Autoimmunity to a liver membrane lipoprotein and liver damage in alcoholic liver disease

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SUMMARY Antibodies reacting with a liver membrane lipoprotein (LSP) have been detected by radioimmunoassay in the sera of 15 (27%) of 55 patients with alcohol-related liver lesions. There was a close association between the presence of the anti-LSP antibody and the finding on liver biopsy of a lymphocytic infiltrate in the portal tracts together with piecemeal necrosis of periportal hepatocytes. These histological features are characteristically found in the autoimmune disorder of chronic active hepatitis, in which anti-LSP antibodies are almost invariably present. It is suggested that in these cases of alcoholic liver disease there is loss of tolerance, and continued production of anti-LSP could promote periportal inflammation and accelerate the progression to cirrhosis. In the cases of acute alcoholic hepatitis without periportal inflammation studied, anti-LSP was not detected demonstrating that production of this autoantibody is not simply secondary to liver damage.

Many mechanisms have been proposed to explain the development of liver damage in patients with a high alcohol intake. Although experimental studies in man and baboons have shown that alcohol itself may be directly damaging to liver cells,¹ epidemiological evidence suggests that only 30% of heavy drinkers develop alcoholic hepatitis and 10% cirrhosis,² and other factors must determine the individual variation in the histopathological response. An increasing number of investigations have shown that potentially tissue-damaging immune responses accompany alcoholic liver injury.³

In one study, peripheral blood lymphocytes from patients with cirrhosis and acute alcoholic hepatitis were found to be cytotoxic to isolated rabbit hepatocytes.⁴ The reaction was shown to be mediated by non-T lymphocytes and could be blocked by the addition of small amounts of a liver membrane lipoprotein (LSP).⁴ One explanation for these findings is that the cytotoxicity was mediated, as in other types of autoimmune liver disease, by an antibody-dependent, cell-mediated mechanism with antibody to LSP playing a major role in determining the specificity.⁵ In the present study a sensitive and specific radioimmunoassay has been used to investigate the frequency of anti-LSP antibodies in alcoholic liver disease and the relationship between the

presence of this antibody and particular histological features in 55 patients with a wide range, both in severity and in type, of alcohol-related liver lesions.

Methods

PATIENTS

All 55 patients investigated had an alcohol intake greater than 80 g/day. Forty-eight of them represented consecutive admissions with alcohol-related liver disease to the Liver Unit over a period of six months. To ensure that the full range of histopathological responses to alcohol was covered, seven additional cases with fatty change alone or inactive cirrhosis were selected from patients attending a follow-up clinic.

Liver histology was available in all cases either from needle biopsy or subsequently at necropsy and serum samples were obtained within two weeks of the time of biopsy. Each biopsy was examined by one of us (BP), without knowledge of the immunological findings, and assessed for the presence and intensity (grade 0 absent, 1 mild, 2 moderate, and 3 severe) of each of the following histological features: fatty change, portal tract mononuclear infiltrate and piecemeal necrosis of periportal hepatocytes, hyaline inclusions, spotty lobular necrosis and polymorphonuclear infiltration, and cirrhosis. From the resultant scores it was possible to classify each biopsy into one

of six categories: fatty liver alone, alcoholic hepatitis, alcoholic hepatitis plus inactive cirrhosis—that is, with minimal portal mononuclear infiltrate and no piecemeal necrosis—alcoholic hepatitis plus active cirrhosis, active cirrhosis without alcoholic hepatitis, and inactive cirrhosis.

RADIOIMMUNOASSAY FOR ANTI-LSP ANTIBODIES

This is described in detail elsewhere.⁶ Serum samples were coded and examined for anti-LSP antibodies without knowledge of the histological findings. Each diluted one in 20 in 0.1M barbitone buffer, pH 8.5 with 1% bovine albumin and 25µl samples incubated in triplicate for three hours at 4°C with 25 µl (2.5 ng) of a purified preparation of human LSP previously radiolabelled with ¹²⁵I. Staphylococcal clumping factor (Sigma), 0.1 ml, was then added to facilitate the precipitation of IgG-¹²⁵I LSP immune complexes and incubation continued for a further hour at 4°C. 0.85 ml barbitone/albumin buffer was then added and the mixture centrifuged at 20 000×g for five minutes. 0.5 ml of the supernatant was removed and this and the remainder of the incubation mixture (including the pellet) counted separately. The per-

centage binding of ¹²⁵I-LSP was then calculated using the following formula:

$$\% \text{ binding} = \frac{\text{cpm remainder} - \text{cpm upper } 0.5 \text{ ml}}{\text{cpm remainder} + \text{cpm upper } 0.5 \text{ ml}} \times 100$$

A normal range was established from the results obtained using 20 control sera from healthy blood donors shown to be negative for HBsAg and anti-HBs. The mean % binding of LSP by these samples was 9.3 with a standard deviation of 2.1, giving an upper limit of the normal range (mean+3 SD) of 15.6%. Values above this level were assumed to reflect the presence of anti-LSP antibodies in the serum under test, an assumption validated in previous specificity studies.⁶

