The Incidence of Australia Antigen/Antibody in Acute and Chronic Liver Disease

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SUMMARY

The Australia (Au/SH) antigen was sought in patients with acute viral hepatitis, chronic hepatitis, cirrhosis and hepatoma of the liver. Thirty per cent of patients with infectious hepatitis and 50% of patients with serum hepatitis had positive tests for Australia antigen. Antibody to the Australia antigen was present in 11·4% of patients with infectious hepatitis, and in 16% of patients with serum hepatitis, thus increasing the over-all positive test to 41·4% and 66% respectively. Screening for antibody is regarded as being of useful diagnostic assistance in patients with hepatitis and also for screening for hepatitis in potential blood donors.

The incidence of positive tests for Australia antigen in chronic hepatitis was 26%, in cirrhosis 6% and in hepatoma 25%.

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An impressive literature has accumulated on the subject of the Australia (Au/SH) antigen since its description by Blumberg *et al.* in 1965.[3] The relationship of this antigen to viral hepatitis was later defined by Blumberg and colleagues,[4] and subsequently many reports have confirmed this relationship, and have extended it from acute liver disease to chronic and neoplastic liver disease[2,10,13,16,19,20,22,25,26,28,29]

While these initial studies were being pursued, a large clinical and epidemiological study was undertaken by Krugman *et al.*[17] at the Willowbrooke State School. These studies defined two types of acute viral hepatitis; a short-incubation-period type designated MS-1 hepatitis (synonymous with catarrhal jaundice or infectious hepatitis), and a long-incubation-period type designated MS-2 hepatitis (synonymous with homologous serum jaundice or serum hepatitis). Subsequently the putative infective agent has been designated virus A and virus B. One of the most important findings to emerge from the clinical study was the clear demonstration that MS-2 hepatitis could be acquired by the faecal-oral route, in addition to its better known acquisition by parenteral administration.

A retrospective analysis of the sera collected during this study by Giles *et al.*[9] and Prince *et al.*[23] demonstrated that the Au/SH antigen was detectable only in those cases of MS-2 hepatitis, by whichever route it was acquired. It is of interest that epidemic hepatitis has been associated with very low number of positive tests for Au/SH antigen.[7,23] The recent demonstration by Del Prete *et al.*[8] of a different antigen found in high incidence early in the course of MS-1 hepatitis, has further defined these two types of infection. On the basis of the available evidence, Prince *et al.*[23] have suggested that the finding of Au/SH antigen in serum from patients with hepatitis is evidence of MS-2 hepatitis, however acquired.

An examination of the incidence of the Au/SH antigen in all forms of liver disease, and in the normal population, is being carried out in view of the high incidence of acute and chronic liver disease in Southern Africa.

MATERIALS AND METHODS

Australia Antigen

Reference Au/SH antigen was kindly supplied by Drs B. S. Blumberg and A. M. Prince. These reference antigens were used initially to screen suitable sera for the presence of anti-Au/SH antibody and later they were used as reference reagents only. We have as a routine used a positive Au/SH serum obtained from a patient who subsequently died of fulminant hepatic necrosis (presumed viral hepatitis).

Anti-Au/SH Antibody

Anti-Au/SH antibody was first obtained from a patient who subsequently died of fulminant hepatic necrosis (presumed viral hepatitis) and later a source of antibody was a patient from the renal dialysis unit who had never experienced overt hepatitis, and a patient, screened as a routine for Au/SH antigen, with no history of hepatitis.

Hepatitis Serum

Serum was obtained from patients admitted to the wards with suspected hepatitis. Blood was drawn by venepuncture, allowed to clot at room temperature for 1 hour and separated by centrifugation. All sera were stored at −20°C until used.

Detection of Au/SH Antigen

The counter-electrophoresis method of Gocke and Howe[11] was used with slight modifications. The essential feature of this method is that antigen and antibody are

electrophoresed against each other so as to bring the reactants together more rapidly than in the usual diffusion methods (Fig. 1). Ten to fifteen millilitres of melted 0·85% Ionagar (Oxoid No. 2) in veronal buffer (0·05 M, pH 8·6) was poured onto glass plates measuring 7·5 cm by 10·0 cm.

