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SERUM AUTOANTIBODIES IN HEPATITIS ASSOCIATED ANTIGEN (HAA) POSITIVE PATIENTS

A POSSIBLE INDEX OF CELL-MEDIATED IMMUNITY TO THE ASSOCIATED-INFECTIVE AGENT

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SUMMARY

Sera from 134 HAA positive patients have been studied for the presence of a variety of autoantibodies. The patients were divided into groups dependent on the clinicopathological severity of the associated liver disease. Both smooth muscle antibody and rheumatoid factor were found in all groups of patients. However, their incidence and titre was higher in the groups of patients who had clinical and pathological evidence of the more active liver cell damage. As autoantibodies in viral infections may be an index of the presence of cell mediated immunity to the causative infective agent these results could support the hypothesis that cell mediated immunity is important in the pathogenesis of liver cell damage in HAA positive patients.

INTRODUCTION

HAA has been found in the sera of a variable proportion of patients with different clinical and histological types of acute and chronic liver disease (Blumberg *et al.*, 1967; Wright McCollum & Klatskin, 1969; Prince, 1971; Sherlock *et al.*, 1970). Its presence in such patients is thought to implicate the infective agent responsible for long incubation, Type B, hepatitis in the pathogenesis of the associated liver disease (Prince, 1968; Giles *et al.*, 1969). Transient presence of HAA is seen in association with either clinical or anicteric acute hepatitis (Krugman & Giles, 1970). Persistence of HAA may be associated with minimal disturbance of liver morphology, chronic persistent hepatitis, chronic aggressive hepatitis (Nielsen *et al.*, 1971), active cirrhosis (Prince, 1968) and possibly liver cell carcinoma (Sherlock *et al.*, 1970). Which clinical course is followed in the individual patient is thought to be determined by differences in both the infective agent and the hosts immune response. However, there is growing evidence to suggest that the host's cellular immune response is of predominant importance in determining the severity of the associated liver cell damage

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(Dudley, Fox & Sherlock, 1972). Such a response will be mediated by thymus-derived (T) lymphocytes.

It has recently been postulated that in viral infections the production of serum autoantibodies depends on the presence of responsive T cells against viral related antigens (Allison, Denman & Barnes, 1971). The consequent T cell stimulation leads to co-operation with antibody-forming B cells which are then stimulated to produce antibody against any host antigen that is intimately associated with the viral specific antigens as on the surface of infected host cells. The production of these autoantibodies would thus depend on competent T cell function directed against the viral antigens.

Hence in HAA positive liver disease one would expect both the titre and presence of autoantibodies to reflect the presence and degree of specific cell mediated immunity against antigenic determinants of the associated infective agent.

In order to study this possibility we have measured a number of autoantibodies in the sera of 134 patients with a variety of HAA positive acute and chronic liver disease.

Patients

134 patients, all HAA positive, were studied. These patients were classified into various groups according to clinical, biochemical and/or, histological findings. Fifty-two patients had acute hepatitis, thirty-five had chronic aggressive hepatitis or active cirrhosis, twenty-three had a chronic persistent hepatitis and fifteen were asymptomatic carriers of HAA who had no biochemical evidence of liver disease. These different groups represent patients with decreasing evidence of liver damage. Nine patients with primary liver cell carcinoma formed a final group. All nine of these patients had evidence of an underlying cirrhosis with variable degrees of liver cell failure.

Sera from thirty-five normal subjects aged 18–55 yr were also examined to act as controls.

MATERIALS AND METHODS

Sera collected from each patient was stored at −20°C until the day of testing. Each serum specimen tested was HAA positive and all were coded and tested without knowledge of their source.

HAA was detected in the sera using a standard human antiserum which gave reactions of identity with antisera from Prince and Blumberg (Fox, Niazi & Sherlock, 1969). This antiserum was used to detect HAA by either two dimensional gel diffusion, counter immunoelectrophoresis or complement fixation.

Antinuclear, mitochondrial, smooth muscle and gastric parietal cell antibodies were detected by the indirect immunofluorescent technique (Holborow & Johnson, 1964). This was modified by the introduction of a 3-min period of fixation in absolute ethanol at room temperature before placing the fluorescein-labelled anti-human immunoglobulin serum on the tissue sections. All sera were tested at dilutions of 1:10 and 1:40 on rat kidney and stomach (fundus) sections cut from material which had been frozen in isopentane chilled to its melting point in liquid nitrogen. The results were read with a Reichert Zetopan microscope using quartz iodine illumination. Weak positive reactions were ignored, moderate reactions recorded as positive and strong reactions at 1:40 further titrated.

