Reprinted from ANNALS OF SURGERY, Vol. 174, No. 4, October 1971

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Immunosuppression, Liver Injury, and Hepatitis in Renal, Hepatic, and Cardiac Homograft Recipients:

With Particular Reference to the Australia Antigen

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RENAL homograft recipients have been reported to have a high incidence of liver disease in the post-transplantation period.[26, 29, 31, 56, 65, 70] It was assumed that the immunosuppressive agents were responsible, either by their hepatotoxicity or because the consequent weakening of the host immune system permitted the frequent development of virus hepatitis. An accurate distinction between these two general possibilities was not feasible until recently. Then, with the description of tests which permitted identification of the hepatitis associated or Australia (Au) antigen,[7, 9, 58] it became possible to decisively study at least one variety of virus hepatitis in transplant recipients.

In this communication, a series of observations related to the problem of hepatic damage with or without hepatitis in a large transplantation program will be presented. These observations will include: (1) The incidence of serologic evidence of Au antigenemia, (2) Comparison of immunodiffusion, immuno-osmoelectrophoresis, electro-immunodiffusion, and complement fixation tests for the detection of hepatitis in immunosuppressed patients, (3) The clinical course followed by immunosuppressed patients infected with the serum hepatitis virus, (4) Evaluation of the anti-complementary activity (ACA) recently described by Shulman and Barker[64] and Purcell *et al.*[60] as an indicator of complexes of Au antigen and antibody, and, (5) Description of the serologic changes and clinical course of a patient dying with chronic aggressive hepatitis, Au positive, who was treated with liver replacement.

Aided by research grants from the Veterans Administration, by grants RR-00051, and RR-00069 from the general clinical research centers program of the Division of Research Resources, National Institutes of Health and by grants AI-10176-01, AI-AM-08898, AM-07772, GM-01686, HE-09110 of the United States Public Health Service.

Presented at the Annual Meeting of the American Surgical Association, March 24–26, 1971, Boca Raton, Florida.

Methods

Specimens were taken from 225 normal volunteers, from 83 recipients of renal homografts, from 10 liver recipients, and from one cardiac recipient. Immunosuppression for the transplant patients was with azathioprine and prednisone[65] to which horse antilymphocyte globulin (ALG) was added in about half the cases.[70]

All the blood samples were allowed to clot for about one hour at room temperature. The sera were then separated by cold

centrifugation and stored at −20 or −70° C. The analyses were subsequently performed independently in two laboratories. In one (hereafter called Laboratory 1) the screening was done with micro-Ouchterlony agarose gel immunodiffusion (AG),[46] or electroimmunodiffusion (EID)[46] technics, reacting the patient's serum against standard sera known to contain Au antigen or anti-Au antibody. The standard serum containing Au antigen was obtained from a patient treated with renal homotransplantation in February, 1968, who did not have obvious liver disease in spite of chronic Au antigenemia. The standard serum containing anti-Au antibody was taken from another renal recipient of approximately the same era who had developed chronic liver disease without persistent Au antigenemia.

In Laboratory 2, fewer recipients were studied, but analysis of the sera was more exhaustive. The Au antigen was detected with the micro-Ouchterlony (AG),[53] immunoelectro-osmophoresis (IEOP),[16, 59] and complement fixation (CF)[63, 64]* tests. In all three systems quantitation was obtained by serially diluting the patient's sera with gelatin-veronal buffer (GVB^{++}); the amount of Au antigen was expressed as the reciprocal of the greatest dilution in which a positive reaction was elicited by that test. The standard sera containing Au antigen were obtained from seven patients whose antigen appeared identical by the Ouchterlony method. One of these serum donors was a renal transplant recipient, another had chronic lymphatic leukemia, two were hemophiliacs and the other three did not have an associated disease; four of the seven patients had received multiple blood transfusions at some previous time. The serum containing anti-Au antibody was donated by the same patient described in the preceding paragraph. However, the IgG was removed from the crude serum by ammonium sulphate fractionation, followed by diethylaminoethyl (DEAE) cellulose chromatography. Almost all the antibody activity was recovered.

*One drop of guinea pig complement was used having a concentration of 1.5 CH 50 units/ml.

TABLE 1. *Incidence of Au Antigen by Random Single Sampling in Homograft Recipients from One Month to 8 Years after Operation, using Immunodiffusion or Electro-immunodiffusion Tests for Screening. Laboratory No. 1**

No.	Au Antigen Positive	Anti-Au Antibody	Negative for Au Antigen and Antibody
89	15 (16.9%)	2 (2.2%)	72 (80.9%)

* The incidence was actually higher than shown since several additional cases were detected in Laboratory No. 2 within the negative group, by the more sensitive method of complement fixation.

In all sera checked for Au antigen, anti-Au antibody also was looked for with AG, IEOP, and CF by reversing the positions of the patient and standard sera and using the Au-containing serum from the renal transplant recipient described earlier as the reference antigen.

The anti-complement activity (ACA) in the sera was quantitated by a hemolysis method.[43] To serial 2-fold dilutions of the patient sera was added an equal volume (one drop) of 1.5 CH50 units/ml of normal human complement. After incubation for 60 minutes at 37° C., one drop of sensitized sheep erythrocyte suspension containing 1×10^8 cells/ml. was added. Hemolysis was graded after an additional 60 minutes incubation and centrifugation at 1,000 rpm for 5 minutes.

An effort was made to distinguish ACA from the other well known heat-stable human complement inhibitor, C3 inactivator.[74] C3 inactivator was looked for with the immune adherence test using the EA 4, 3 cell system.[74] In addition, rheumatoid factor (RA) possesses anticomplementary activity.[30, 34] RA was detected with the latex fixation test.*

In addition, the sera of four renal patients who were known to possess both ACA and

*Latex RA Kit, Hyland Corporation, Los Angeles, California.

TABLE 2. *Comparison of Australia Antigen Detection with AG, EID, IEOP, and CF in 50 Cases Studied Repetitively after Transplantation*

No. Positive			
AG	EID	IEOP	CF
15	15	15	23*

* These 23 included 8 detected only by CF plus the 15 diagnosed with all the other methods.

C3 inactivator were submitted to DEAE-cellulose ** column chromatography[57] after special cellulose washing.[75] In addition, further purification of ACA elutions from the DEAE column was obtained by gel filtration with Sephadex G-200 and the refined product was re-examined serologically for ACA activity.

The decomplemented (heated at 56° C. for 30 minutes) sera from four other patients who possessed high titer ACA were examined by electronmicroscopy. To two of the sera of renal recipients which had Au antigen detectable by all the methods used, purified anti-Au IgG was added; the resulting precipitate was examined ultrastructurally. In addition, the ACA in the sera of two liver recipients was examined ultrastructurally after being purified by ammonium sulphate precipitation, DEAE chromatography, Sephadex G-200 gel filtration, polyvinyl chloride (Pevikon) block electrophoresis* and recycling through Sephadex G-200. One of these hepatic patients had an ACA titer ranging as high as 1/1,024 but no serologic evidence of Au antigenemia. The other had an ACA titer of 1:48; Au antigen became detectable by CF only (Table 6).

Results

Incidence of Au Antigen or Anti-Au Antibody

In Laboratory 1 the sera from 83 renal, five hepatic, and one cardiac transplant recipients were studied with electroimmunodiffusion (EID) and/or with AG (Table 1). The results were negative for Au antigen and Anti-Au antibody in 72 cases (80.9%). Fifteen (16.9%) of the patients had Au antigenemia including one of the five liver and 14 of the 83 kidney recipients. The final two (2.2%) did not have detectable Australia antigen but there was unequivocal evidence of anti-Au antibody.