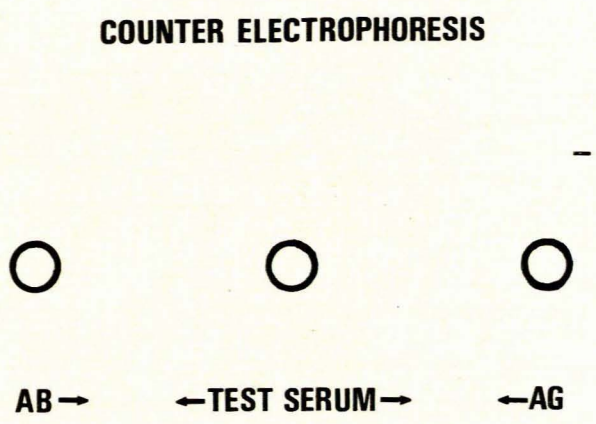

Fig. 1. Schematic representation of counter-electrophoresis. Antibody is placed in the well nearest the anode and antigen in the well nearest the cathode. Arrows indicate the direction of migration of the proteins. Serum in the centre well migrates in both directions.

After the gel had set, wells were punched in the agar in triplicate (well diameter 3 mm, distance between wells 5 mm) with a template. The plate was transferred to an electrophoresis tank. Then 0·01 - 0·02 ml of serum containing Au/SH antigen was placed in the well nearest the cathode and a similar volume of serum containing anti-Au/SH antiserum was placed in the well nearest the anode. The unknown serum was placed in the centre well. An electric current of 15 mA/plate was then applied for 90 minutes at room temperature (22°C), using a bridge buffer of veronal acetate (pH 8·6). Four plates with 10 rows of triplicate wells were prepared for each electrophoretic run. It was thus possible to test 40 serum samples each time.

At the completion of the electrophoresis, precipitin lines could be seen between some wells. The plates were then placed in 0·9% saline to wash out excess protein. During this period further precipitin lines were seen to have developed. Finally, to define poorly visible precipitin lines more clearly, the plates were dipped into 1% tannic acid (M+B reagents) for 10 minutes. This procedure was most useful when doubtful lines were seen. All sera giving doubtful lines were concentrated by pressure dialysis and retested against the routine standard reagents and against a panel positive sera (antigen and antibody).

RESULTS

Infectious Hepatitis

Seventy sera from patients with infectious hepatitis were examined. There were 64 adults and 6 children in this group. All the patients gave a typical short history preceding the onset of the jaundice, except the solitary patient with anicteric hepatitis. All denied any contact with blood or blood products. Nineteen (27%) of 64 adult sera were positive for the Au/SH antigen, and 8 (11·4%) were positive for anti-Au/SH antibody. Two (33%) of 6 children were positive for Au/SH antigen, and no antibody was found in this group. There were 7 deaths in this group of patients (6 adults, 1 child). In one of these patients anti-Au/SH antibody was found and in another the Au/SH antigen was found. In most patients the tests represented a single determination in the course of the illness. In 6 patients several tests were done over the course of 2 - 3 weeks and showed either antigen or antibody persistence. In one patient the initial test was negative but became positive after two weeks (Table I).

TABLE I. THE INCIDENCE OF AU AND ANTI-AU ANTIBODY IN ACUTE AND CHRONIC LIVER DISEASE

Classification	No. tested	Total positive	Positive Au antigen	Antibody
Infection hepatitis:				
Adults	64	27	19	8
Children	6	2	2	—
Serum hepatitis	24	16	12	4
Blood donors*	94	5	1	4
Chronic hepatitis	15	6	4	2
Cirrhosis	31	2	2	—
Hepatoma	8	2	2	—

* Suspected of having transmitted hepatitis.

Serum Hepatitis

Twenty-four patients were classified in this group on the grounds of a history of exposure to blood or blood products 1 -3 months previously, and clinical/biochemical evidence of acute liver dysfunction. In 12 (50%) sera precipitin lines developed against the standard antibody, while 4 (16%) showed the presence of anti-Au/SH antibody. Ninety-four blood donors, suspected of having transmitted the infection to these patients with serum hepatitis, were recalled for testing retrospectively. One of these donors was shown to carry the Au/SH antigen and repeated testing has shown this antigenaemia to have persisted for 6 months. Four donors had anti-Au/SH antibodies in their serum. None of these donors had any illness suggestive of hepatitis, either before they donated blood, or subsequently before their recall for testing.

