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Australia antigen and primary biliary cirrhosis

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SYNOPSIS Sera from 64 patients with primary biliary cirrhosis have been examined for Australia antigen (Au). On immunodiffusion and immunoelectrophoresis all sera were negative. Using a radioimmunoprecipitation technique 15·6% of sera contained antigen compared with an incidence of 3·1% in matched controls, a significant difference ($P = 0.015$). Anti-Au was found in 9·4% of patients and in 7·8% of controls. In lymphocyte transformation studies lymphocytes from one of 24 patients with primary biliary cirrhosis transformed on stimulation with an Au-rich serum.

Australia antigen (Au) and antibody (anti-Au) have been looked for by a number of workers in patients with primary biliary cirrhosis (Fox, Niazi, and Sherlock, 1969b; Wright, McCollum, and Klatskin, 1969; Kaboth, Schober, Arndt, Vido, Selmair, Gallasch, Verma, Thomssen, and Creutzfeldt, 1970; Reinicke and Nordenfelt, 1970; Vischer, 1970; Kaplan and Grady, 1971; Alarcón-Segovia and Fishbein, 1971; Doniach, del Prete, Dane, and Walsh, 1972; Maddrey, Saito, Shulman, and Klatskin, 1972). These investigations have in the main produced negative findings. However, Doniach and her colleagues (1972) reported that in 69 cases of primary biliary cirrhosis Au was demonstrable in 13%, using a radioimmunoassay method for its detection (Walsh, Yalow, and Berson, 1970), an incidence similar to that found in 60 cases of Hashimoto's thyroiditis with no prior history or evidence of acute liver disease. Coyne (Zavatone), Millman, Cerda, Gerstley, London, Sutnick, and Blumberg (1970), using an immunofluorescent technique, demonstrated Au in the liver cell nuclei of one patient with primary biliary cirrhosis.

In the present study we have sought evidence for an association between Au and primary biliary cirrhosis (*a*) by examining sera from patients with primary biliary cirrhosis and matched controls using immunodiffusion, immunoelectrophoresis, and radioimmunoassay methods; (*b*) by seeking evidence of cell-mediated immunity to Au using lymphocyte transformation in response to Au-rich serum.

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Materials and Methods

SEROLOGICAL EXAMINATION

Test sera were available from 64 patients (table I) in whom the clinical, biochemical, and serological criteria for a diagnosis of primary biliary cirrhosis had been established (Goudie, MacSween, and Goldberg, 1966; Scheuer, 1967; Sherlock, 1971). In 23 of these patients the diagnosis was confirmed from examination of liver biopsy material. These sera had been referred to the regional immunopathology laboratory (Glasgow) over the period 1967-71 and had been stored at −20°C. Age- and sex-matched control sera were selected from the same source, and the clinical details of these patients are summarized in table II. Care was taken to ensure that each control specimen had been stored at −20°C for a similar period ± two months as that for each matching test serum.

Each serum was examined for Au and anti-Au by immunodiffusion (Blumberg, London, and Sutnick, 1970a), by immunoelectrophoresis (Pesendorfer, Krassnitsky, and Wewalka, 1970), and by radioimmunoprecipitation assay (Coller, Millman, Halbherr, and Blumberg, 1971).

LYMPHOCYTE TRANSFORMATION STUDIES

These were performed on peripheral blood lymphocytes in 24 patients with primary biliary cirrhosis and 24 age- and sex-matched controls (table I), using as transforming agent a serum rich in Au and which had been shown to produce lymphocyte transformation in patients who had recovered from a previous clinical attack of Au-positive serum hepatitis

	A Primary Biliary Cirrhosis Group and Matched Controls	B Primary Biliary Cirrhoris Subgroup and Matched Controls—Lymphocyte Transformation Studies
No.	64	24
Sex	59 Female	22 Female
Mean age (yrs)	57·5 (range 30-78)	55·8 (range 30-75)
Au ID[1]	All negative	PBC sera only tested—all negative
Au IEOP[2]	All negative	PBC sera only tested—all negative
Au RIA[3]	See table III	[4]/18 (Au); [1]/18 (Au and anti-Au)

Table I *Details of patients and controls used in present study*

[1]ID —immunodiffusion
[2]IEOP —immunoelectrophoresis
[3]RIA —radioimmunoprecipitation assay: only 18 of the 24 sera in subgroup B were examined.