The foregoing statistics were collected by studying single serum samples obtained from the individual patients from one month to 8 years after transplantation, at re-hospitalization or at routine clinic visits. It was conceivable in some cases in which the results were negative that evidence of serum hepatitis had been present with these tests earlier, but with the resolution of the positive serologic findings. To evaluate this possibility, serial stored sera were examined both in Laboratories 1 and 2 from 35 of the 72 patients pronounced to be Au negative on the basis of their single samples, including the two recipients who had anti-Au antibody. In these 35 recipients (of 33 kidneys, one liver, and one heart), three to 31 serial samples were examined over collection intervals that ranged from 2 to 35 months, with an average of 14.4 ± 9.1 (S.D.) months. None of the 35 patients had the Au antigen in any of their specimens as detected with the AG, EID, and IEOP examinations. Thus the single sample analysis with the precipitating technics appeared to provide adequate gross screening for post-transplantation epidemiologic studies since no patient who exhibited the Au antigenemia with those tests ever had disappearance of the positive serologic findings. However, it will be pointed out in the next section that the incidence derived with the precipitating tests was an under estimation since several additional cases were detected by the more sensitive method of complement fixation.

** Lot No. 2407, Brown Company, Berlin, New Hampshire.

* Pevikon C870, Fosfatbolacet, Stockholm (5), Sweden.

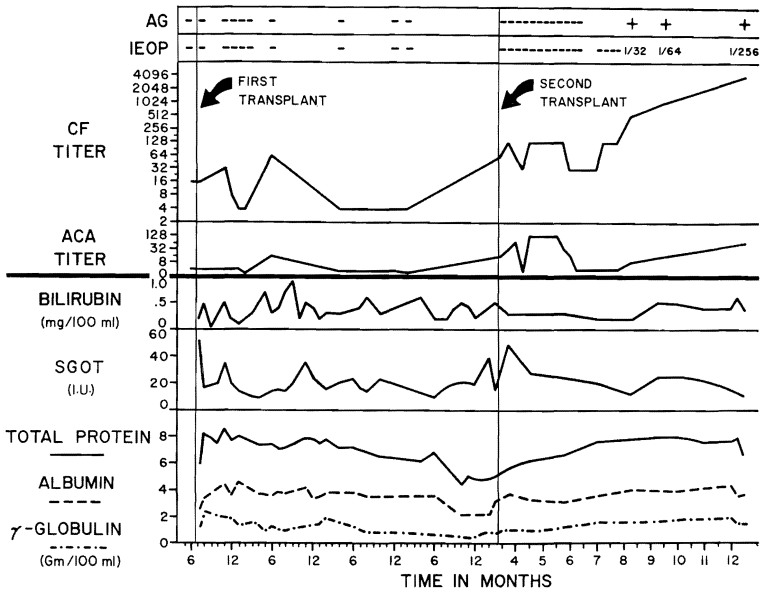

FIG. 1. The course of a patient who received two kidney homografts almost a year apart. Hepatitis was never diagnosed on clinical grounds or by biochemical criteria; the depressed serum protein concentration prior to the second transplantation was due to homograft nephrosis and proteinuria. Yet, the complement fixation was positive at all times, even preceding the first transplantation. After the second transplantation at which time immunosuppression was intensified, all Au tests became positive. See text for explanation of abbreviations.

Comparative Sensitivity of Detection Methods

In the 15 recipients in the preceding section who were known to be Au positive and in one heart, one liver, and 33 renal recipients who were thought to be Au negative, serial serum samples collected over periods of one to 54 months were studied by all four of the methods shown in Table 2. An average of 13.4 ± 11.1 (S.D.) specimens were examined from each patient. The 15 recipients who were found to have the Au antigen with the precipitating tests (AG, EID, and IEOP) were also positive with complement fixation (CF) as defined by a titer of 1:4 or higher. In addition, 8 patients of the 35 who were negative for Au antigen by the precipitating tests had significant increases in CF at some time indicating that Au antigen had been present even though this had been missed by AG, EID, or IEOP.

In addition to providing a higher yield, the CF was invariably the most sensitive way to follow individual patients. It became positive earlier and evolved more dramatically than any other test (Fig. 1).

The 225 people who were thought to be normal were examined with AG, IEOP, and CF. None had positive tests.

Clinical Manifestations of Au Antigenemia

Forty-five of the non-hepatic transplant recipients (44 renal and one cardiac) who were studied serologically at frequent postoperative intervals also had concomitant liver function tests. Consequently, it was possible in these 45 cases to correlate abnormalities in hepatic function with the presence or absence of Au antigenemia.

Au Positive by All Tests. Three of the 12 kidney recipients who were repeatedly studied (Fig. 2) had definite Au antigenemia prior to transplantation detected by the precipitating methods (AG and IEOP) as well as by CF. Two other patients were positive preoperatively with CF only; 16 and 50 months postoperatively the AG and IEOP in these two patients became positive. The details of one of the latter cases is shown in Figure 1. In this patient, the CF test was elevated in variable titers on every occasion for more than 4 years before the diagnosis of Au antigenemia could be confirmed by AG and IEOP. The seven

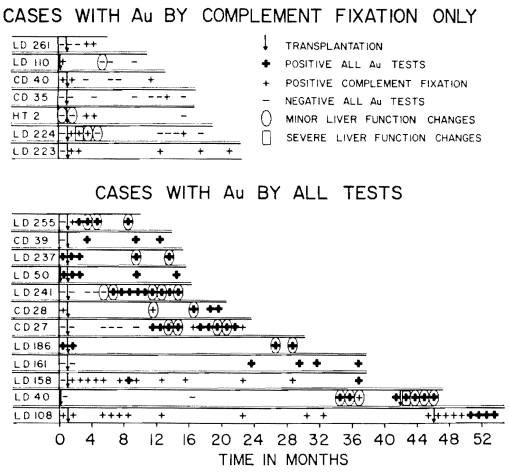

FIG. 2. The serologic findings and liver function tests in one cardiac and 18 renal recipients who were diagnosed as having Au antigenemia. In the 7 cases shown above, only the complement fixation test became positive. An antigen was detectable by all the diagnostic methods in the bottom 12 cases. When an arrow is at the extreme left of an individual patient's schematic summary, the first studies were obtained sometime after the transplantation. Otherwise, the symbols to the left of the arrows represent preoperative determinations. In Cases LD 40 and LD 50, the first examinations were more than 5 years after a first transplantation, but before a second transplantation. LD 108 was studied both before and after each of his 2 transplantations which were almost 4 years apart; details of this case are also summarized in Figure 1.

patients who had no evidence of the Au antigen before transplantation or at the time of the first examination postoperatively developed positive serologic tests from one to 35 (average 12½) months later (Fig. 2).

Once the full battery of analyses showed the Au antigen, these findings did not permanently disappear in any instance in the subsequent followup intervals of 4 to 30 (average 13.7) months (Fig. 2). The liver function tests of the 12 patients obtained at the same time as the various serologic examinations are summarized in Figure 2. There was only one isolated example (Case LD 40) of serious acute hepatic malfunctions, defined as two SGOT tests exceeding 250 I.U. (normal < 50) or bilirubin of greater than 2 mg./100 ml. Minor function abnormalities were observed at some time in six other cases, almost invariably consisting of relatively trivial increases in SGOT (50 to 250 I.U.). The latter transaminase abnormalities are still present in five of the 12 recipients. On the other hand, five of the patients who have had serologic evidence of hepatitis for one to more than 4 years had no evidence whatsoever of hepatic dysfunction at any time.

Au Positive by C.F. Only. One patient had an elevated CF titer before operation and another one was positive when first tested 3½ years post-transplantation. The other five recipients developed positive CF examinations from 1 to 13 months (average 4.2) after operation. In contrast to the precipitating tests, a positive CF result did not necessarily imply chronicity of Au antigenemia since reversion to normal occurred in four of the seven patients (Fig. 2).

Abnormal liver function tests of a minor nature were seen transiently in two of the seven patients (Fig. 2). A third recipient (LD 224) became deeply jaundiced at the same time as there was evidence of hepatic necrosis and very low grade increases in the CF titers (Fig. 3). Except for this case, there was no correlation between abnormal CF tests and hepatic dysfunction.