Results

Anti-LSP antibodies were detected in sera from 15 (27%) of the 55 cases tested. Analysis of the results in relation to the categories of disease as defined histologically showed striking differences in the frequency of anti-LSP in the different groups. The antibody was detected most often in sera from cases with active cirrhosis with or without alcoholic hepatitis (60% and 60% respectively) and was only found in one (11%) of the nine patients with acute alcoholic hepatitis alone and none of the six with inactive cirrhosis (Figure). This finding would suggest a close association between anti-LSP and periportal inflammatory activity (mainly found in, and characteristic of, active cirrhosis) and this was confirmed when the findings were related to the presence or absence of individual histological features (Table). Thus, anti-LSP was detected in 10 (56%) of 18 patients whose biopsies showed a portal mononuclear infiltrate with piecemeal necrosis of periportal hepatocytes, but in only five (14%) of the remaining 37 cases (χ^2 10.8, $p < 0.005$). Weaker associations, which were not statistically significant, with alcoholic hyaline and cirrhosis probably reflected the tendency for both these lesions to be associated with periportal inflammation.

As individual histological features had been scored with respect to their severity, it was also possible to examine the relationship between the frequency of anti-LSP antibodies in serum and the

Figure Percentage ¹²⁵I LSP bound by sera from the 55 patients with alcoholic liver disease divided into groups according to the histological appearances on liver biopsy. The dotted line indicates the upper limit of the normal range.

Table Anti-LSP and histological features on liver biopsy in alcoholic liver disease

	Periportal activity			Lobular inflammation			Hyaline inclusions			Fatty change		
	Anti-LSP			Anti-LSP			Anti-LSP			Anti-LSP		
	(no.)	(%)		(no.)	(%)		(no.)	(%)		(no.)	(%)	
Absent	37	5	14	9	3	33	21	4	19	11	2	18
Present	18	10	56	46	12	26	34	11	32	44	13	30

intensity of each histological lesion. The results again underlined the specificity of the association between anti-LSP and portal and periportal inflammation. In the majority of those patients with this histological feature, the lesion was mild and anti-LSP was detected in 50%. Two cases had a moderate lymphocytic portal infiltrate with piecemeal necrosis of periportal hepatocytes and anti-LSP was present in both. None of the patients had severe periportal changes, comparable with those found in most patients with chronic active hepatitis.

There was no significant association between anti-LSP antibodies and abnormalities in any of the standard biochemical tests of liver function, including serum levels of aspartate transaminase, alkaline phosphatase, gamma glutamyl transpeptidase, and bilirubin, or with total globulin, individual immunoglobulins, or autoantibodies reacting with smooth muscle or nuclear antigens. There was no significant difference in the sex ratio between those with and those without anti-LSP in the serum.

Discussion

The most striking finding in the present study is the close association between the presence of anti-LSP antibodies in serum and the histological features of portal and periportal lymphocyte infiltration together with piecemeal necrosis of periportal hepatocytes. In our previous study of lymphocyte cytotoxicity to rabbit hepatocytes in patients with alcoholic liver disease, there was also a close correlation between the presence of cytotoxic lymphocytes and portal and periportal inflammation.⁴ In contrast with the results of the present study a significant correlation with histological features of alcoholic hepatitis was also found, but all but two of those patients had cirrhosis and a separate group with acute alcoholic hepatitis alone were not studied. Nine such patients were investigated in the present study and anti-LSP antibodies were detected in only one. This argues against the possibility that an anti-LSP response is simply a reflection of liver cell damage, and additional evidence against such a view is the lack of any correlation between the presence of anti-LSP and serum aspartate aminotransferase levels.

Detailed analysis of the relationship between anti-LSP antibodies and histological appearances in liver tissue have now been reported by us in three conditions—chronic active hepatitis,⁷ primary biliary cirrhosis,⁸ and alcoholic liver disease. In all three there is a close relationship between the presence of this autoantibody and portal and periportal lymphocyte infiltration with piecemeal necrosis. Thus, anti-LSP, although not disease specific, was seen to be

'lesion specific' and, as similar histological features can be induced in rabbits by immunisation with human liver fractions containing LSP,⁹ it seems increasingly likely that immune responses to antigen(s) in the LSP preparation are the cause, rather than the result, of periportal liver cell necrosis.

It is now clear that there are several antigenic determinants within the LSP preparations,¹⁰⁻¹² one of which is liver specific but species cross-reactive, a second which may be liver and species specific, and possibly a third which cross-reacts with a component in other tissues.¹³ All LSP determinants seem to be present in or on the hepatocyte membrane and controlled fragmentation of the cell membrane or the LSP complex followed by isolation of the relevant components will be necessary before the specificity of the anti-LSP response in different liver diseases can be analysed in detail.

The immunological interactions which lead to the production of anti-LSP antibodies in association with increased alcohol intake are unclear. In acute viral hepatitis high titres of anti-LSP are found during the acute stages and persist in those showing progression to chronic liver disease.⁷ A similar autoimmune response may in some patients be triggered by acute alcoholic hepatitis, and if this continues the resulting piecemeal necrosis could favour the development of cirrhosis. Inactive cirrhosis could represent a terminal stage in which immune reactivity becomes depressed as a consequence of the cirrhotic process, but another possibility is that the cirrhosis in these cases developed directly from a preceding alcoholic hepatitis without triggering any autoimmune response. Serial studies documenting the changes in immune responses to alcohol-altered and self antigens over prolonged periods in patients with varying degrees of liver damage and alcohol intake will clearly be of considerable importance in clarifying the pathogenetic mechanisms determining the progression to cirrhosis.

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