Various arthritides	14
Non-toxic goitre	12
Healthy members of nursing and medical staff	7
Various skin diseases	5
Connective tissue disease	5
Iron-deficiency anaemia	5
Cardiovascular disease	4
Neuromuscular disorders	3
Miscellaneous group	9
Total	64

Table II *Clinical details of 64 controls for patients with primary biliary cirrhosis*

(Yeung Laiwah, 1971). The method used for lymphocyte transformation studies has previously been described (Yeung Laiwah, 1971) and has been adhered to except that all cultures were set up in triplicate. In all 24 patients immunodiffusion and immunoelectrophoresis testing for Au and anti-Au was performed, and in 18 of the 24 radioimmunoprecipitation screening was carried out.

Results

AU AND ANTI-AU

On immunodiffusion and immunoelectrophoresis testing all 64 primary biliary cirrhosis sera and controls gave negative results for antigen and antibody. The results obtained on radioimmunoprecipitation are summarized in table III. The difference in the frequency of Au between patients and matched controls is highly significant ($P = 0.015$ using Fisher's exact 2×2 test).

	No. Studied	Au Positive	Anti-Au Positive
Primary biliary cirrhosis	64	10[1] (15·6%)	6[1] (9·4%)
Controls	64	2[2] (3·1%)	5[2] (7·8%)

Table III *Au and anti-Au results in primary biliary cirrhosis patients and controls using radioimmunoprecipitation*

[1]Two of these positive for both Au and anti-Au
[2]One of these positive for both Au and anti-Au

LYMPHOCYTE TRANSFORMATION STUDIES

In only one of 24 patients with primary biliary cirrhosis did the peripheral blood lymphocytes transform in response to an Au-rich serum, the lymphocytes showing a 3·5-fold increase in ^{14}C-thymidine incorporation as compared with unstimulated controls. This patient was a male aged 55, and on radioimmunoprecipitation there was no demonstrable Au or anti-Au in the serum. No lymphocyte transformation was obtained in lymphocytes from the 24 control patients using Au-rich serum. The results of Au and anti-Au screening in the sera of this subgroup of patients with primary biliary cirrhosis is summarized in table I.

Discussion

Since the discovery of Au by Blumberg, Gerstley, Hungerford, London, and Sutnick (1967) and the demonstration of its association with hepatitis, there have been a number of studies in which an association has been sought between occurrence of the antigen and the development of chronic liver disease. Primary biliary cirrhosis is one type of chronic liver disease in which Au has been sought by several workers, but in the main the use of immunodiffusion, immunoelectrophoresis, complement fixation, haemagglutination, haemagglutination inhibition, and electron microscopy have produced negative results (Fox *et al*, 1969b; Wright *et al*, 1969; Kaboth *et al*, 1970; Reinicke and Nordenfelt, 1970; Vischer, 1970; Kaplan and Grady, 1971; Alarcón-Segovia and Fishbein, 1971; Doniach *et al*, 1972; Maddrey *et al*, 1972). Alarcon-Segovia and Fishbein (1971), using complement-fixation methods, reported Au in five of seven patients with primary biliary cirrhosis. Kaplan and Grady (1971) investigated 10 cases and Maddrey *et al* (1972) 97 cases, and by immunoelectrophoresis and complement fixation these workers found no and one positive case respectively. Furthermore, Maddrey *et al* examined 52 primary biliary cirrhosis sera electron microscopically with negative results. Sama, Benz, Aach,

Hacker, and Kaplan (1973) found that five of 24 patients with primary biliary cirrhosis were positive for Au by both electron microscopy and radioimmunoassay, but eight were false positive by electron microscopy and suggested that electron microscopy alone is not a reliable way to detect Au.

Using haemagglutination and haemagglutination inhibition, Maddrey *et al* (1972) found three of their 97 cases positive for Au and 12 of 97 cases positive for Anti-Au, results, however, which were not different from their control populations and on the basis of which they suggested that serum hepatitis virus was not implicated in the pathogenesis of primary biliary cirrhosis, its occasional occurrence in the serum of such patients being attributable to coincidental intercurrent infections with the virus.