Au Negative by All Tests. Of the 24 patients who never had Au antigen despite frequent testing (Fig. 4), 14 (58.3%) had

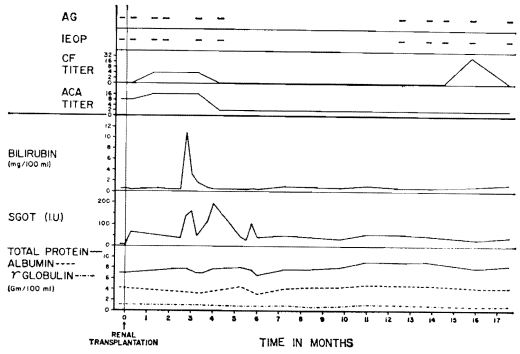

FIG. 3. The course of a patient (LD 224) who developed evidence of severe hepatitis after transplantation. At the same time, he had low grade increases in complement fixation titers of Au antigen as well as slightly anticomplementary activity in the serum. Note the transient secondary complement fixation titer rise more than a year later.

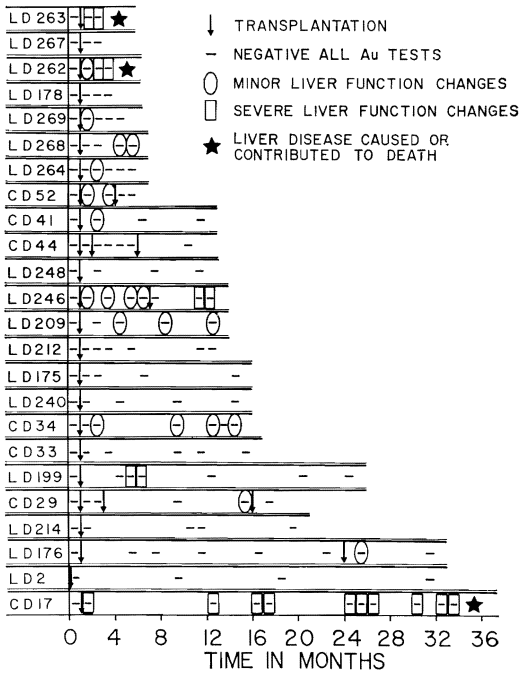

FIG. 4. The negative serologic findings (AG, IEOP, and CF) and contemporaneous liver function tests in 24 patients who did not have Au antigenemia. The explanation for the arrows is the same as in the caption for Figure 2. Note that liver disease caused or contributed to three deaths.

evidence of hepatic dysfunction at some time after transplantation. The incidence of functional abnormalities was thus actually slightly higher than in the recipients shown in Figure 2 whose sera had evidence of the Au antigen but who had hepatic dysfunction at a rate of only 10 in 19 (52.6%).

The liver disease in the Au negative patients was usually trivial. However, there were five patients who had *serious* hepatic malfunction as defined earlier in terms of SGOT and bilirubin rises. Liver disease either caused or contributed importantly to the deaths of three of these five recipients (Fig. 4, 5).

In one of the five patients with serious liver disease, azathioprine toxicity was so strongly considered that this drug was stopped and replaced with cyclophosphamide (Fig. 6). A marked improvement in hepatic function was noted in the ensuing 7 weeks. These observations were not thought either to weaken or strengthen the hypothesis of azathioprine hepatotoxicity in this patient.

Anti-Au Antibody. In all the patients who had Au antigen by any test, anti-Au antibody was looked for with AG, IEOP, and CF, but it was never detected. In addition, all the patients with negative Au tests were examined. Two of these latter recipients had unequivocal evidence of anti-Au antibody (Table 3) which developed one week and one year respectively after transplantation, without ever having detectable

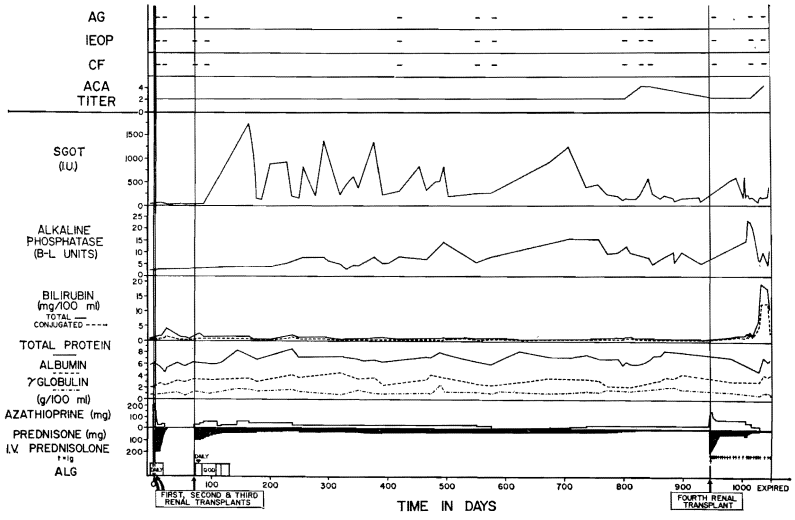

FIG. 5. The course of a recipient (CD 17) of 4 successive renal homografts who despite consistently negative serology for Au antigen showed significant aberrations of liver function tests over a 3-year period. Following the fourth transplantation, she became deeply jaundiced and died 3 weeks later.

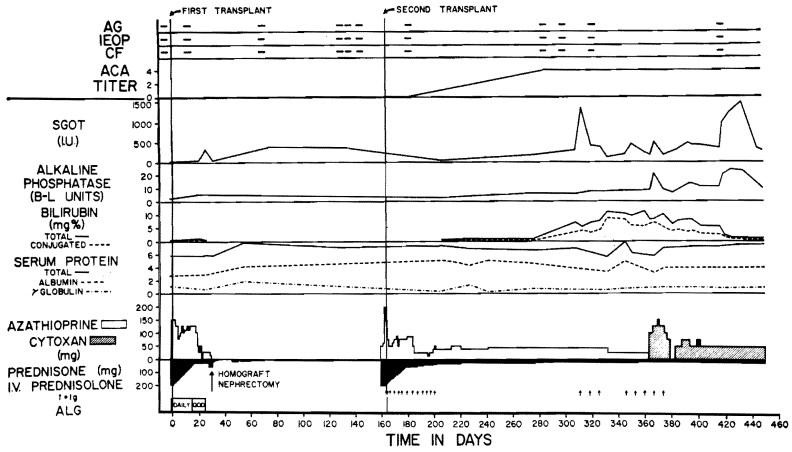

FIG. 6. A renal recipient (LD 246) whose hepatic functional derangements following a second transplant were suspected to be caused by azathioprine toxicity. Cyclophosphamide (Cytoxan®) was substituted for azathioprine. The serum bilirubin receded over the next 7 weeks, and subsequently it has returned nearly to nomal.

Au antigenemia. The first of these patients has had no hepatic functional abnormalities. The second one has had very severe liver disease which has become chronic. A biopsy in August, 1970 was read as chronic aggressive hepatitis with early cirrhosis.