Chronic Hepatitis

This group of patients comprised 15 patients with features suggestive of active chronic hepatitis, or with persistent hepatitis. Four (26%) sera contained Au/SH antigen and 2 (13%) contained anti-Au antibody.

Cirrhosis of the Liver

The sera of 31 patients with cirrhosis were tested. Evidence of cirrhosis was confirmed clinically, biochemically and histologically. These patients represent a heterogeneous group of cirrhotics, the majority of which was on an alcoholic basis. The Au/SH antigen was present in 2 (6·4%).

Hepatoma of the Liver

Eight sera from patients with hepatoma were examined. Positive tests for the Au/SH antigen were detected in 2 (25%). As far as could be ascertained from the protocols none of these patients had been exposed to blood or blood products. None of these patients was receiving immunosuppressive or cytotoxic therapy before testing. None of the serum samples studied contained both antigen and antibody.

DISCUSSION

The reported incidence of Au/SH antigen in viral hepatitis has varied from 4·5% in the west of Scotland[24] to 80% in New York.[10] There are several reasons for this wide variation. Blumberg *et al.*[6] have suggested that a genetically determined host response may influence the number of positive tests in the same way that it appears to be operative in determining persistent antigen carriage. The timing of the tests in the course of viral hepatitis also appears to be important, thus several workers have demonstrated that the earlier tests are done in the course of the illness the greater the incidence of positive results.[5,10] It has also been shown that antigen may be present for only one day during the course of the illness.[27] The age of the patients tested may also be relevant since some workers have demonstrated a low yield of positive results in young patients.[24] A further difficulty has been the realization that a given case of hepatitis cannot be readily categorized as either MS-1 or MS-2 type infection.

We have found an incidence of 30% in infectious hepatitis and 50% in serum hepatitis. These findings are significant when viewed against a 1·9% prevalence of antigen in a sample of 1 800 healthy volunteer blood donors.[21] Our findings in chronic hepatitis, cirrhosis and in patients with hepatoma are similar to other reported series.

The incidence of antibody to Au/SH antigen in patients from the USA who have had multiple transfusions has been reported at 18 - 28%,[10,14] which stands in marked contrast to the low incidence reported from Australia and Britain.[20] In a prospective study of subjects who have had multiple transfusions, Holland *et al.*[15] found that 6% of patients who developed jaundice, and 12% of patients who did not develop jaundice, had antibodies to Au/SH antigen. In chronic liver disease Guardia *et al.*[12] have found that 20% of patients with cirrhosis of the liver had antibody to Au antigen. The incidence of antibodies in acute liver disease has not been reported with any frequency. Gocke and Kavey[10] mention the finding of antibody which gave poor precipitin lines in a few patients. We have found antibodies in 11·4% of our patients with infectious hepatitis and in 16% of the patients with serum hepatitis. In some patients the antibody has been weak and has produced poor precipitin lines, which have, however, been consistent when retested.

The finding of antibody in relatively large numbers of the patients with acute hepatitis increased the over-all incidence of positive tests from 30% to 41·4% in infectious hepatitis and from 50% to 66% in serum hepatitis. It is tempting to suggest that the finding of antibodies to Au/SH antigen represents direct antigenic stimulation because the antibodies have been related temporarily to disease activity. The immunological advantage to the host of these antibodies is, however, not clear. The antibodies have been shown to cause clumping of virus particles,[2] but have not been shown to protect against the development of serum hepatitis.[15] The formation of complexes between antibody and Au/SH antigen have been suggested as a possible explanation of the acute nephritic illness which occasionally complicates acute viral hepatitis.[1]

Although there is evidence which relates these antibodies to antigenic stimulation, it is possible that the detection of antibody represents a non-specific response to the virus causing hepatitis. Whatever the mechanism of their production, we believe that screening for antibody has diagnostic and epidemiological importance. From the data presented here we believe that routine screening for antibody is relevant when assessing population groups for the prevalence of hepatitis. It is also of importance when assessing the suitability of blood donors.

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