

## A Serum Antigen (Australia Antigen) in Down's Syndrome, Leukemia, and Hepatitis

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WE HAVE PREVIOUSLY REPORTED the presence of an isoantigen of human sera, rare or absent in normal U. S. and northern European populations but relatively common in patients with leukemia (1, 2). The "new" isoantigen has been called "Australia antigen" because it was first found in the serum of an Australian aborigine. It appears to be different from the low-density lipoprotein isoantigen system first described in our laboratory (3-5). Hemophilia patients and others who have received multiple transfusions may develop precipitating isoantibodies against Australia antigen. These precipitating isoantibodies react with a protein in the blood of some normal and some sick individuals. This isoantigen (Australia antigen) could not be identified as being any of the known serum proteins.

Australia antigen (Au(1)) has the immunoelectrophoretic mobility of an alpha globulin and a specific gravity of less than 1.21 (6). It stains faintly with the lipid-specific dye Sudan black, indicating the presence of some lipid. It can be distinguished from the serum alpha- and beta-

lipoproteins by its staining characteristics, flotation properties, and immunological specificities. Specific antibodies against Australia antigen have been produced in rabbits by immunization with whole serum that contains Australia antigen followed by absorption with the sera of individuals without the protein (7).

Recent studies of blood collected in Cebu, Peru, and Rongelap Atoll show significant family clustering of the trait (8). In addition, the segregation data are consistent with the hypothesis that individuals homozygous for an allele  $Au^1$  (genotype  $Au^1/Au^1$ ) have detectable Australia antigen (phenotype Au(1)) while those homozygous for an alternative allele ( $Au/Au$ ) and heterozygotes ( $Au^1/Au$ ) have no detectable antigen (phenotype Au(0)). Pending the acquisition of additional data, this genetic hypothesis has been proposed to explain the family clustering in the populations tested.

With the use of human and rabbit antisera, the distribution of Au(1) in several normal and patient populations has been determined (2, 8). Au(1) is absent in normal U. S. populations and rare in north European populations (approximately 1/1,000) but is fairly common in some Southeast Asian and Mediterranean populations. It is also relatively common in patients with leukemia, particularly in those with acute granulocytic leukemia (see below).

The original finding of Au(1) in very low frequency in normal individuals and in

Received November 2, 1966; accepted for publication January 24, 1967.

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This study was supported by research grants CA-06551, CA-08069, and FR-05539, the National Cancer Institute, the National Institutes of Health, Bethesda, Md.

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relatively high frequency in some kinds of leukemia suggested that individuals with this trait (which they may have inherited) may be more susceptible to leukemia than those without it. A corollary of this hypothesis is that individuals with a high risk of developing leukemia would comprise a population having a higher frequency of the trait than subjects drawn from the normal populations. Several such groups are known. These include patients with Down's syndrome (mongolism), polycythemia vera, co-twins of an identical twin having leukemia, individuals exposed to radiation, and others (for a review, see Miller (11)). Several of these groups have been tested for the presence of Au(1), and the highest frequency in any U. S. population so far tested has been found in a group of patients with Down's syndrome (9, 10).

In the present paper, we describe our further studies on the prevalence of Au(1) in Down's syndrome patients.

#### MATERIAL AND METHODS

Blood specimens were obtained from 75 male residents of the State Colony at New Lisbon, N. J., who were admitted with the diagnosis of mongolism. For a control group, blood specimens were obtained from 76 male residents with the diagnosis of "epilepsy." The age distribution was approximately the same as in the Down's syndrome group, and they had been institutionalized for approximately the same length of time. The admitting diagnoses of residents at New Lisbon State Colony are supplied by the patient's local physician, and these in general are not altered after admission. Chromosome studies were performed on 17 of the Down's syndrome patients and 4 of the controls (see below).

After the identification of Down's syndrome patients with and without detectable Australia antigen, a second collection was undertaken in which blood was withdrawn from 19 residents with Au(1) and 26 residents without it. A series of tests were performed on these samples. The white blood cell, red blood cell, and platelet counts, the white blood cell differential count, hemoglobin concentration, and hematocrit volume were determined using standard laboratory methods. We are indebted to Dr. R. A. Donato