In both cases, anti-complementary activity (ACA) has been present in low titers

TABLE 3. *Two Patients Who Developed Anti-Au Antibody without Ever Manifesting Au Antigenemia*

	Au Antigen				Anti-Au Antibody			Hepatic Function		
Date	AG	IEOP	CF** Titer	ACA** Titer	AG	IEOP** Titer	CF** Titer	SGOT (I.U.)	Bilirubin (mg./100 ml.)	Albumin (Gm./100 ml.)
					Case I					
2-19-68*	—	—	<2	8	—	<2	<2	27	0.6	3.2
3-12-68	—	—	<2	8	—	<2	<2	60	0.5	2.8
5-23-68	—	—	<2	8	—	<2	<2	120	0.3	3.1
3-6-69	—	—	<2	4	—	<2	2	86	2.0	2.8
6-24-70	—	—	<2	4	—	<2	4	55	1.7	2.7
7-5-70	—	—	<2	4	—	<2	4	90	2.1	2.7
7-16-70	—	—	<2	8	+	2	16	70	2.0	3.1
8-14-70	—	—	<2	8	+	16	128	40	6.3	3.0
9-7-70	—	—	<2	8	+	16	128	42	2.8	2.7
9-12-70	—	—	<2	8	+	16	128	44	1.4	2.6
9-29-70	—	—	<2	8	+	16	128	60	1.0	2.7
12-8-70	—	—	<2	8	+	8	32	68	1.1	3.1
					Case 2					
8-21-70*	—	—	<2	4	—	—	<2	7	0.7	3.0
8-31-70	—	—	<2	4	+	8	32			
9-7-70	—	—	<2	8	+	4	8			
9-21-70	—	—	<2	8	+	2	8	27	0.7	3.2
9-25-70	—	—	<2	8	+	2	8			
10-23-70	—	—	<2	8	—	1	4			
11-2-70	—	—	<2	4	—	1	4	50	0.7	3.9
11-27-70	—	—	<2	4	+	4	8			
12-7-70	—	—	<2	4	+	2	8	38	0.3	3.5

* These were pre-transplantation studies. The operations were on February 19, 1968 (Case I) and August 27, 1970 (Case 2).

** The titers are expressed as the reciprocal of the greatest dilution giving a positive reaction.

TABLE 4. *Relation of Anticomplementary Activity (ACA) and Latex Fixation Tests for Australia (Au) Antigen in 48 Renal and One Cardiac Transplant Recipients*

	Au+ by all Tests	Au+ by CF only	Au−
No. with ACA titer ⩾ 1:16	14/14	6/7	1/28*
No. with Positive Latex Fixation Test	5/14	1/7	2/28

* Includes the two patients who had anti-Au antibody; neither had significant ACA.

during most of the period of study. The interpretation of this finding will be considered later on.

Anticomplementary Activity

Renal and Cardiac Recipients. One cardiac and 48 renal recipients who had repeated examinations for Au antigen also had ACA determination at these times. In each of the patients who was Au positive by all the detection methods, ACA was increased to or above a titer of 1:16 (Table 4). The same was true for six of the seven patients who had the Au antigen detectable only by CF. In contrast, only one of 28 patients in whom there was no evidence of the Au antigen had an ACA titer as high as 1:16 (Table 1).

In the patients with Au antigen, the significant ACA titers usually developed at or about the same time as one or more of the Au test became positive, as exemplified in Figure 1. However, this temporal association was sometimes not precise, with the ACA rising either before or after the other serologic changes. While there was a good general correlation between ACA and Au antigenemia, there was no clear association with the rheumatoid factor as detected by the Latex fixation test (Table 4).

Hepatic Recipients. Of the hepatic recipients, there were eight whose ACA was determined serially before and after operation. All eight had significant increases of ACA titer. The elevations occurred both in patients with and in those without Au antigenemia. In fact, the highest ACA titer (1:1,024) was in a child who never had any other serologic evidence of the Au antigen (Patient 5, Table 5). Similarly, the rheumatoid factor could not be correlated with Au antigenemia.

Normal People. There were 225 normal controls of whom none had any serologic evidence of Au antigen (see earlier). Of this group, 208 had no ACA and the other 17 had titers of 1:2 to 1:8, too low to be considered significant.

The Nature of ACA. The evidence in the preceding sections (Tables 4 and 5) showed that ACA was *not* rheumatoid factor. In addition, the titers of C3 inactivator were determined in seven of the eight liver recipients shown in Table 5 and in 30 renal recipients (15 Au positive, 15 Au negative). These fluctuated remittently, but not in relationship to ACA. A difference between ACA and C3 inactivator was proved by the DEAE cellulose chromatography and Sephadex G-200 gel filtration which permitted separation of the two substances. With both chromatographic technics, the ACA and C3 inactivator were at widely separated peaks (Figure 7).

The electron micrographic studies with the precipitates or purified ACA from the sera of four recipients (two renal and two hepatic) who possessed high ACA titers are summarized in Table 6. An example of what was construed as a complex of virus and antibody is shown in Figure 8. The only

TABLE 5. *Relation of ACA to the Latex Fixation and Au Antigen Tests in Eight Liver Recipients*

Patient	Highest ACA Titer	Au Tests	Latex Fixation
1	1:256	All+	−
2	1:128	All+	+
3	1:32	CF+	−
4	1:96	CF+	+
5	1:1024	−	+
6	1:32	−	−
7	1:32	−	−
8	1:32	−	+

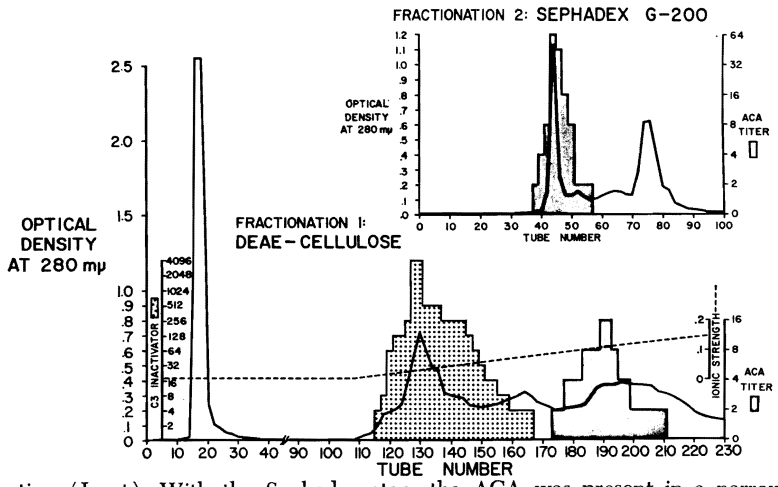

FIG. 7. Elutions of ACA and C3 inactivator from the serum of a liver recipient known to have a high ACA titer, but Au negative by all tests. The first fractionation was with a DEAE-cellulose column, using phosphate buffer, the ionic strength of which was adjusted with sodium chloride. The individual tubes were analyzed for protein content (expressed as optical density), and titered for C3 inactivator and ACA. Tubes 176–210, containing the ACA, were then pooled and submitted to Sephadex G-200 fractionation (Inset). With the Sephadex step, the ACA was present in a narrow band of the eluate, but C3 inactivator could not be detected in any of the samples.

specimen that did not contain easily identifiable virus-like particles came from the purified ACA of the liver recipient who was serologically Au negative.

Liver Replacement for Chronic Aggressive Hepatitis, Au Positive

A 27-year-old woman with end-stage hepatic disease and remittent encephalopathy was treated on August 9, 1970 by total removal of a 688 Gm. cirrhotic liver and replacement with a cadaveric homograft. Between 1959 and 1964 she had been an intravenous drug user and was admitted to a Los Angeles Hospital in 1964 with a bout of acute hepatitis from which she was thought to have promptly recovered. However, in July, 1969, she was seen by Dr. Allen Rediker of the University of Southern California, Los Angeles, who diagnosed chronic aggressive hepatitis after liver biopsy. Moreover, from then until the time of transplantation almost a year later, six blood samples were Au positive by the AG test performed in California. Using stored sera, these findings were the same in the Colorado Laboratories 1 and 2. In addition, there was confirmatory evidence of persistent Au antigenemia by the IEOP, EID, and CF examinations (Fig. 9) immediately before transplantation. The ACA was also elevated (Fig. 9).

At the time of operation, she had 9 liters of ascites, bilateral pleural effusions and jaundice. Liver function tests were typical of terminal hepatic disease including hypoalbuminemia (<2.0 Gm./100 ml.) and a depressed prothrombin time (<20%). She also had striking hypergammaglobulinemia (4 Gm./100 ml. or more).

In spite of her poor condition prior to the transplantation, the early postoperative convalescence was uncomplicated. Immunosuppressive treatment included the use of azathioprine, prednisone, and horse ALG (Fig. 9). Hepatic function was excellent from the beginning. The high serum concentration of gamma globulin promptly fell (Fig. 9).