The most sensitive method yet available for detection of Au and anti-Au appears to be that of radioimmunoassay, in which radioisotope labelling of Au is used (Walsh *et al*, 1970; Coller *et al*, 1971). Using this method Doniach *et al* (1972) found Au in nine of 69 (13%) of primary biliary cirrhosis cases, and found anti-Au in 15 (22%). As a control population these workers used sera from 60 patients with Hashimoto's thyroiditis in whom there was no previous evidence or history of hepatitis, and in these, Au was present in seven (12%) and anti-Au in two (3%). Like Maddrey and his colleagues they concluded that Au is probably not an aetiological factor in primary biliary cirrhosis.

In the present study we confirmed the previous experience of other workers in that all 64 primary biliary cirrhosis sera gave negative results using immunodiffusion and immunoelectrophoresis methods. However, in contrast to this, on radioimmunoprecipitation detectable quantities of Au were present in 10 of 64 sera (16%), an incidence significantly different from the 3% in controls. As Maddrey and his colleagues (1972) correctly point out, patients with primary biliary cirrhosis experience a high frequency of blood transfusion and opportunities for infection with hepatitis virus during hospital admissions, and many of them have been subjected to laparotomy. Of the 10 patients positive in the present series two had received blood transfusions, two others had had surgical operations, but the remainder, while having been in hospital on a number of occasions, gave no relevant history as to a possible infective source. Neither of the two positive control patients gave a history of jaundice, neither had been transfused, both had normal liver function tests, but both had nursed family members suffering from jaundice.

There is thus considerable uncertainty regarding the relationship between Au and primary biliary cirrhosis. It seems to us that the finding of a statistically increased frequency of Au in the patients with primary biliary cirrhosis is of biological significance, and that there are several possible explanations for this association. The explanation could be trivial in that the radioimmunoassay technique is measuring some blood constituent present in primary biliary cirrhosis but less commonly in controls, and which reacts non-specifically in the assay. Most of the available evidence indicates, however, that the technique is specific but it is nevertheless difficult to rule out the non-specific hypothesis.

Theoretically the association could reveal a direct aetiological role for Australia antigen in the genesis of primary biliary cirrhosis. This seems unlikely in view of the failure to demonstrate the association in several of the previous studies. In the studies of acute viral hepatitis and other forms of hepatitis in which Au may have an aetiological role, the associations are usually more striking and the antigen can be detected using relatively insensitive methods.

It is of course possible that the pathogenesis of the disease is a consequence of Au/anti-Au complex formation. Evidence for such an incriminating mechanism for Au has been produced in polyarteritis nodosa (Gocke, Morgan, Lockshin, Hsu, Bombardieri, and Christian, 1970; Couleru, German, Bousquet, and Sarrazin, 1972) and in a case of chronic glomerulonephritis (Combes, Shorey, Barrera, Stastny, Eigenbrodt, Hull, and Carter, 1971). If primary biliary cirrhosis were due to complexes some of these might be detected by a radioimmunoassay technique, and in fact in the present study two cases had both Au and anti-Au in their serum.

We have previously noted that a proportion of sera from patients with primary biliary cirrhosis have anticomplementary activity (unpublished observations). There is a variety of evidence that the presence of anticomplementary activity in populations in which Au is common could be due to the presence of Au/anti-Au complexes. Shulman and Barker (1969) produced evidence for the presence of immune complexes in their anticomplementary sera. Mazzur, Vanstory, London, Sutnick, and Blumberg (1972) have recently shown a striking association between anticomplementary activity and the presence of Au or anti-Au in the sera from several populations in which Au is common. The presence of complexes might account for the inconsistent results in the detection of Au and anti-Au in primary biliary cirrhosis sera, since the complexes may bind all the antigen and antibody and render it undetectable by insensitive methods.

A third possibility is that primary biliary cirrhosis is related to the 'Au affinity group' of diseases. Blumberg, Sutnick, and London (1970b) suggested

that patients with certain diseases characterized by impairment of immune mechanisms are more likely to become persistently infected with Au if they are exposed than are controls. These diseases include lymphocytic leukaemia, Down's syndrome, lepromatous leprosy, and others. This implies that Au is not the aetiological agent but that in some diseases the aetiological agent is similar to Au in that it is related to the same susceptibility factors. It is noteworthy in this context that Doniach *et al* (1972) found a 12% incidence of Au in patients with Hashimoto's thyroiditis, and more recently it has been reported that 41% of sera from patients with systemic lupus erythematosus were Au positive (Alarcón-Segovia, Fishbein, and Díaz-Jouanen, 1972).

