzA, azathioprine; CyA, ciclosporin; OLT, orthotopic liver transplantation.
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Hepatitis C virus RNA quantitation and degradation studies in whole blood samples in vitro

Hepatitis C virus (HCV) qualitative and quantitative polymerase chain reaction (PCR) tests have evolved from specialist research tools into tests that are widely used in routine clinical practice. Clinical therapeutic decisions are based on HCV RNA titre; hence if the result is inaccurate, patients may be given, or alternatively denied, treatment inappropriately. Little data exist on the effect of environmental conditions on HCV RNA titre after blood has been taken from the patient.[1–3]

We therefore decided to evaluate the variation in HCV RNA titre after whole blood samples were taken from patients, to determine whether time at room temperature, temperature variation and blood collection systems affect the result obtained by the clinician.

Patients were recruited to the study from the liver clinic at the John Hunter Hospital in Newcastle, New South Wales, Australia. From each of 10 patients who were known to be HCV RNA positive from previous HCV qualitative PCR testing, 24 ml blood was collected in EDTA tubes. Immediately after each sample was taken, it was transported to the laboratory. Serial plasma samples were tested using the Roche Amplicor (Roche Laboratories, Sydney, Australia) HCV quantitative PCR kits to assess HCV RNA titres. Samples were tested for HCV RNA titre after standing between 0 and 24 h at room temperature (24°C). Comparisons of HCV RNA titre were also made after one freeze–thaw cycle, after collection in serum or EDTA tubes, and after 1:10 dilution of the 0-h samples.

HCV RNA titre was stable at room temperature for >24 h (fig 1). It was also stable over one freeze–thaw cycle, and no difference was seen in HCV RNA titre between blood collected in EDTA and that collected in serum tubes. Some patients had high HCV RNA levels, above the recommended upper limit for accuracy of the Roche Amplicor kit, but a 1:10 dilution of these samples and subsequent comparison with the neat samples revealed a high correlation coefficient of 0.91 (fig 2), indicating that the kit may still provide accurate HCV quantitation above the recommended upper limit.

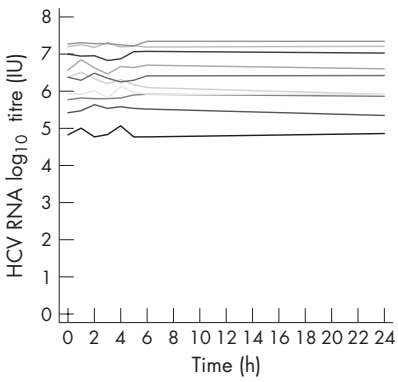

Figure 1 Hepatitis C virus (HCV) RNA degradation assays for samples at 0–24 h (individual patients shown).

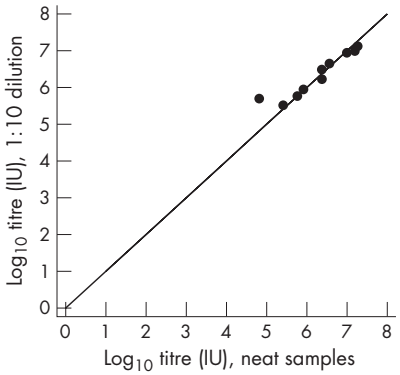

Figure 2 Hepatitis C virus RNA degradation assays, comparison of neat samples with 1:10 dilution.

Covariance analysis of the variability of the $\log_{10}$ RNA titre in two separate tests on the same frozen plasma samples showed covariance levels to be similar to those reported by Roche Laboratories (Sydney, Australia) in their reproducibility data.[4]

Hence, from this study, we can conclude that HCV RNA is stable within the parameters tested, at least for the highly conserved 244 base target sequence in the 5′ untranslated region of the HCV genome, which is used during the reverse transcription-PCR amplification stage in the Roche Amplicor kit.[4] It is possible that the long single strand HCV RNA molecule may fragment during the first 24 h after collection such that the virus is no longer viable for infection, but this theory cannot be tested within the limits of this study.

If HCV RNA does not fragment, the results of this study suggest that it may remain viable for at least 24 h at room temperature, which has public health implications for transmission of the virus through needle sharing, razors and household contact. Education of patients and their close contacts should emphasise that the virus may remain viable for at least 24 h at room temperature with little or no RNA degradation, hence care should be exercised with all potential body fluid contact.

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