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AUTHORS' INTRODUCTION

In 1969 we were requested by the publishers of *CRC Critical Reviews in Clinical Laboratory Sciences* to prepare a comprehensive review of the literature on Australia antigen. The paper was published in 1971 and included all papers on Australia antigen of which we were aware that had been published up to July 1970.

The original studies on Australia antigen began in 1963 and until 1968 essentially all the publications on the subject emanated from the Division of Clinical Research of The Institute for Cancer Research. Several papers from other laboratories appeared in 1968 and in subsequent years large numbers were published from laboratories throughout the world.

The present bound publication is essentially the same as that published in the CRC Critical Reviews with some minor changes. It also includes a supplementary list of some publications which have appeared before and after July 1970 but which for the most part are not discussed within the text of this review. We plan to prepare a more comprehensive book on Australia antigen sometime in the future.

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INTRODUCTION

The first publication referring to what was later known as Australia antigen [Au(1)] appeared in 1964.[1] In 1963 a precipitating antibody which reacted with a serum from an Australian aborigine had been detected in the blood of a hemophilia patient. Following a practice common in human genetics, a geographic term "Australia antigen" was applied to the reacting material in the blood of the aborigine.

After this initial finding we initiated a survey of sera from normal and diseased populations and established that the agent in the blood was persistent and that it could be stored for weeks, months, or years, and retain its reactivity. Serial samples from many subjects had been stored at -25°C in the blood collection of the Institute for Cancer Research for up to 10 years. These were tested and some of the initial results are shown in Table 1. From this early survey of populations we determined that Australia antigen is rare in normal Americans and North Europeans, but relatively common in some tropical and Southeast Asian populations. We also found that Australia antigen was not common in most of the diseases in which it was tested, but it was relatively common in patients with leukemia. In 1966 we found its association with hepatitis[2] and began to test the hypothesis that it was an infectious agent which causes hepatitis in man.

The results of these studies, most of which have now been amply confirmed in laboratories other than our own, will be summarized briefly here in Table 2, and are discussed in detail in later sections. Australia antigen is associated with acute viral hepatitis, both "serum" and "infectious," and with various forms of chronic hepatitis. Isolated Australia antigen has an appearance compatible with that of a virus particle of about 200 Å in diameter.[3] The preparations may contain sausage-shaped figures and larger (400 Å) particles.[3,4] Millman and his colleagues[5,6] have prepared fluorescent anti-Au(1) antiserum. With this they have shown that the liver cells of patients with Australia antigen in their peripheral blood

TABLE 1

a. Australia Antigen in Normal Populations

Population	No. Tested	Australia Antigen Present	
		No.	%
Aborigines, Australia	208	12	6
Chinese, U. S., and Taiwan	65	0	0
Eskimo, Alaska	24	0	0
Greeks, Greece	179	8	4
Indians, Canada	78	0	0
Indians, Mexico	100	1	1
Israelis	96	2	2
Japanese U. S.	48	0	0
Koreans	1	1	
Micronesians, Rongelap	193	7	4
Negroes, U. S.,	241	0	0
Newborn children, white	18	0	0
Polynesians, Bora Bora	24	1	4
Samaritans, Israel	125	2	2
Taiwanese	23	3	13
Tristan da Cunha Islanders	42	0	
Vietnamese	24	1	4
Whites, U. S.,	215	0	0
	1704	38	

b. Australia Antigen in Patients

Disease		No. Tested		Australia Antigen Present	
Abetalipoproteinemia		4		0	0
Amyotrophic laternal sclerosis		15		0	0
Anemia		18		0	0
Arthritis, various*		15		0	0
Cancer (other than leukemia)		47		0	0
"Connective tissue" disorders†		5		0	0
Diabetes		96		0	0
Hemophilia		24		0	0
Hyperchloesterolemia		17		0	0
Leukemia		70		8‡	11.4
Acute myelogenous	17		4		
Acute lymphocytic	38		2		
Chronic myelogenous	10		0		
Chronic lymphocytic	3		1		
45 chromosomes	2		1		
Lupus erythematosis		69		0	0
Multiple myeloma and macroglobulinemia		93		1‡	1.1
Myasthenia gravis		11		0	0
Rheumatic fever		124		0	0
Tangiers Island disease		3		0	0
Thalassemia		48		2	4.2
Total		659		10	

*Includes eight patients with rheumatoid arthritis, three with psoriatic arthritis, and four with Sjögren's disease.
†Other than lupus erythematosis.
‡One patient had both chronic lymphocytic leukemia and multiple myeloma, and is included in both categories.