Anti-immunoglobulin G antibody (rheumatoid factor) was measured by the slide latex

test using human F 11 coated latex particles (Hyland RA test) added to 1:20 dilutions of sera. The degree of agglutination after one minute was graded as negative, +, ++ or +++.

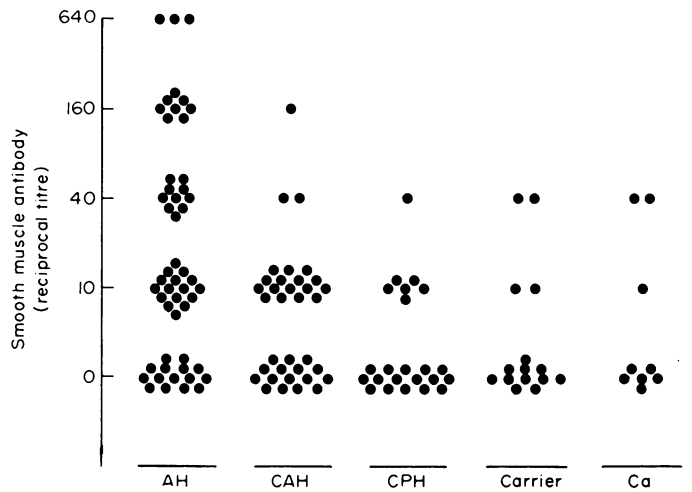

FIG. 1. The reciprocal titre of smooth muscle antibody in the serum of different groups of HAA positive patients. AH = acute hepatitis; CAH = chronic aggressive hepatitis; CPH = chronic persistent hepatitis; Ca = primary liver cell carcinoma.

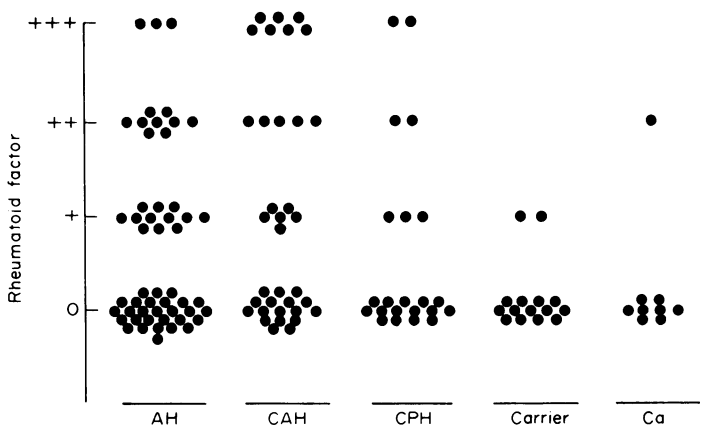

FIG. 2. Serum rheumatoid factor in different groups of HAA positive patients. AH = acute hepatitis; CAH = chronic aggressive hepatitis; CPH = chronic persistent hepatitis; Ca = primary liver cell carcinoma.

RESULTS

Autoantibodies were looked for in the serum of all patients except for one patient with chronic persistent hepatitis in whom the presence of rheumatoid factor was not tested. Antinuclear factor and mitochondrial antibody were not detected in any of the patients

studied. Gastric parietal cell antibodies were found in only three patients, all at a titre of 1:10. Two of these patients had acute hepatitis and the other chronic aggressive hepatitis without cirrhosis. Both smooth muscle antibody and rheumatoid factor were found in all groups of patients (Figs 1 and 2).

Smooth muscle antibody was found most frequently and of highest titre in the patients with acute hepatitis. It was less common and of lower titre in the patients with either chronic aggressive hepatitis or active cirrhosis and least common and also of low titre in all other three groups of patients. Smooth muscle antibody at a titre of 1:10 was found in six of our thirty-five control subjects (17%). This was a slightly lower incidence and titre than was found in the last three groups of patients, those with chronic persistent hepatitis, primary liver cell carcinoma and the healthy HAA carriers.

The incidence of rheumatoid factor also differed in the various groups. It was found most commonly and of highest titre in patients with either active cirrhosis or chronic aggressive hepatitis, slightly less often in patients with acute hepatitis, still less in patients with chronic persistent hepatitis and least in both patients with primary liver cell carcinoma and healthy HAA carriers. Rheumatoid factor was only present in one of our thirty-five controls.

No definite correlation was found between the presence of smooth muscle antibody and rheumatoid factor.