A further possibility is that the radioimmunoassay method is detecting host material antigenically related to Australia antigen. It has been shown that Au particles appear to contain serum proteins antigenically related to human serum proteins (Millman, Hutanen, Merino, Bayer, and Blumberg, 1971). Blumberg, Millman, Sutnick, and London (1971) have suggested that there are shared antigenic specificities in the host and on the Australia antigen, and that these are involved in the observed differences in response to infection with Au. This concept implies that the aetiological agent stimulates the production of host material antigenically related to Australia antigen, and it is possibly this which is being detected by the radioimmunoassay method. This could account for the apparent association of Au with a variety of liver diseases, the most recent example of which has been its implication in alcoholic cirrhosis (Pettigrew, Goudie, Russell, and Chaudhuri, 1972).

Finally the results of our lymphocyte transformation studies indicate that in only one of 24 patients with primary biliary cirrhosis could a state of delayed hypersensitivity to Au be demonstrated. Yeung Laiwah (1971) has previously shown that the lymphocytes of patients who have recovered from Au-associated viral hepatitis, but who no longer have demonstrable Au, will transform when exposed to a serum rich in the antigen. While the present results could be adduced as evidence against a possible pathogenetic role for Au in primary biliary cirrhosis it seems more likely that failure to show delayed hypersensitivity to Au in these patients could be a manifestation of the reported impairment of cellular immunity in this disease (Fox, James, Scheuer, Sharma, and Sherlock, 1969a), and could offer an explanation for the apparent persistence of the virus in some instances.

References

Alarcón-Segovia, D., and Fishbein, E. (1971). Australia antigen in systemic lupus. *New Engl. J. Med.*, **284**, 448.

Alarcón-Segovia, D., Fishbein, E., and Díaz-Jouanen, E. (1972). Presence of hepatitis-associated antigen in systemic lupus erythematosus. *Clin. exp. Immunol.*, **12**, 9-19.

Blumberg, B. S., Gerstley, B. J. S., Hungerford, D. A., London, W. T., and Sutnick, A. I. (1967). A serum antigen (Australia antigen) in Down's syndrome, leukemia and hepatitis. *Ann. intern. Med.*, **66**, 924-931.

Blumberg, B. S., London, W. T., and Sutnick, A. I. (1970a). Viral hepatitis and tests for Australia (hepatitis associated) antigen and antibody. Section 3. Immunodiffusion test. *Bull. Wld Hlth Org.*, **42**, 964-966.

Blumberg, B. S., Sutnick, A. I., and London, W. T. (1970b). Australia antigen as a hepatitis virus: variation in host response. *Amer. J. Med.*, **48**, 1-8.

Blumberg, B. S., Millman, I., Sutnick, A. I., and London, W. T. (1971). The nature of Australia antigen and its relation to antigen-antibody complex formation. *J. exp. Med.*, **134**, 320-329s.

Coller, J. A., Millman, I., Halbherr, T. C., and Blumberg, B. S. (1971). Radioimmunoprecipitation assay for Australia antigen, antibody, and antigen-antibody complexes. *Proc. Soc. exp. Biol. (N.Y.)*, **138**, 249-257.

Combes, B., Shorey, J., Barrera, A., Stastny, P., Eigenbrodt, E. H., Hull, A. R., and Carter, N. W. (1971). Glomerulonephritis with deposition of Australia antigen-antibody complexes in glomerular basement membrane. *Lancet*, **2**, 234-237.

Couleru, O., German, A., Bousquet, O., and Sarrazin, A. (1972). Immune complexes in large particles of Australia antigen in polyarteritis. *Lancet*, **1**, 445-446.

Coyne (Zavatone), V. E., Millman. I., Cerda, J., Gerstley, B. J. S., London, W. T., Sutnick, A. I., and Blumberg, B. S. (1970). The localization of Australia antigen by immunofluorescence. *J. exp. Med.*, **131**, 307-319.