TABLE 2

Evidence in Support of Australia Antigen Being A Virus
Or An Antigen On A Virus Which Can Cause Hepatitis In Man

1. Association with acute viral hepatitis.
2. Association with chronic hepatitis.
3. Virus-like appearance under the electron microscope (200 A particles).
4. Transmission of Au(1) from man to man.
5. Transmission and passage of partially purified Au(1) to an animal host (infant African green monkey).
6. Localization with fluorescent anti-Au(1) to the nuclei of liver cells of patients with hepatitis and/or Au(1) in their blood.
7. Distribution in institutions is consistent with the distribution of an infectious agent.
8. Detection of small amounts of RNA (5%) in isolated Au(1).
9. Evidence of replication of Au(1) in liver cells in tissue culture.

and/or hepatitis have, in general, fluorescent granules in their nuclei. This has been confirmed by studies of liver cells of leukemia patients with Australia antigen.[7] Patients who are transfused with blood containing Australia antigen will often develop hepatitis accompanied by Australia antigen in the blood.[8] Isolated and partially purified Australia antigen has been transmitted to nonhuman primates (infant African green monkeys) and passaged two times. The amount of antigen present in the final passage is greater than would have been expected by simple dilution.[9]

Liver cells from patients with Australia antigen, which contain material which reacts with fluorescent anti-Au(1), have been grown in tissue culture. After six passages, the cells still contained Australia antigen in their nuclei, and Au(1) was also found in the tissue culture fluid.[10] Epidemiologic studies have shown that the distribution of Australia antigen in institutions is consistent with the distribution of an infectious agent.[11] It has been reported that RNA is present in Australia antigen isolated from blood in a proportion of about 5%.[12] This is an extremely small amount if Australia antigen is a complete virus. It is still not certain if the RNA is a portion of the particle or a contaminant, although its presence has been confirmed by other methods.[13]

In addition to the characterization of Au(1), our original interest in differences in human response to disease led us to characterize those individuals who have persistent Au(1) (in whom it was first found) in contrast to those who get it transiently with or without the development of acute hepatitis. These differences (genetics, sex frequency, immunologic response, type of disease, etc.) also will be discussed in this review.

Tests for the Detection of Australia Antigen

Immunodiffusion

Australia antigen was defined as a precipitin band in agar gel that formed as the result of a reaction between a serum from a multiply – transfused patient with hemophilia and an Australian aborigine.[14] All antisera used in our laboratory since then have been compared either directly or indirectly with this original hemophilia serum, and all sera found to have Au(1) are serologically similar (giving lines of identity) to the antigen in the aborigine's serum. Furthermore, most investigators who have published studies of Australia antigen have received antisera from the Philadelphia laboratory and compared their antisera with our reference standards. The Division of Biologics Standards has established a standard reference antibody and a panel of reference antigens. This antibody, which was produced in guinea pigs, also gives a line of identity with our human antibody.

The immunodiffusion (ID) method used in our laboratory is given in detail elsewhere.[15] In brief, we coat 3¼" x 4" glass lantern slides with 1.1% agarose in a buffer that has a pH of 7.4 to 8.4. The particular buffer used, phosphate buffered saline or veronal buffer, etc. does not seem to be important so long as the pH does not fall below 7.4. In the past we have used Noble agar and Ionagar and these work satisfactorily, especially with strong antibodies. Agarose is more uniform and the results obtained more reproducible. Seven hole patterns, one center well and six peripheral wells, are punched in the gel. Serum containing anti-Au(1) is placed in the center well. Serum known to contain Au(1) (positive control) is placed in the upper and lower wells. In this