DISCUSSION

Our results with smooth muscle antibody compare variably with the incidence found in other reports in HAA positive hepatitis. In acute hepatitis smooth muscle antibody has been found in up to 87% of cases and would appear to occur in equal frequency and titre independent of the presence or absence of HAA (Farrow *et al.*, 1970; Ajdukiewicz *et al.*, 1972). The lower incidence of 25% positive in another report (Wright, 1970) may have been due to the use of specific anti-human IgG fluorescein conjugate to detect the antibody. It has been demonstrated that, at least initially, the smooth muscle antibody seen in patients with acute hepatitis is predominantly IgM (Farrow *et al.*, 1970). In patients with chronic liver disease and HAA positive sera our results appear to correlate less well with those of other workers. Vischer (1970) found no smooth muscle antibody in any of his patients with HAA positive chronic liver disease but was only accepting as positive titres of 1:40 or greater. Wright (1970) also found no smooth muscle antibody in six patients with HAA positive chronic aggressive hepatitis and only one of three patients with HAA positive subacute hepatic necrosis. In this study the fluorescein conjugated antibody was again specific for IgG. The difference in these results may be partly due to the fact that we have recorded as positive titres greater than or equal to 1:10 and that our fluorescein conjugated antibody was not specific for IgG only.

Rheumatoid factor has also been reported in the serum of patients with HAA positive acute hepatitis (Ziegenfuss, Miller & Rossman, 1971) and our findings corroborate this report.

None of our patients had antinuclear factor in their serum. This contrasts with the high correlation between the presence of both antinuclear factor and HAA in acute hepatitis reported by Farrow *et al.*, 1970. There appears to be uniformity in the results for antinuclear factor in HAA positive chronic hepatitis, its presence being unusual in all such reports (Vischer, 1970; Bulkley *et al.*, 1970). The absence of mitochondrial antibodies in

these different groups of patients also agrees with other studies (Farrow *et al.*, 1970; Vischer, 1970).

The interesting point about our findings in this study is the varying incidence of autoantibodies in our different groups of patients. As the pathological evidence for active liver cell destruction becomes less so does the incidence of autoantibodies. It would appear unlikely that the mechanism of production of these autoantibodies was related non-specifically to the liver cell damage as one would then expect their occurrence in all active liver disease independent of aetiology and also a higher incidence in our group of patients with primary liver cell carcinoma.

The formation of autoantibody in viral infections has recently been suggested to be dependent on T and B cell co-operation (Allison *et al.*, 1971). If T cell sensitization develops against viral dependent antigens closely associated with host antigens as in the surface membrane of infected cells, B cells capable of recognizing that host antigen will be stimulated to produce antibody. This system is analogous to the immunological response to an hapten-carrier antigen (Benacerraf, 1971; Mitchison, 1971). A strong antibody response to the hapten requires the presence of immunity of the T cells against the carrier. If such a scheme operates in viral infections then the development of autoantibodies during the infection should be a marker of the presence of specific T cell immunity against the responsible virus. Hence the variable incidence of autoantibodies in our patients could indicate the presence of extensive specific T cell immunity against viral-dependent antigens in patients with acute hepatitis, less in patients with chronic aggressive hepatitis and active cirrhosis, still less in those with chronic persistent hepatitis and least in those with primary liver cell carcinoma and the healthy carriers. The lack of adequate T cell function is well documented in patients with a variety of malignant neoplasms (Alexander & Hamilton Fairley, 1967).

If such a scheme is applicable in our patients one should be able to demonstrate in HAA positive patients a close association between viral-specific antigens and the antigenic determinants found in smooth muscle and IgG.

Sera containing smooth muscle antibody is capable of reacting with a normal constituent of the membranes of certain human cell types including the liver (Farrow, Holborow & Brighton, 1971). This reaction can be blocked by prior absorption of the antibody by a variety of smooth muscle cells. Hence there is a cross-reacting antigen in both liver cell membrane and smooth muscle. During infection with viruses, virus-specific antigens can be found on the surfaces of infected host cells (Allison *et al.*, 1971). Hence in HAA positive patients where the infective agent presumably proliferates in the liver cell there should be a close relationship of virus specific antigen and an antigen immunologically similar to that found in smooth muscle cells.

A humoral antibody response to virally related antigens could also be expected in HAA positive patients. The circulating antibody produced would be expected to complex with viral-dependent antigens and hence lead to intimate contact between both immunoglobulin and the viral antigens. If T cell function against these viral antigens is present this should predispose to autoantibody formation against the complexed immunoglobulin. As rheumatoid factor is antibody against IgG this could explain the slightly lower incidence of rheumatoid factor in patients with acute hepatitis compared to chronic aggressive hepatitis and active cirrhosis as the antibody to the viral antigens produced early in the disease is more likely to be IgM (as in the primary immune response) becoming predominantly IgG later in the chronic phase of the disease.

These results would thus support the concept that normal T cell function against the infective agent associated with HAA is necessary to produce acute hepatitis and that impairment of such immunity is related both to persistence of the infective agent and continuing liver damage whereas absence of specific T cell immunity will be related to persistence of the antigen without continuing liver cell damage.

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