Doniach, D., Del Prete, S., Dane, D. S., and Walsh, J. H. (1972). Viral hepatitis related antigens in 'automimmune' hepatic disorders. *Canad. med. Ass. J.*, **106**, 513-518.

Fox, R. A., James, D. G., Scheuer, P. J., Sharma, O., and Sherlock, S. (1969a). Impaired delayed hypersensitivity in primary biliary cirrhosis. *Lancet*, **1**, 959-962.

Fox, R. A., Niazi, S. P., and Sherlock, S. (1969b). Hepatitis-associated antigen in chronic liver disease. *Lancet*, **2**, 609-612.

Gocke, D. J., Morgan, C., Lockshin, M., Hsu, K., Bombardieri, S., and Christian, C. L. (1970). Association between polyarteritis and Australia antigen. *Lancet*, **2**, 1149-1153.

Goudie, R. B., MacSween, R. N. M., and Goldberg, D. M. (1966). Serological and histological diagnosis of primary biliary cirrhosis. *J. clin. Path.*, **19**, 527-538.

Kaboth, U., Schober, A., Arndt, H. J., Vido, I., Selmair, H., Gallasch, E., Verma, P., Thomssen, R., and Creutzfeldt, W. (1970). Australia (SH)-antigen-befunde bei Leberkranken und Blutspendern. *Dtsch. med. Wschr.*, **95**, 2157-2165.

Kaplan, M. M., and Grady, G. (1971). Serum-hepatitis antigen in chronic hepatitis and primary biliary cirrhosis. *Lancet*, **1**, 159-161.

Maddrey, W. C., Saito, S., Shulman, N. R., and Klatskin, G. (1972). Coincidental Australia antigenemia in primary biliary cirrhosis. *Ann. intern. Med.*, **76**, 705-709.

Mazzur, S. R., Vanstory, L., London, W. T., Sutnick, A. I., and Blumberg, B. S. (1972). Serological studies on populations associated with Australia antigen. *Res. Commun. chem. Path. Pharmacol.*, **3**, 435-454.

Millman, I., Hutanen, H., Merino, F., Bayer, M. E., and Blumberg, B. S. (1971). Australia antigen: physical and chemical properties. *Res. Commun. chem. Path. Pharmacol.*, **2**, 667-686.

Pesendorfer, F., Krassnitsky, O., and Wewalka, F. G. (1970). Viral hepatitis and tests for Australia (hepatitis associated) antigen and antibody. Section 5. Immunoelectrophoretic methods. *Bull. Wld Hlth Org.*, **42**, 974-975.

Pettigrew, N. M., Goudie, R. B., Russell, R. I., and Chaudhuri, A. K. R. (1972). Evidence for a role of hepatitis virus B in chronic alcoholic liver disease. *Lancet*, **2**, 724-725.

Reinicke, V., and Nordenfelt, E. (1970). Hepatitis-associated antigen in chronic liver disease. (Letter). *Lancet*, **1**, 141-142.

Sama, S., Benz, W., Aach, R., Hacker, E., and Kaplan, M. (1973). False-positive Australia-antigen particles in primary biliary cirrhosis. *Lancet*, **1**, 14-17.

Scheuer, P. J. (1967). Primary biliary cirrhosis. *Proc. roy. Soc. Med.*, **60**, 1257-1260.

Sherlock, S. (1971). *Diseases of the Liver and Biliary System*, 4th ed. (revised 3rd printing). Blackwell, Oxford.

Shulman, N. R., and Barker, L. F. (1969). Virus-like antigen, antibody, and antigen-antibody complexes in hepatitis measured by complement fixation. *Science*, **165**, 304-306.
Vischer, T. L. (1970). Australia antigen and autoantibodies in chronic hepatitis. *Brit. med. J.*, **2**, 695-698.
Walsh, J. H., Yalow, R., and Berson, S. A. (1970). Detection of Australia antigen and antibody by means of radioimmunoassay techniques. *J. infect. Dis.*, **121**, 550-554.
Wright, R., McCollum, R. W., and Klatskin, G. (1969). Australia antigen in acute and chronic liver disease. *Lancet*, **2**, 117-121.
Yeung Laiwah, A. A. C. (1971). Lymphocyte transformation by Australia antigen. *Lancet*, **2**, 470-471.

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