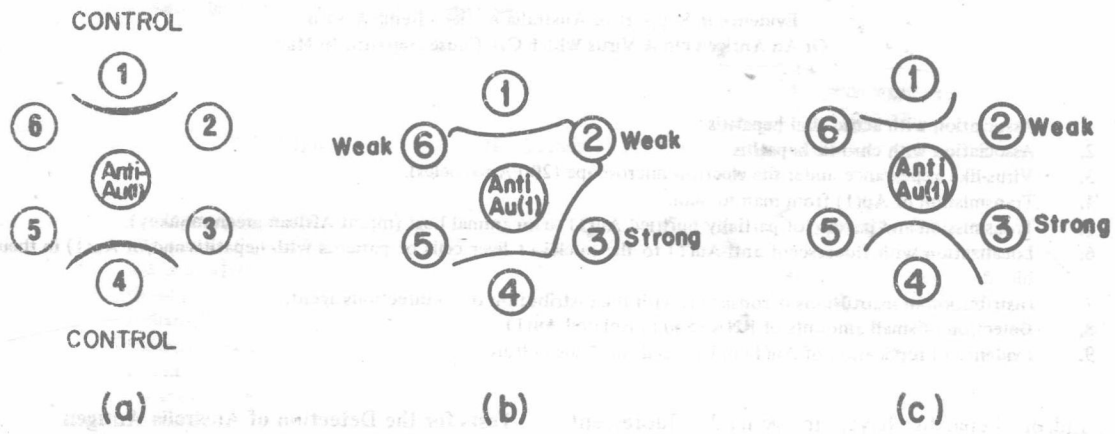

FIGURE 1. Double immunodiffusion in agar gel. a. Controls in wells 1 and 4, all test sera negative. b. Controls in wells 1 and 4. Test sera in wells 2, 3 and 6 positive. c. Control in wells 1 and 4. Sera in wells 2 and 3 contain antibody.

manner all sera tested are placed adjacent to the positive control and a serum is only called positive if it forms a line of identity with the positive control precipitin (Figure 1). Also, in this method sera are simultaneously tested for the presence of antibody to Au(1). It should be noted that we do not put additives such as protamine in the gels and we do not ordinarily concentrate sera or double fill wells. These techniques all introduce some measure of nonspecificity and although they may have use as research procedures, they should not be used routinely in clinical testing. If the procedures described herein are followed, all results can be directly related to the original Australia antigen-antibody reaction.

None of the other methods currently in use (e.g. complement fixation, reaction electrophoresis) have the assurance of specificity present in the immunodiffusion reaction. Furthermore, sera scored positive by one of these other methods but negative by immunodiffusion may or may not be detecting Au(1). This will have to be evaluated by duplicating studies we have already completed with the immunodiffusion method.

Complement Fixation (CF)

Complement fixation is a method commonly used to quantitate antibody titers following virus infections. Shulman and Barker[17] and Purcell et al.[18] adapted this method for the detection and quantitation of Australia antigen. The method used by most workers is a modification of the microtiter method. The test serum is diluted in twofold dilutions from 1:4 to 1:512. Two units of guinea pig complement with 2 to 4 units of antibody, antigen, or saline are incubated overnight at 4°C, followed by the addition of sensitized sheep red blood cells and an incubation of 15 to 30 minutes at 37°C. If complement is fixed only in the antigen or antibody series the reaction is considered specific. If serum with saline alone fixes complement the test serum is considered anticomplementary (AC). If the AC titer is as high as the specific CF titer the serum is recorded as AC and the interpretation of this result is uncertain. If the titer of CF activity in the antigen or antibody series is detectable in a fourfold or greater dilution beyond the AC activity, then the serum can be scored positive for antigen or antibody.

Shulman and Barker[17] suggested that sera from hepatitis patients with AC activity contained Au(1) – anti-Au(1) complexes. At this writing this hypothesis has not been rejected nor has it been substantially supported. Some sera with strong AC activity form typical Au(1) precipitins on immunodiffusion, others will show antigen-antibody complexes on radioimmunoassay, but

the majority do not give Australia antigen reactions by other methods (immunodiffusion, reaction electrophoresis). AC activity is commonly encountered in blood from hepatitis patients (see discussion of common source epidemics) and it is important to subject this hypothesis to further testing.

