Autoimmune reaction to a liver specific membrane antigen during acute viral hepatitis

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SUMMARY Lymphocyte cytotoxicity for isolated hepatocytes has been demonstrated in 93% of cases of acute viral hepatitis tested within two weeks of the onset of symptoms. The frequency of cytotoxicity during this time was similar for HBsAg positive and negative cases. However, after this time it was significantly higher in HBsAg positive than negative cases, 90% and 25% respectively ($P < 0.01$). Cytotoxicity was found in B-cell, but not T-cell, enriched fractions of lymphocytes, compatible with an antibody-dependent K-cell mediated reaction. In two cases the assay remained positive on retesting six months later, and follow-up liver biopsies showed the features of chronic aggressive hepatitis. These findings suggest that, in addition to the known immunological reactions against viral antigens that occur during the acute phase of viral hepatitis, an autoimmune reaction directed against a liver specific protein is also initiated; and if this reaction persists then chronic hepatitis may develop.

Recent studies using the lymphocyte transformation (Laiwah, 1971) and leucocyte migration tests (Irwin *et al.*, 1974; Reed *et al.*, 1974) have shown that sensitization to the hepatitis B surface antigen can be detected in the majority of cases of antigen positive hepatitis. This, together with the recent demonstration of HBsAg on the surface of hepatocytes during acute hepatitis B infection (Alberti *et al.*, 1975), supports the concept that a T-cell mediated immune reaction directed against virus bearing hepatocytes is responsible for the liver damage (Dudley *et al.*, 1972).

In addition to destroying infected hepatocytes, we have suggested that this T-lymphocyte response could lead to the activation of B cells responsive to a normal hepatocyte surface antigen, the liver-specific membrane lipoprotein (Eddleston and Williams, 1974). With removal of the infected liver cells the stimulus to this B-cell activation would disappear but if, for some reason, the virus persists or if there is a genetic defect in the control of immune responses then continuation of the reaction could be the mechanism underlying progression to chronic liver disease. To investigate this possibility further we have studied lymphocyte cytotoxicity for isolated hepatocytes both in patients with HBsAg positive and negative acute viral hepatitis.

Methods

Of the 36 patients investigated, 18 (10 males and eight females) were HBsAg positive, the remainder (six males and 12 females) being HBsAg negative—both initially and throughout the course of the illness. All patients had been admitted to hospital, although the timing of the admission from the onset of symptoms, the severity of the illness, and the degree of abnormality in liver function tests varied (Table). In eight patients the course was that of a fulminant hepatitis with the development of encephalopathy, and six of these died. Serum levels of bilirubin, aspartate aminotransferase, alkaline phosphatase, smooth muscle antibody, and anti-nuclear factor were also measured on the day of cytotoxicity testing. Liver biopsies performed during the acute illness in 21 of the patients were assessed 'blind' by a pathologist, for features of liver cell necrosis and cellular infiltration, each graded on a point scoring system from zero to nine, to give an index of the severity of liver damage. Twenty-eight of the patients were investigated only once, but cytotoxicity in the other eight cases was measured both during the acute state and again after an interval of three to six months.

PREPARATION OF LYMPHOCYTES

Leucocyte-rich plasma was obtained from 20 ml

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Table Clinical details of the patients studied