Within 30 minutes after host hepatectomy, all serologic evidence of persistent hepatitis had disappeared by the AG, EID, IEOP, CF, and ACA tests. Almost 6 weeks later, the CF, EID, and IEOP returned to positive, followed almost a month later by the same change in the AG.

One week after all the hepatitis detection tests had become positive and 80 days after the transplantation, the patient was readmitted with increases in the transaminases,

TABLE 6. *Electronmicrographic Studies of Heated (56° C. for 30 minutes) Sera from Patients Who Had High ACA Titers*

Patient No.	Type Recipient	Au	Anti-Au IgG* Added to Sera	Fraction Examined by EM	Virus-like Particles Seen
1	Kidney	+All	Yes	Precipitate	Yes***
2	Kidney	+All	Yes	Precipitate	Yes***
3	Liver	−	No	Purified ACA	No
4	Liver	+CF**	No	Purified ACA	Yes***

* By EM, this IgG did not contain virus particles.

** The positive CF test became evident 4 days after the serum with high ACA was collected, analyzed, and found to contain the virus-like particles seen in Figure 8.

*** These virus-like particles were often surrounded by halos that were interpreted to be antibody (Fig. 8) as described by Almeida *et al.*[1]

jaundice, alkaline phosphatemia, and depression of the prothrombin time to 50%. For several days she had marked loss of appetite. The diagnosis of recurrent hepatitis was made, rather than that of delayed homograft rejection. Consequently, immunosuppression was not increased. The biochemical abnormalities of the presumed hepatitis developed and largely receded over a period of 8 weeks (Fig. 9). However, the evidence of Australia antigenemia has persisted to the present time 8 months post-transplantation.

Discussion

It has not yet been established that the Australia antigen is the actual serum hepatitis virus.[5, 14, 47] The most fundamental reason for withholding judgment on this question is that DNA or RNA has not been proved to be present in the Au antigen[47]; one or the other of these nucleic acids must be present if it is a virus. Nevertheless, the evidence suggests either that the Au antigen is the serum hepatitis virus or something very closely associated with it. The work of Krugman and Giles[36] and of Barker *et al.*[4] has shown that the Au antigen is infectious. Moreover, Au positive serum usually contains particles that look like viruses of the Picorna class,[5, 51] approximately 200 Å in diameter.[1, 2, 4, 5, 20, 28, 35, 47, 51, 58]

Conceding for the moment that the Au antigen is the serum hepatitis virus, it is hardly surprising to find an increased incidence of this agent in the transplant population. In Blumberg's initial studies and in a number of subsequent ones, the Au antigen was found with increased frequency in patients with a variety of natural immunologic deficiency states including Down's syndrome,[9, 13, 71, 72] leukemia,[7, 9, 12] leprosy[10, 11] and uremia.[24, 37, 39, 40, 77] In our patients, an analogous host background of partial immunologic invalidism was provided by iatrogenic immunosuppression. Although reports of documented serum hepatitis under this latter circumstance have been few in number,[26, 37, 41, 51, 62, 66, 70] this is undoubtedly because technics for Au antigen detection have been widely employed only in the last year or so. It can be expected that serum hepatitis will be found in every large transplantation program providing the appropriate survey is conducted.

The incidence is only the beginning of the total problem in assessing the significance of serum hepatitis under conditions of chronic immunosuppression. Of greater import is the fact that a patient once infected under these circumstances cannot recover in the traditional sense and rid himself of the microorganism, at least as its presence is measured by the precipitating methods (AG, EID, and IEOP). It is of some theoretical interest to note that serologic recovery can occur if the Au antigen is detected *only* by complement fixation (CF). Possibilities for this divergent find-

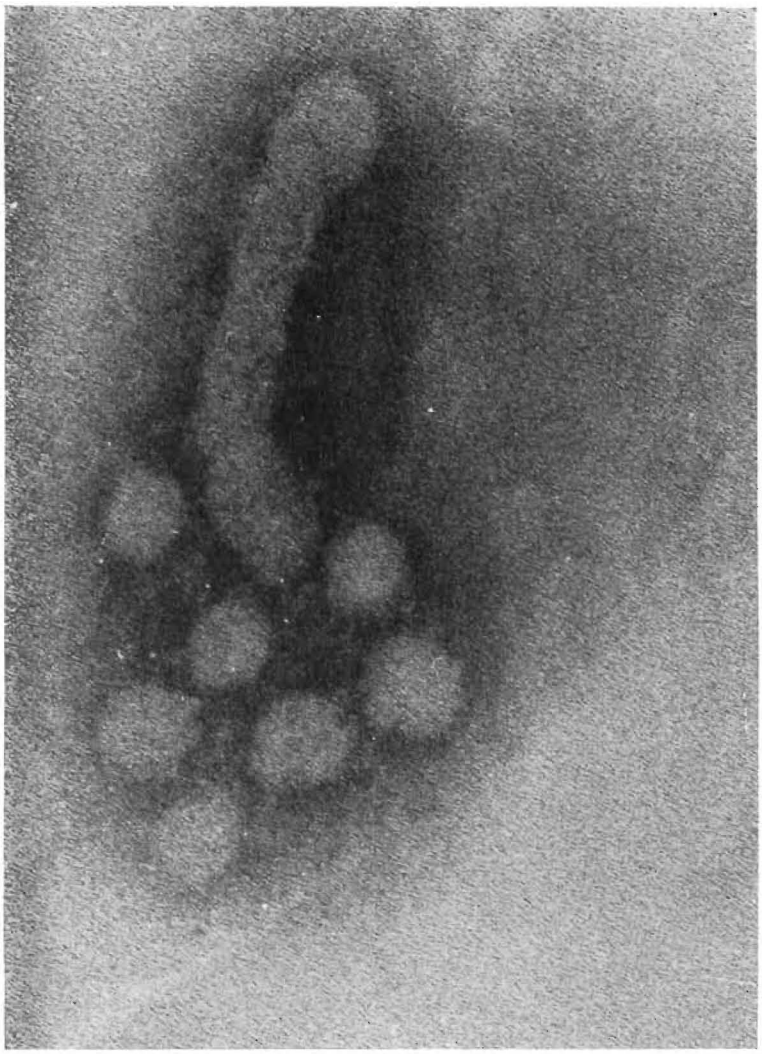

FIG. 8. Virus-like particles isolated from the anticomplementary activity (ACA) purified from the serum of a liver recipient who was Au antigen negative but whose serum had a high ACA titer. The isolated ACA was negatively stained with the conventional phosphotungstic acid method. The virus-like particles had diameters of 160–250 Å. The electron micrography was performed by Paul Nakane, M.D. (×500,000).

ing are, first, that the virus doses being measured by the CF method are small enough to be cleared by the residual host defenses or, second, that the CF determines something that is qualitatively, not just quantitatively, different than that measured by the precipitating techniques.

The observation "*once Au positive, always Au positive,*" if the detection has been with a precipitation technic, has important consequences. An infected patient becomes a carrier capable of infecting other organ recipients or the immunologically normal people who are providing ongoing care. For example, both parents of the renal recipient whose serum serves as our Au antigen standard have developed serum hepatitis. In the last 9 years, more than a dozen of the professional team working in our transplantation unit have developed hepatitis and one, a research technician, has died. Au tests were performed only on the last three afflicted staff personnel, and these were positive, including the fatal case. By personal communication,[49, 78] it is known that deaths of staff members from serum hepatitis have been observed at other transplantation and dialysis centers.