Mazzur et al.[16] in our laboratory have made a systematic comparison of CF with ID tests. Six hundred and forty-four sera were selected from groups with increased frequencies of Au(1) including sera from patients with hepatitis, Down's syndrome, leprosy, leukemia and thalassemia, and sera from normal populations. Of these, 159 were positive by both tests, 430 were negative by both tests, 38 were positive by CF and negative by ID, and 22 were positive by ID and negative by CF. The 22 that were positive by ID and negative by CF were AC and the 38 that were positive by CF and negative by ID had, in general, low titers of Au(1) (1:16 or below). This study suggests that there is a small increase in sensitivity by the CF method but this advantage may be cancelled out by the inability to interpret AC bloods.

Counterelectrophoresis

This method for the detection of Australia antigen (also known as crossover electrophoresis, reaction electrophoresis and immunoelectro-osmophoresis) was first introduced in Europe by Bedarida, Trinchieri, and Carbonara[19] and Pesendorfer, Krassnitzky, and Wewalka.[20] Its use in the U. S. was reported initially by Gocke and Howe[21] and later by Prince and Burke.[22] The method combines the rapid migration of serum proteins achieved by electrophoresis with the usual immunodiffusion precipitin reaction in agar gel. Under the appropriate electrophoretic conditions Au(1) migrates toward the anode while anti-Au(1), a gamma globulin, migrates toward the cathode. Thus when serum containing Australia antigen is placed in a well towards the cathode, and antibody to Australia antigen is placed in an adjacent well on the anodal side and an electrophoretic current is applied, antigen and antibody will migrate toward each other, and when they meet at equivalence a precipitin will form. The conditions used by investigators employing this method vary with respect to the type of agar, the buffer system, pH, voltage, amperage and duration of electrophoresis. Systematic comparative studies have not been done to evaluate these factors. Furthermore, the specificity of precipitin bands formed during this procedure cannot be ascertained directly. That is, lines of identity with control positive sera do not occur.

There is general agreement that this method is rapid; precipitin bands form in less than one hour. The proponents of reaction electrophoresis have stated that it is more sensitive than the immunodiffusion method. In our hands, reaction electrophoresis has about the same sensitivity as immunodiffusion. It is somewhat less sensitive if only the readings of wet plates are considered, but it detects a few more precipitins on stained plates. We do not know whether or not the additional precipitins noted on stained plates are Australia antigen.

Thus far the primary advantage of the method appears to be speed and this makes it suitable for use by blood banks. We recommend, however, in situations not requiring a rapid test, immunodiffusion be done either in conjunction with or instead of reaction electrophoresis.

Radioimmunoassay

Radioimmunoassays have been reported by Walsh, Yalow, and Berson,[23] Purcell,[15] and Coller and Millman.[24] The methods have in common the labeling of purified Au(1) with ^{125}Iodine, the development of a standard precipitin curve of anti-Au(1) with the radioactive labeled Au(1). The methods differ in the technique used to detect and quantitate Au(1) – anti-Au(1) binding.

In the method of Coller and Millman,[24] Au(1) is isolated from plasma treated with pronase by column chromatography and sucrose and cesium chloride density gradient ultracentrifugation. ^{125}Iodine is conjugated with the purified Au(1) by the method of Greenwood, Hunter and Glover.[25] The assay of Au(1) involves the coprecipitation of soluble complexes of Au(1) ^{125}I, and human anti-Au(1) with rabbit anti-human IgG and 25% saturated ammominum sulfate, and counting the radioactivity in the precipitate. Less than 0.1 mμg of Au(1) is detected by this method as are antibody titers of 1:320,000. The technique permits: 1) detection of Au(1) and anti-Au(1) present in the same serum; 2) quantitative estimation of Au(1); 3) the detection of Au(1) and anti-Au(1) in concentrations too low to be detected by other means.

At present the radioimmunoassay methods are laborious, take several days to complete, and have