Case no. and sex	Weeks from onset	Cytotoxicity	Aspartate transaminase (IU/l)	Bilirubin (μmol/l)	Alkaline phosphatase AP (IU/l)	HBsAg	Autoantibodies		Hepato-cyte necrosis	Cellular infiltrate †
							ANA	SMA		
1 M	1	39	350	80	—	+	NT	NT		
2 M	1	48	500	120	—	—	NT	NT		
3 F	1	49	730	90	89	+	—	—	6	4
4 M	1	51	1100	104	—	—	NT	NT		
5 F	1	45	320	400	204	—	—	—	6	2
6 F	1	71	232	420	245	—	—	—	6	7
7 M	2	25	450	275	—	—	—	—		
8 M	2	34	300	180	204	—	NT	NT		
9 M	2	36	—	—	—	+	NT	NT		
10 F	2	48	98	270	118	+	—	—	5	3
11 M	2	60	1800	232	139	+	—	10	6	5
12 M	2	74	1600	190	210	+	—	—		
13 F	2	66	380	610	1000	—	—	—	6	5
14 F	2	37	64	13	—	—	NT	NT		
15 F	3	0	360	336	100	—	10	10		
16 M	3	45	1900	210	51	+	—	—		
17 F	3	63	107	395	207	—	—	—		
18 F	4	0	1100	312	217	Ab	40	40	7	7
19 F	4	6	1500	227	32	—	—	320	9	7
20 F	4	9	53	148	139	Ab	—	320	6	2
21 F	4	30	206	390	106	—	—	40	9	3
22 F	5	8	248	187	105	—	10	—	7	3
23 M	6	60	255	96	87	+	—	—		
24 M	6	79	184	180	355	—	—	—	5	3
25 F	6	13	147	460	241	—	—	—	6	5
26 F	6	32	216	65	115	+	—	10	8	7
27 F	6	36	1450	49	610	+	120	—	4	3
28 M	6	43	57	60	109	+	—	10	5	3
29 F	8	16	246	95	118	—	10	—	7	2
30 F	8	31	243	127	278	—	1280	80	6	5
31 F	8	67	65	7	37	+	20	—	1	2
32 M	8	34	350	40	168	+	—	20	6	2
33 M	8	50	350	510	109	—	10	10	4	4
34 M	8	48	245	18	65	+	—	—	4	4
35 F	8	5	380	370	272	+	—	—		
36 M	8	54	1100	281	79	+	—	—		

Ab: HBs antibody. NT: Not tested

peripheral venous blood by sedimentation with 2 ml 6% Dextran and 100 units preservative-free heparin. After diluting 1:1 with RPMI 1640, the plasma was incubated in a cotton-wool column at 37°C for 15 minutes to remove macrophages (Rocklin *et al.*, 1970). The resulting leucocytes were centrifuged over a Ficoll Triosil density gradient to remove the remaining neutrophils and red blood cells (Böyum, 1968). The interface lymphocytes which contained less than 1% neutrophils and 5% monocytes were used for cytotoxicity testing.

In some patients enriched populations of B and T lymphocytes were prepared. The method of separation has been described in detail elsewhere (Cochrane *et al.*, 1976). It entailed rosetting T-cells with sheep red blood cells, and B cells with sheep red cells coated with antibody and complement, followed by separation of the rosetted from non-rosetted cells by centrifugation over a Ficoll Triosil density gradient.

MICROCYTOTOXICITY ASSAY

This was carried out as described elsewhere (Thomson *et al.*, 1974) except that the enzyme solution for the preparation of isolated hepatocytes did not contain deoxyribonuclease. A suspension of lymphocytes in RPMI 1640, containing 1 Molar Hepes 2.3%, foetal calf serum 10%, penicillin 200 units per ml, and streptomycin 100 μg/ml was added to the hepatocytes in microtest plates to give a final ratio of 400:1. After 48 hours of incubation at 37°C plates were inverted for two hours and detached hepatocytes and lymphocytes were removed by gentle washing. The number of hepatocytes remaining in the test compared with control wells was expressed as the per cent cytotoxicity. Each plate, consisting of six rows of 10 wells each, had 20 control wells and each test suspension of lymphocytes was added to at least 10 wells.

The upper limit of normal for cytotoxicity in this

assay was 32%, this being two standard deviations above the mean value of 4% obtained in 25 healthy control subjects.

Results

Significant cytotoxicity for isolated hepatocytes was demonstrated in 13 (93%) of the 14 patients tested within two weeks from the onset of their illness (Fig. 1). These included all of the six cases who were positive for HBsAg at this time and seven of the eight who were not. Of the 22 patients tested between three and eight weeks after admission, cytotoxicity was present in 12 (55%) including nine (90%) of the 10 who were HBsAg positive, but only three (25%) of the 12 who were antigen negative ($p < 0.01$, Chi squared test).

Enriched populations of B and T lymphocytes were prepared from five patients, three HBsAg negative and two HBsAg positive, whose peri-

pheral blood lymphocytes showed significant cytotoxicity (mean $42\% \pm 8$). When B-cell enriched fractions were tested, cytotoxicity was present in all five cases, mean $49\% \pm 6$. In contrast, when T-cell enriched fractions were used, the results were all within the normal range (mean $14\% \pm 13$) (Fig. 2). In five further patients, whose peripheral blood lymphocytes did not show significant cytotoxicity, the results obtained using B-cell and T-cell enriched populations ($4\% \pm 8$ and $5\% \pm 9$ respectively) were not significantly different from those obtained using the combination of T and B lymphocytes ($12\% \pm 8$).

