Histocompatibility antigens, autoantibodies, and immunoglobulins in alcoholic liver disease

R J BAILEY, N KRASNER, A L W F EDDLESTON, ROGER WILLIAMS, D E H TEE, DEBORAH DONIACH, L A KENNEDY, J R BATCHELOR

Summary

Determination of histocompatibility antigens in 63 patients with alcoholic liver disease showed that HLA-B8 was more prevalent in patients with cirrhosis than in controls, but among those with fatty liver and minimal fibrosis the prevalence of this antigen was normal. Another noticeable difference was the absence of HLA-A28 in the cirrhotic group. In the total series of 219 patients the prevalence of antinuclear and smooth muscle antibodies was raised; they were especially prevalent in patients with cirrhosis. Raised serum IgA and IgG concentrations were also common (found in 50% and 37%, respectively) and were again significantly associated with cirrhosis. In contrast, serum IgM levels, which were raised in 46%, of cases, were not significantly related to the presence of cirrhosis but correlated significantly with the degree of portacaval shunting.

These results support recent evidence suggesting that immune responses may be implicated in alcohol-induced liver damage, particularly in its progression to cirrhosis.

Introduction

Although the development of cirrhosis in the alcoholic is directly related to the quantity of alcohol and the time taken to consume it, an individual susceptibility also seems to be present. This...
may be based on genetic predisposition, and the occasional progression to cirrhosis after alcoholic hepatitis despite abstinence from alcohol, also suggests that factors other than direct toxicity must play a part. The recent demonstration of lymphocyte sensitisation to liver antigens and direct cytotoxicity to hepatocytes in patients with alcoholic liver disease suggests that immune reactions are implicated at some stage in pathogenesis.

Similar immune responses occur in chronic active hepatitis, and in this there is an increased prevalence of the histocompatibility antigen HLA-B8. We have studied the distribution of histocompatibility antigens in patients with various types of alcoholic liver damage. Serum autoantibodies and immunoglobulins were also measured for further indirect evidence of an immune process and of a relation between the titre of autoantibodies and particular histocompatibility antigens as in active chronic hepatitis.

Patients and methods

Histocompatibility antigens were determined by a microcytotoxicity technique in 63 patients with alcoholic liver disease. Fifty-three patients had cirrhosis with or without features of simultaneous alcoholic hepatitis, and 10 were fatty liver with early portal tract fibrosis, alcoholic liver damage being confirmed by liver biopsy in each patient. None of the patients were persistently HBsAg positive by radioimmunoassay, although the antigen was detected transiently in two patients, one of whom developed acute type B hepatitis. The prevalence of histocompatibility antigens among these patients was compared, by a test with continuity correction, with that in a control group of 95 subjects from the same area of south-east England.

These 63 patients together with a further 156 (219 in all) formed the series in which the prevalence of autoantibodies and immunoglobulin levels were determined. Alcoholic liver disease was histologically confirmed in every case. Antinuclear, smooth muscle, and antimitochondrial antibodies were detected by indirect immunofluorescence. The ages of these patients ranged from 31 to 74 years (mean age 52 years), and results were compared with those from 260 healthy controls aged from 5 to 86 years (mean 44 years). IgG, IgA, and IgM concentrations were determined by the method of Mancini. In 77 of the 219 patients an estimate of portosystemic shunting was obtained from liver scintiscans with technetium sulphur colloid by determining the peak count rate over the spleen.

Results

Twenty-four (45%) of the 53 patients with cirrhosis had HLA-B8 compared with 24 (25%) of the 95 controls (P <0.025). In the 10 patients with fatty liver and minimal fibrosis there was no increase in prevalence of HLA-B8 (table I). The first series antigen A28 was completely absent in the cirrhotic group but present in 14 (15%) of the controls (P <0.001 by Fisher’s exact test). The distribution of ABO and rheus blood group antigens was normal in all the groups examined.