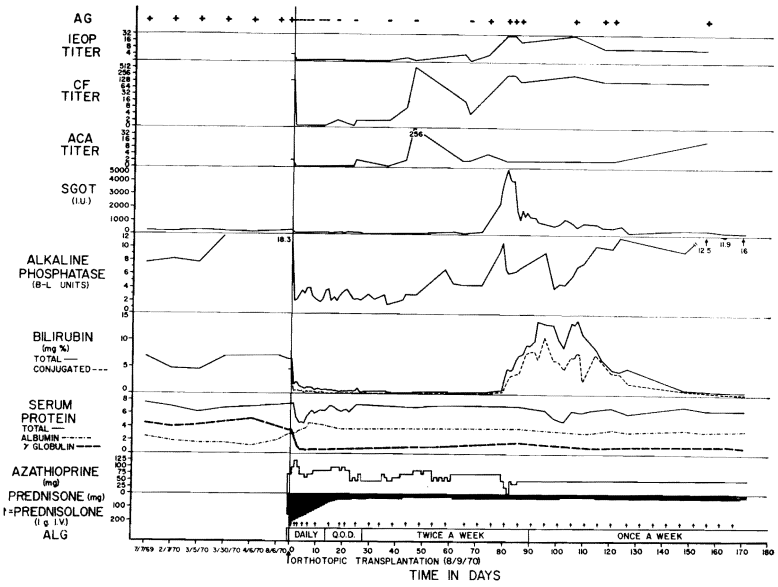

FIG. 9. The course of a patient with chronic aggressive hepatitis, Au positive, who was treated with liver replacement. Immediately after transplantation, all the serologic evidence of hepatitis disappeared, only to return subsequently. Note the profound hypergammaglobulinemia (4 Gm./100 ml. or more); albumin which was 2 Gm./100 ml. or less preoperatively rose to and remained at normal levels after placement of the new liver.

With this particular virus, the risks imposed by infecting hospital personnel are relatively predictable, as judged by the enormous literature concerned with the mortality and morbidity of serum hepatitis. The immunologically normal person with florid disease has a mortality of 1 to 12%.[50, 61] Another small per cent develop chronic and progressive hepatic disease. The vast majority recover fully.

The implications of serum hepatitis in the chronically immunosuppressed recipient who has continuous viremia from which he cannot hope to recover are by no means so well known in terms of liver function over a period of years. It has been suggested by some authorities that the virulence of hepatitis under conditions of immunosuppression is considerably less than in a normal person,[37, 62] presumably by the coincidental prevention of autoimmune sequellae of hepatocellular injury. Such opinions have been used to rationalize the wisdom of immunosuppressive treatment of patients with chronic aggressive hepatitis. Stated differently, the proposition would predict that serum hepatitis would be less dangerous to the immunosuppressed transplant patient than to his nurses or physicians.

The foregoing hypothesis cannot be refuted by the observations in the present investigation since there was no mortality from proven serum hepatitis amongst the studied transplantation patients whereas a non-immunosuppressed technician died during the same interval. Nevertheless, the notion that immunosuppressive agents are liver-protective can hardly be strongly supported by a group of organ recipients of whom more than half had hepatic injury from whatever cause. The most that can be said is that serum hepatitis was not the major cause of the high incidence of hepatic damage in this patient population since the biochemical indices of injury were essentially the same with or without Au antigenemia. Because of this latter fact, exclusion of candidates for renal transplantation because of an Au positive test cannot be justified on the basis that the recipient would be thereby placed at an exorbitant risk.

The same conclusion applied to the patient in the present report who was dying of hepatic failure secondary to chronic aggressive hepatitis, Au positive. In this case, removal of the diseased organ was followed within a few minutes by the com-

plete disappearance of all serologic evidence of the Au antigen from the peripheral blood, indicating that the primary source feeding the antigenemia had been the liver. That it was not the sole repository of the virus was suggested by the subsequent recurrence of the hepatitis at about the same time as would have been expected with an initial exposure to virus at the time of transplantation.

For infectious diseases, the incubation period is often thought of in terms of the host immunologic apparatus. A somewhat different view is necessary in our patient whose liver was replaced. In this case, the latent period seemed intrinsic to the target organ, the liver. When the liver finally exhibited injury, the extrahepatic manifestations of serum hepatitis also became evident, including such complaints as excruciatingly painful joints. Recovery then followed in much the same way as after her first attack of acute serum hepatitis 6 years previously.

The long-term prognosis of this hepatic recipient remains guarded. However, there are two reasons to hope that her new organ will not suffer the same fate as her native liver. First, as discussed earlier, immunosuppression may help prevent the ravages of progressive hepatic disease, at least as these are dependent upon an autoimmune component. Second, the homograft may be less susceptible to chronic aggressive hepatitis if the recently propounded theories of genetic predisposition to serum hepatitis [8, 22] have validity. Hepatic homografts have been proved to retain donor genetic specificity for years and probably permanently after transplantation.[3, 33, 38, 45, 68, 70] Consequently, the transplantation of the liver from a donor who possessed genetically determined resistance to hepatocellular injury could be a major advantage. Third, the patient has had no sign of the hypergammaglobulinemia that was such a prominent feature before transplantation.

Although potential renal and hepatic recipients cannot logically be denied transplantation on grounds that a positive Australia antigen tests would subject them to a prohibitive risk, a decision against the undertaking could be justified on the basis that the creation of a dangerous endemic hepatitis pool within the hospital is unwarranted. After much discussion within our group, we have decided that chronic Au antigenemia should not *a priori* exclude patients for transplantation, providing the appropriate infectious precautions are carried out including training the patient to be impeccable about personal hygiene. It seems to us that the major problem in the past has been the unknowing entry of hepatitis carriers into the program. The single most important identifiable source of hepatitis in our transplantation patients has been pretransplantation renal hemodialysis, accounting for almost half our cases. It may be assumed that at least some of the other half were infected by members of the first group in the course of sharing the ward facilities.

Now that Au tests are available for screening, this kind of hazard can be minimized since at least it will be known in advance which patients constitute a threat and require special isolation care. If hepatitis is to be kept to a minimum, other screening procedures will be essential, including examination of blood donors. Au antigen tests should also be performed on all prospective organ donors. Recently, a cadaveric donor, who had been pronounced dead in one of our hospitals on the basis of irreversible neurologic injury, was discovered to have high titer Au antigenemia. If this had not been detected either 3 or 4 of his organs would have been transplanted and in addition his corneas would have been placed in the eye bank.

Although control of the serum hepatitis problem is predictably going to be one of the major tasks of any large transplantation program, there is no assurance that

such efforts will influence the high incidence of liver disease in these patients since so many recipients with negative Au tests develop abnormal hepatic function. Other viruses, such as those responsible for infectious hepatitis (IH), could be responsible. If this were so, it would have been expected that the anticomplementary activity (ACA) might have been elevated in consequence of the formation of complexes of the virus antigen and antibody. Increases of ACA were observed by Chang and O'Brien during the IH epidemic of the Holy Cross football team.[18] However, in our patients, ACA increases were confined essentially to those renal patients with the Au antigen or to all hepatic recipients with or without Australia antigenemia. This constituted indirect evidence that infectious hepatitis was not a major factor in the liver injury found in our patients.

It is conceivable that further studies of ACA will lead to a better understanding either of serum hepatitis or of the complex immunologic events in the transplant patient. The ACA examined in the present study was shown to be separate from several other recognized complement inhibitors. It was demonstrated to be distinct from C_1 esterase inhibitor[21] by virtue of its heat stability, to have different physical characteristics (Fig. 7) than C3 inactivator,[74, 76] and to have no relationship to rheumatoid factor.

