Australia antigen and primary biliary cirrhosis

R. N. M. MacSWEEN, A. A. C. YEUNG LAIWAH, A. A. BUSUTTIL,
MAIR A. THOMAS, SHEILA K. ROSS, G. WATKINSON, I. MILLMAN, AND
B. S. BLUMBERG

From the University of Glasgow, Departments of Pathology and Medicine, Western Infirmary, Glasgow,
and the Institute for Cancer Research, Fox Chase, Philadelphia, Pennsylvania, USA

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 Klatkin,
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
Klatkin, 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 Hashi-
moto’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, de-
monstrated 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.

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 radio-
immunoprecipitation assay (Coller, Millman,
Halbherr, and Blumberg, 1971).

LYMPHOCYTE TRANSFORMATION STUDIES

These were performed on peripheral blood lympho-
cytes 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 transforma-
tion in patients who had recovered from a previous
clinical attack of Au-positive serum hepatitis

1Present address: Genetics Laboratory, Department of Biochemistry,
University of Oxford, Oxford.

Received for publication 1 March 1973.
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 radioimmuno-precipitation 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.

Table I Details of patients and controls used in present study

<table>
<thead>
<tr>
<th></th>
<th>A Primary Biliary Cirrhosis Group and Matched Controls</th>
<th>B Primary Biliary Cirrhosis Subgroup and Matched Controls—Lymphocyte Transformation Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>64</td>
<td>24</td>
</tr>
<tr>
<td>Sex</td>
<td>59 Female</td>
<td>22 Female</td>
</tr>
<tr>
<td>Mean age (yrs)</td>
<td>57.5 (range 30-78)</td>
<td>55.8 (range 30-75)</td>
</tr>
<tr>
<td>AU/IEOP</td>
<td>All negative</td>
<td>1/18 (Au); 1/18 (Au and anti-Au)</td>
</tr>
<tr>
<td>RIA</td>
<td>See table III</td>
<td></td>
</tr>
</tbody>
</table>

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 radioimmuno-precipitation screening was carried out.

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 radioimmuno-precipitation 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; Alarcon-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 haemaggulation and haemaggulation 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 pointed 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, Vanstony, 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


**Australia antigen and primary biliary cirrhosis**


---

**Reports and Bulletins prepared by the Association of Clinical Biochemists**

The following reports and bulletins are published by the Association of Clinical Biochemists. They may be obtained from The Administrative Office, Association of Clinical Biochemists, 7 Warwick Court, Holborn, London, WC1R 5DP. The prices include postage, but air mail will be charged extra. Overseas readers should remit by British Postal or Money Order. If this is not possible the equivalent of 50p is the minimum amount that can be accepted.

**SCIENTIFIC REPORTS**


4 An Evaluation of five Commercial Flame Photometers suitable for the Simultaneous Determination of Sodium and Potassium March 1970 P. M. G. BROUGHTON and J. B. DAWSON 80p ($2)

**SCIENTIFIC REVIEWS**

1 The Assessment of Thyroid Function March 1971 F. V. FLYNN and J. R. HOBBS 60p ($1.50)

2 Renal Function Tests Suitable for Clinical Practice January 1972 F. L. MITCHELL, N. VEALL, and R. W. E. WATTS 60p ($1.50)

**TECHNICAL BULLETINS**

9 Determination of Urea by AutoAnalyzer November 1966 RUTH M. HASLAM 40p ($1)

11 Determination of Serum Albumin by AutoAnalyzer using Bromocresol Green October 1967 B. E. NORTHAM and G. M. WIDDOWSON 40p ($1)

13 An Assessment of the Technicon Type II Sampler Unit March 1968 B. C. GRAY and G. K. MCGOWAN 40p ($1)

14 Atomic Absorption Spectroscopy. An outline of its principles and a guide to the selection of instruments May 1968 J. B. DAWSON and P. M. G. BROUGHTON 40p ($1)


16 A Guide to Automation in Clinical Chemistry May 1969 P. M. G. BROUGHTON 60p ($1.50)

17 Flame Photometers (2nd edition) 1969 P. WILDING 60p ($1.50)

18 Control Solutions for Clinical Biochemistry (4th edition) March 1970 P. M. G. BROUGHTON 60p ($1.50)

19 Spectrophotometers. A comparative list of low-priced instruments readily available in Britain May 1970 C. E. WILDE and P. SEWELL 60p ($1.50)

20 Quantities and Units in Clinical Biochemistry June 1970 P. M. G. BROUGHTON 60p ($1.50) More than 30 copies in units of 10 at 20p

21 Filter Fluorimeters: A comparative list of 18 instruments September 1970 H. BRAUNSBERG and S. S. BROWN 60p ($1.50)

22 Bilirubin standards and the Determination of Bilirubin by Manual and Technicon AutoAnalyzer Methods January 1971 BARBARA BILLING, RUTH HASLAM, and N. WALD 60p ($1.50)

23 Interchangeable Cells for Spectrophotometers and Fluorimeters September 1971 E. S. BROWN and A. H. GOWENLOCK 60p ($1.50)

24 Simple Tests to Detect Poisons March 1972 B. W. MEADE et al. 60p ($1.50)

25 Blood Gas Analysers May 1972 K. DIXON 60p ($1.50)

26 Kits for Enzyme Activity Determination September 1972 S. B. ROSALIK and D. TARLOW 80p ($2.00)

27 Assessment of Pumps Suitable for Incorporation into Existing Continuous Flow Analytical Systems November 1972 A. FLECK et al 60p ($1.50)