Autoantibodies—Antinuclear antibody was present in 13% of the 219 patients compared with 4.6% of the controls, a statistically significant difference (P <0.001). In most patients titres were 1/10 or 1/20 but were over 1/80 in four. Similarly, smooth muscle antibody was present in 21% of patients compared with only 1.9% of controls (P <0.001). Again, although most had low titres (1/10 and 1/20), eight patients had smooth muscle antibody titres of 1/40 to 1/80. Antimitochondrial antibody was present in four (2%) patients, a prevalence not significantly different from that of the control group.

Immunoglobulins—Increases in serum immunoglobulin concentrations were also common in the 219 patients (raised IgG in 37%, IgA in 59%, and IgM in 46%). Increases in two or three of the immunoglobulins were often found in the same patient, although increases in the concentration of a single immunoglobulin were also observed. IgA concentrations alone were raised above normal in 11% of patients, while solitary increases in IgM and IgG concentrations were found in fewer patients (6% and 5.5%, respectively). The index of collateral shunting obtained from the peak count rate over the spleen on the scintiscan showed a significant association with IgM levels (r = 0.4138; P <0.01) but not with IgG or IgA concentrations. When examined in relation to the type of liver disease present, there was a significant association between increased concentrations of IgG and IgA and the presence of cirrhosis (table II). A similar association was found for smooth muscle antibodies but not for antinuclear antibodies.

Unfortunately there were too few patients with autoantibodies who had their histocompatibility antigen phenotypes determined to allow analysis of any association between autoantibody titres and the presence of HLA-B8 or any other histocompatibility antigens.

Discussion

If immune reactions are important in the pathogenesis of alcoholic liver disease then patients with the most severe damage—that is, cirrhosis—would be expected to have evidence of a more intense immune response, and our results suggest that they do.

In several studies it has been suggested that the increase in immunoglobulin concentrations in alcoholic cirrhosis results from collateral shunting allowing antigens derived from the gut to bypass the liver and stimulate production of antibodies in the spleen. Our finding of a correlation between the increase in serum IgM and a measure of portosystemic shunting supports

<table>
<thead>
<tr>
<th>TABLE I—Percentage distribution of histocompatibility antigens, including HLA-A28 and HLA-B8</th>
</tr>
</thead>
<tbody>
<tr>
<td>First series antigens</td>
</tr>
<tr>
<td>Controls Patients with alcoholic liver disease and cirrhosis</td>
</tr>
<tr>
<td>Patients with alcoholic liver disease without cirrhosis</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TABLE II—Relation of serum autoantibodies and immunoglobulins to type of alcoholic liver damage. Results are percentages of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autoantibodies</td>
</tr>
<tr>
<td>No tested</td>
</tr>
<tr>
<td>Patients with fatty change with or without fibrosis</td>
</tr>
<tr>
<td>Patients with cirrhosis</td>
</tr>
</tbody>
</table>

| Significance of difference between those with and those without cirrhosis | NS | P <0.05 | NS | P <0.05 | P <0.01 | NS |

ANA = Antinuclear antibody. SMA = Smooth muscle antibody. AMA = Antimitochondrial antibody. NS = Not significant.
this hypothesis, but the lack of a similar correlation for IgG and IgA suggests that other factors may be important, one possibility being a general increase in immune responsiveness.

The prevalence of autoantibodies in the present series belies the generally accepted view that they are rarely, if ever, found in alcoholic cirrhosis.\(^7\) They were less prevalent in our patients than in those with chronic active hepatitis\(^7\) but more prevalent than in the controls. Furthermore, although the titres were generally low, similar titres are found in some patients with chronic active hepatitis.\(^3\)