In renal patients, the association of ACA with the Australia antigen could be made with nearly perfect reliability. If, as seems likely, the ACA in these patients consists of Au-antigen-anti-Au antibody complexes, these circulating complexes could contribute to the glomerulonephritis commonly found in the transplants[44, 69] by a mechanism comparable to that described by Oldstone and Dixon in the lymphocyte choriomeningitis virus experimental model.[52]

In contrast, all the recipients of liver homografts possessed ACA, whether or not Au antigen was diagnosed. The purified ACA of one Au positive patient was shown to contain virus-like particles and surrounding halos that looked like antigen-antibody complexes. However, the purified ACA of another patient who was Au negative did not have detectable virus ultrastructurally despite even higher titers. Consequently, the ACA in liver patients may have a special significance over and above any association with the Au antigen. This would not be suprising in liver recipients, inasmuch as high ACA titers have been seen in several diseases that have in common abnormalities of protein metabolism, including hypergammaglobulinemia, cryoglobulinemia, and multiple myeloma.[17, 19]

In the absence of any evidence of an infectious etiology for progressive hepatic disease in the post-transplantation period of a given patient, serious consideration must be given to the possibility of drug hepatotoxicity with particular reference to azathioprine.[67, 70] In such cases, a change in therapy should be considered. As a replacement for azathioprine, cyclophosphamide has received inadequate attention despite its impressive advantages. It has little hepatotoxicity,[48] is one of the best immunosuppressive agents in rodents,[6, 15, 23, 25, 32, 42, 73] and was even shown on a limited trial basis a number of years ago to be capable of preventing renal homograft rejection in humans.[23, 27, 54, 55] In one of our patients, a change was made from azathioprine to cyclophosphamide in December, 1970. After many weeks the seriously deranged liver function tests began to improve. No deterioration in renal function has occurred during the 3 months of cyclophosphamide therapy.

Summary

The hepatitis associated or Australia (Au) antigen has been found with precipitating detection methods in the sera of almost one-fifth of a group of 89 chronic survivors after renal, hepatic, or cardiac transplantation. An additional two recipi-

ents were shown to possess anti-Au antibody. Using a more sensitive complement fixation test, several additional diagnoses of Au antigenemia were made, so that 28% of the studied patients had serologic evidence of having had or, more commonly, of having contact with the serum hepatitis virus.

Once Au antigenemia developed in the immunosuppressed patient, it seldom disappeared and these organ recipients became infectious carriers. Before detection methods for the Au antigen were available, a number of Au positive patients were unknowingly entered into the transplantation program already infected from dialysis or blood transfusion. In turn, staff members treating these carriers frequently developed acute hepatitis and in one instance the consequence was the death of a research technician.

In the recipients of renal homografts who had serial hepatic function as well as serologic tests, there was an incidence of liver malfunction at some time in the post-transplantation period exceeding 50%. However, the frequency of either minor or major derangements in hepatic function was essentially the same with or without Au antigenemia, suggesting that serum hepatitis was not an overriding or even the most important factor in the production of the well-documented liver disease that occurs after renal transplantation.

Anticomplementary activity (ACA) has been said to reflect the presence of circulating antigen-antibody complexes. In both the symptomatic and asymptomatic renal recipients with chronic Au antigenemia, the ACA titers were essentially always elevated. Their sera contained virus-like particles. In contrast, ACA titers were almost never abnormal in the Au negative renal recipients. This finding was construed as evidence against the possibility that liver disease in the Au negative patients was being caused by a different kind of virus, such as the infectious hepatitis (IH) variety. In some instances, drug hepatotoxicity may have been responsible. In one case, azathioprine was so strongly suspected that it was discontinued and replaced altogether with cyclophosphamide. This kind of change in therapy should probably be considered more frequently in such cases.

In liver recipients, the increases in ACA were not confined to patients with Au antigenemia but were always present. In two recipients with high ACA titers, one Au negative and one Au positive, the ACA was highly purified with chromatographic techniques. The product from the Au positive serum contained virus-like particles, whereas the ACA in the other case did not have this finding.

On the basis of much of the foregoing information about serum hepatitis under conditions of chronic immunosuppression, a patient dying of chronic aggressive hepatitis, Au positive, was submitted to liver replacement and then treated with azathioprine, prednisone, and heterologous ALG. Within minutes after host hepatectomy, the Au antigen disappeared from the serum suggesting that the native liver had been its primary source. The antigen returned many weeks later just before the onset of a moderately severe bout of hepatitis affecting the homograft. The patient has almost completely recovered from this incident and is now 8 months post-transplantation.

Acknowledgments

We wish to thank Dr. Masami Sato, First Department of Internal Medicine, Okayama University School of Medicine, Okayama, Japan for kindly supplying Au antigen reference sera.

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Discussion

Dr. John S. Najarian (Minneapolis): I would like to compliment Dr. Starzl on an excellent study on this group of patients. I would like to make one comment, and ask him two questions.

(Slide) We transplanted a patient about a year and a half ago who had a subacute hepatitis with decreasing hepatic function and who went into hepatic-renal failure; eventually we did a hepatic transplant on this patient. This led to many problems, including yours truly, who developed hepatitis. I would like to show you what occurred prior to the time of this transplant.

Here is the hepatic transplant; it was done at this point. Prior to that time the total number of cases of hepatitis among hospital and laboratory personnel, transplant personnel, dialysis personnel, was about this rate; and yet we had several patients with positive Australia antigens, and at least in most instances, subicteric hepatitis.

The transplant was performed at this point, and you can see the tremendous number of cases that resulted—a total of 46. It was pointed out to me by Dr. Simmons, who made this slide, that I am one of these little black squares.

The total cases is shown here, over periods extending from approximately two and one-half months from the time of transplant on, most of which could be directly related to this transplant in this patient with hepatitis.

Who was affected? It was the hospital personnel, the transplant personnel; interestingly enough, the dialysis personnel were much more careful than the rest of us, and didn't have an increased incidence of hepatitis. There was no increase of hepatitis in the dialysis patients. There was only one case in a posttransplant patient. The number of transplants that we were doing on a monthly basis was about at the same rate as it was before this time, so we did not decrease our activities.

I simply bring this out to corroborate what Tom said; and that is that the ones not at risk are the dialysis patients and the transplant patients—the dialysis patients perhaps because they are immunosuppressed from their uremia, and the transplant patients, perhaps because they were immunosuppressed as well. All of us nonimmunosuppressed patients fit in this category (active hepatitis).

I would like to ask Tom two questions. The first relates to the claim (sic) that he has transplanted one patient with hepatitis. I am wondering if the data that is accumulating now will indicate that, perhaps, we should consider the possibility of immunosuppressing a patient who contracts hepatitis, bad as that sounds? We have toyed with this for some time because immunosuppressed patients do so well. Perhaps the major destruction to the liver occurs because of the host response to the virus in the liver. If immunosuppression is used, it may be a method of handling patients with hepatitis—specifically, the older patients in whom the mortality, as you know, goes up rather precipitously.

The second question has to do with an observation that he may or may not have made on some of these patients. It has been recently reported in *The Lancet* that a group of patients that had hepatitis on dialysis showed an increase in erythropoitin. These authors discovered this effect by accident, as you know, when they saw that the requirement for transfusion in some patients was diminishing and they correlated this finding with patients who developed hepatitis on dialysis. They made the suggestion that under these circumstances the liver is not degrading erythropoitin if this occurs and there were increased erythropoitin levels, increased hemoglobin, and a decreased need for transfusion.

I wonder if this was seen in any of the patients that Dr. Starzl described with positive Australia antigen, or hepatitis.

DR. J. GARROTT ALLEN (Palo Alto): This has been a fascinating presentation, and on a subject that I have been interested in for many years. One of the most difficult things to explain in transfusion hepatitis is the existence of the carrier donor. How does such an individual manage to harbor the infectious agent of hepatitis (presumably a virus) for so many years without himself developing evidence of liver disease? The carrier and his virus seem to adapt themselves very well to each other.

One cannot escape the concept of tolerance in such a state—tolerance of the carrier for the virus and tolerance of the virus for the carrier. If we understood this mechanism, the rejection problem of transplantation would also be solved.