There was no correlation between the presence or absence of cytotoxicity and the levels of serum aspartate aminotransferase, bilirubin, or alkaline phosphatase, measured on the day of testing. Neither was there a correlation between the presence of cytotoxicity and the histological features of liver cell necrosis nor the degree of lymphocyte, plasma cell, and monocyte infiltration, in the 21 patients in whom a liver biopsy was performed within two weeks of testing.

Fig. 1. *Lymphocyte cytotoxicity for isolated hepatocytes in patients with HBsAg positive (○) and HBsAg negative (●) acute viral hepatitis separated according to time of initial measurement in relation to onset of symptoms. The horizontal dotted line indicates the upper limit of the normal range.*

Fig. 2 Cytotoxicity obtained using peripheral blood lymphocytes, T and B, B cell enriched, and T cell enriched fractions of lymphocytes, in six of the patients. HBsAg positive (○) and HBsAg negative (●) cases.

Fig. 3 Results of the cytotoxicity assay in eight patients during the acute phase of viral hepatitis and on repeat testing three to six months later. HBsAg positive (○) HBsAg negative (●).

In two patients, one HBsAg positive and one negative, the acute hepatitis progressed to chronic active hepatitis. Both showed significant cytotoxicity when first tested during their acute illness, and on repeat testing six months later both again gave positive results (Fig. 3). A further biopsy in each case showed chronic inflammatory cell infiltration of portal tracts and piecemeal necrosis of periportal hepatocytes typical of chronic aggressive hepatitis. Six further cases, all of whom showed cytotoxicity during their acute illness, have so far been retested three to six months later, but in these cytotoxicity results were now within the normal range. Tests for HBsAg, which were initially positive in four of the cases, were all negative, and liver function tests had returned to normal.

Discussion

In recent studies using this assay of lymphocyte-mediated cytotoxicity for hepatocytes we have found consistently positive results in patients with un-

treated chronic active hepatitis. The reaction appears to be mediated by cells bearing Fc-receptors (K-cells) (Cochrane *et al.*, 1976), and can be blocked by the addition of small amounts of a purified liver specific membrane lipoprotein prepared from either rabbit or human liver, suggesting that the hepatocyte damage is due to an immunological reaction directed specifically at this antigen (Thomson *et al.*, 1974).

The present study demonstrates that a similar autoimmune response occurs in the majority of patients during the early stages of acute viral hepatitis. This reaction in both antigen positive and negative cases is clearly not due to autoreactive T-cells but is more likely to be of the antibody-dependent cell-mediated type as in the patients with chronic active hepatitis. If this autoimmune reaction is due to activation of B lymphocytes by T-cells reacting with viral determinants on the hepatocyte membrane then it is possible to envisage two ways in which the reaction could continue and so produce chronic active hepatitis (Eddleston and Williams, 1974). Firstly, there might be an inability to suppress the autoreactive B-cells even though the virus had been cleared, and we have recently shown that the histocompatibility antigens HLA-B8 and HLA-B12 in patients with HBsAg negative chronic active hepatitis seem to be closely linked to genes promoting such an immune hyper-responsiveness (Galbraith *et al.*, 1976). Secondly, if the viral stimulus remains, continued T-cell destruction of infected hepatocytes will constantly stimulate the autoreactive B lymphocytes. This may have been the situation in the one patient who remained HBsAg positive after six months. He continued to show lymphocyte-mediated cytotoxicity for hepatocytes and had gone on to develop chronic active hepatitis. The other patient who developed this condition also showed persistence of the cytotoxic reaction but was HBsAg negative throughout, supporting recent reports of chronic liver disease occasionally after HBsAg negative acute hepatitis (Alter *et al.*, 1975; Galbraith *et al.*, 1975).

The absence of a correlation between cytotoxicity and the various clinical estimates of the degree of liver damage during the acute illness suggests that the autoimmune reaction to the liver-specific protein plays a minor role in the production of hepatocellular necrosis at this stage. However, the finding of liver lesions, resembling those found in chronic active hepatitis, in rabbits repeatedly immunized with liver-specific antigen (Meyer zum Büschenfelde *et al.*, 1972), some of which show progression to cirrhosis (Meyer zum Büschenfelde *et al.*, 1974), clearly shows that persistence of sensitization to the membrane lipoprotein could be responsible

for the development of chronic active hepatitis in man.

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