We have suggested that the higher prevalence of HLA-B8 in chronic active hepatitis occurs because this antigen is linked to genes that promote abnormally raised and prolonged antibody responses.\(^8\) While HLA-B8 is also fairly prevalent (45%) in alcoholic cirrhosis, the association is not as great as in HBsAg-negative chronic active hepatitis, in which more than 60% of the patients have this histocompatibility antigen.\(^9\) Similarly, the higher prevalence of serum autoantibodies and raised immunoglobulin concentrations is not as striking as in chronic active hepatitis,\(^10\) and thus immunologically these two groups of patients seem to differ in the intensity of their immune responses. The biological significance of the absence of HLA-A28 is not clear, but this antigen was also not found in any of our patients with HBsAg-negative chronic active hepatitis\(^11\) nor in those originally reported by MacKay and Morris.\(^9\)

In spite of these genetic similarities there are obvious clinical and histological differences between the two diseases, and there are probably qualitative, as well as quantitative, differences in their immunopathogenesis. Thus, T lymphocytes were recently found to predominate (91%) in the portal tract infiltrates in alcoholic liver disease, whereas the proportion in patients with chronic active hepatitis seemed to be much lower.\(^12\) Possibly these T lymphocytes are reacting to the changes that alcohol may induce in hepatocyte membranes\(^10\) and are actively implicated in the destruction of these changed liver cells. The intensity of this reaction may determine the rate of progression to cirrhosis, and any factor that may be associated with unusually intense immune responses, such as high immunoglobulin concentrations, autoantibodies, and possibly HLA-B8, would therefore be expected to be particularly common in those who have developed cirrhosis.

We are indebted to the Wellcome Trust for their continued generous support and to Dr Bernard Portmann for the histological assessments. RJB was supported by the Canadian Hepatic Foundation.

References

Effect of ethyloestrenol on fibrinolysis in the vessel wall

U HEDNER, I M NILSSON, S ISACSON

British Medical Journal, 1976, 2, 729-731

Summary
Forty-nine patients with decreased fibrinolytic activity in the vessel walls or a decreased release mechanism, or both, were treated with ethyloestrenol for three to 17 months. Forty-five of the patients had had recurrent, phlebographically verified, deep venous thrombosis (DVT) and four had arterial thrombosis. Ethyloestrenol 8 mg/day was given to 31 patients and 4 mg/day was given to 12. The remaining six patients had been treated with a combination of phenformin and ethyloestrenol. The phenformin was withdrawn but they were kept on ethyloestrenol 8 mg/day. Another 15 patients with a normal fibrinolytic system—four with recurrent DVT and 11 with severe arteriosclerosis—were given ethyloestrenol 8 mg/day.

Coagulation Laboratory, Allmänna Sjukhuset, Malmö, and Department of Medicine, Halmstad, Sweden
U HEDNER, MD, associate professor, coagulation laboratory
I M NILSSON, MD, professor of medicine, coagulation laboratory
S ISACSON, MD, associate professor, department of surgery

The spontaneous fibrinolytic activity, local fibrinolytic activity during standardised venous occlusion of the arms, and fibrinolytic activity of the vessel walls increased significantly after treatment with ethyloestrenol 8 mg/day for three months. No further increase occurred after three months, and ethyloestrenol 4 mg/day had no effect. No values rose significantly in the patients with a normal fibrinolytic system. One patient suffered a recurrence within three months of treatment, before the fibrinolytic system became normal. In one patient the fibrinolytic defect reappeared after 10 months in spite of continued treatment. Two of the three women of fertile age developed irregular cycles and intermenstrual bleeding, which disappeared when the treatment was withdrawn. No other side effects were observed.

Introduction
A defective fibrinolytic defence mechanism (decreased fibrinolytic activator activity in the vein walls or a defective release of fibrinolytic activator from the vein walls, or both) has been found in about 70% of patients with recurrent deep venous thrombosis (DVT) and no known predisposing condition.\(^1\) Fearnley et al\(^3\) showed that phenformin (100 mg/day) combined with ethyloestrenol (8 mg/day) increased the spontaneous fibrinolytic activity of the blood. The same treatment was later