The possibility of a carrier state and its relationship to impaired host immunity in hepatitis first occurred to me in 1947, when in the course of treatment of the first patient with hypo- or agammaglobulinemia, we discovered that this patient did not develop hepatitis despite our supplying her with gammaglobulin in pooled plasma and blood for the next 12 years. A similar observation has more recently been reported by a number of workers who have noted that posttransfusion hepatitis acquired in patients on immunosuppressive therapy usually runs a very mild course. In the dialysis units and on transplantation services few if any of these patients have died of hepatitis, but this is not true of the staff taking care of these patients. As Dr. Najarian and others have noted, the fatalities have tended to occur among the personnel, people not on immunosuppressive agents. With a normal immune response, they slowly produce antibodies, and eventually four to eight weeks later, there is a "confrontation" of the virus with the antibodies in the hepatic cells that leads to cellular destruction.

In this discussion, one is reminded of the significance and eloquence of von Pirquet and Schick's book, "Serum Sickness," first published in 1905, and translated from German into English by Schick in 1951. The point of their study that is relevant to this discussion can be paraphrased as follows: *No antibody formation—no disease.* The same concept probably applies to many organisms that are nonpathogenic for man, simply because they do not provoke an antibody response. This, in a sense, is what we are approaching, it seems to me, in patients with posttransfusion hepatitis on immunosuppressive therapy, an antigen in man unable to produce antibodies. In many instances there is no point in keeping these patients in the hospital for their hepatitis, though regulation of their immunosuppressive therapy requires it. These patients should be considered highly infectious to hospital personnel and visitors.

Dr. Najarian mentioned the possibility of treatment of hepatitis with immunosuppressive agents. We have wondered about this too, but usually by the time the patient has hepatitis, his disease is far enough advanced that immunosuppressive therapy may carry a much greater hazard than when the disease develops in a patient already on immunosuppressive therapy. As far as I know, we have had no deaths from hepatitis among patients on immunosuppressive therapy.

I would like to raise a point with Dr. Starzl: The best results for the detection of the Au antibody that I know of, are those from Dr. Prince of the New York Blood Center. As of last week, I had a letter from him to the effect that by whatever test his laboratory has attempted to use, a maximum of only twenty-five per cent of infectious donors was detected. Similar reports have come from the NIH and others. It makes one begin to question if three fourth of the cases are not due to some other agent than the Au antigen. Although Au screening is recommended, it seems rather a futile method of reducing the incidence of the disease at this time.

And this is a serious consideration, it seems to me, when one realizes that the average patient, leaving out the heart cases, gets about four units of exposure. To take one of those four possibilities out, I don't believe is going to do anything to our present attack rate.

DR. ALAN G. BIRCH (Boston): We have had the unfortunate task of reviewing the long-term data on 37 cases of clinical hepatitis which occurred on our dialysis and transplant unit during a 16 month span in 1969 and 1970. There were 14 cases occurring in dialysis patients, out of a pool of approximately 150 patients at risk, 13 cases among the 70 transplanted patients during this time, and ten cases out of about 80 staff exposed.

Our experience substantiates the impression and points made by both Dr. Starzl and Dr. Najarian and others, that there certainly is a gradation in the clinical severity in these cases. The dialysis and transplant patients have mild cases. They do, however, usually show significant enzyme rises, whereas the cases in the staff have been far more severe clinically, and one dialysis nurse and one patient's wife have succumbed from hepatic failure.

Our experience does not substantiate the contention made by Dr. Starzl that persistent Australian antigenemia is inevitable in the immunosuppressed patient. It has been our policy to decrease or discontinue Imuran when hepatitis is clinically diagnosed, and we have discontinued Imuran for 20 to 380 days, for an average of 160 days, in 12 such patients.

The Australian antigen, measured by the somewhat crude immunodiffusion technic, has remained negative in five of these patients, including one who had a positive antibody titer. Three of four patients who started with a positive Australian antigen when Imuran was discontinued, have converted to negative during the subsequent 3 to 6 months. Two patients in whom Imuran was not

discontinued were, and have continued to be, Australian antigen positive.

While the patient is off Imuran, we have not added additional immunosuppressive drugs, but have merely slightly increased the prednisone dose. Yet only three episodes of rejection have occurred in a total of 1700 days at risk off Imuran. This very low rejection rate makes us wonder if there is indeed some protective effect engendered by the deranged hepatic function, or possibly due to the rise in anticomplementary activity described by Dr. Starzl.

Finally, we would like to say that the contention by Dr. Starzl that decreasing the Imuran indeed may be good for the liver itself—is suggested, in that six of the 12 patients in whom Imuran was discontinued, three with and three with negative Australian antigen, have had recurrent enzyme rises when Imuran was reinstituted. These rises have quickly fallen back to normal when Imuran was again discontinued, and have, in five of the six cases, allowed Imuran to be started again only after some months of further followup.

DR. THOMAS E. STARZL (Closing):

Dr. Najarian, I can't help but note that those most concerned with epidemiologic studies of hepatitis, have an interest that correlates with their own previous serum bilirubins.

Dr. Najarian raised two questions. The first was whether or not immunosuppression should be used for patients who become ill with serum hepatitis. This question was asked of me by Dr. Francis Moore in Los Angeles recently, and I will now give the same answer as I gave him, but perhaps with somewhat less assurance. The answer was no.

It may be, as Dr. Najarian has suggested, that the chance of dying from acute hepatitis under conditions of immunosuppression is less than in the normal patient. However, the penalty of immunosuppression appears to be chronic Australia antigenemia. I suspect that in the long run somebody with chronic Australia antigenemia will be subject to a whole variety of risks, of which some are not even yet known. Chronic aggressive hepatitis would be one possibility, and this would be unacceptable in a disease that has 90% total recovery rate, as you yourself experienced, Dr. Najarian.

Moreover, there are other chronic hepatic diseases in the etiology of which Australia antigenemia has been implicated or speculated about. These include primary biliary cirrhosis and even hepatoma. These are some of the reasons my answer is no.

I'm sorry to say that I have no information on the second question which concerned erythropoitin.

As to Dr. Prince's observations, I think that Dr. Prince believes that his IEOP method is nearly 100% accurate if the diagnosis is serum hepatitis and if samples can be obtained at the appropriate stage of the disease. Of course, there are other kinds of hepatitis than that detectable by the Au testing, which is the point made by Dr. Allen. Consequently, the mere use of Au screening will not eliminate hepatitis, although certainly the testing is worthwhile.

Dr. Allen, it seems to me, has raised the really key issue about the reason for chronic Australia antigenemia in immunosuppressed patients. The tolerance theory mentioned by Dr. Allen was described a number of years ago by Professor Burnett of Australia as an explanation of chronic viremia. With immunosuppression, the conditions are propitious for the development of tolerance, including as they do the continuous presence of the antigen, plus immunologic weakening. In turn, this combination of circumstances is precisely that which Dr. Robert Schwartz, of Boston, thought important for tolerance induction in his first reports about Imuran in 1959. In this classic paper he showed that tolerance to bovine serum albumin (BSA) could be produced by administering BSA and Imuran together. It is not hard to similarly envision the hapatitis virus plus immunosuppression leading to tolerance to the virus at the same time as renal antigens plus the same immunosuppression lead to kidney graft acceptance.

Concerning the whole question of tolerance, I didn't want to leave that subject without complimenting Dr. Pierce and Dr. Marchioro for what I think may well be a hallmark paper given at this meeting. They have shown us how we can get at some of the mechanisms of graft acceptance in a way that is different and easier than in those pioneer studies presented by Dr. Joe Murray to this association in his paper of 1964 on canine renal transplantation. With the technics of Pierce and Marchioro, the essential inquiries can be made in a test tube, or on a plate, rather than requiring complicated experimental protocols such as those previously used.

Finally, I think there's nothing essentially inconsistent about the observations at Denver and at the Peter Bent Brigham Hospital. I think if one really wanted to get rid of chronic Australia antigenemia, the only reasonable way to do it would be to stop immunosuppression. It just happens that we have not done that, and the group at the Brigham have. Since they have, they have coincidentally performed a tolerance experiment in those dozen or so patients who have had their immunosuppression stopped. It is heartening to see in the human that the rate of continuing graft function after discontinuation has been higher than that in the experimental animal, or more specifically the dog.