of Jeanes Hospital, Fox Chase, Philadelphia, Pa., for performing these tests. The following red blood cell antigens were identified using the appropriate antisera: B, A<sub>1</sub>, A, C, D, D<sup>a</sup>, E, c, e, Le<sup>a</sup>, Le<sup>b</sup>, Kp<sup>a</sup>, Kp<sup>b</sup>, M, N, S, s, Vel, K, k, Fy<sup>a</sup>, Fy<sup>b</sup>, Jk<sup>a</sup>, Lu<sup>a</sup>, Lu<sup>b</sup>, Js<sup>a</sup>, P, Tj<sup>a</sup>, and V<sup>a</sup>. We are indebted to Dr. Neva M. Abelson of the Hospital of the University of Pennsylvania for performing these tests. Uric acid levels were determined by the method of Liddle, Seegmiller, and Laster (12) through the courtesy of Dr. L. A. Healey of the University of Washington. The white blood cell glucose-6-phosphate dehydrogenase and galactose-1-phosphate uridyl transferase levels were determined by Drs. W. J. Mellman and F. A. Oski of the University of Pennsylvania using the methods described by them (13). The vitamin B<sub>12</sub> levels were determined by the method of Hutner, Bach, and Ross (14). We are indebted to Dr. W. J. Williams of the University of Pennsylvania for performing these tests.

Blood specimens from nine Down's syndrome residents (five girls and four boys) of a Maryland institution for mentally retarded children were also tested. Blood from patients with polycythemia vera was obtained at three different clinics. These included 14 patients from Philadelphia, 36 from Portland, Ore., and 32 from San Francisco. Other sera from patients were collected primarily in the Philadelphia area, as noted in the Acknowledgments section. Venous blood was collected into dry Vacutainers (Becton, Dickinson Co.) or test tubes. The blood was allowed to clot overnight and the serum, removed by centrifugation. In some cases, small amounts of sera were separated from the blood immediately after collection and used in the double diffusion tests. Sera were stored at -20°C for later testing.

Double diffusion in agar gels was done by a micro-Ouchterlony technique described previously (15). Double diffusion in agar gel tubes was done by the method of Preer and Preer (16). Chromosome studies were performed on leukocyte cultures from 17 of the Down's syndrome patients using a method recently described (17).

The chi-square determinations were done as described by Snedcor (18). When any of the two-by-two table cells contained numbers lower than five, Fisher's exact method was used. Student's *t* test was used for comparing the quantitative results obtained on the antigen-positive and -negative bloods. Where appropriate, the method for comparing unpaired series of numbers was used.

TABLE 1. Distribution of Australia Antigen in Patients and U. S. Normals

Disease	No. Tested	No. Positive	% Positive
A-beta-lipoproteinemia	6	0	0
Amyotrophic lateral sclerosis	15	0	0
Anemia, various	26	0	0
Arthritis*	70	0	0
Cancer, other than leukemia	95	0	0
Diabetes†	303	0	0
Down's syndrome (mongolism)‡	84	25	29.8
Fanconi's anemia (hypoplasia bone marrow)	2	1	—
Hemophilia	60	3	5.0
Hepatitis, virus§	48	5	10.4
Hodgkin's disease	12	1	8.3
Hypercholesterolemia	17	0	0
Leukemia and related diseases (see Table 2)	177	16	9.0
Lupus erythematosus	69	0	0
Multiple myeloma and macroglobulinemia	95	1	1.1
Myasthenia gravis	11	0	0
Polycythemia vera	82	0	0
Rheumatic fever	124	0	0
Tangier disease	3	0	0
Thalassemia	84	2	2.4
U. S. normal population**	1,524	0	0
Total	2,907	54	

\* Fifty rheumatoid arthritis, 3 psoriatic, 4 Sjogren's, 8 ankylosing spondylitis, 5 "connective tissue" disease.

† Includes 96 from the United States and 207 from Brazil.

‡ Includes 75 male patients from the New Jersey institution, and 5 females and 4 males from the Maryland institution.

§ These sera were from patients suspected of having infectious or serum hepatitis.

|| One patient with Au(1) had both chronic lymphocytic leukemia and multiple myeloma and is included in both categories.

\*\* This includes 607 Negro and 917 white persons.

## RESULTS

### DISTRIBUTION OF AUSTRALIA ANTIGEN IN PATIENTS

Nearly 30% of the Down's syndrome patients had detectable Australia antigen in their serum. This includes (Table 1) 19 of the 75 males from the New Jersey institution and 3 of 5 females and 3 of 4 males from the Maryland institution. This represents the highest frequency found in any patient group so far tested. Four of the 76 control patients had Australia antigen. Of these, three had some stigma of Down's syndrome, that is, prominent epicanthal folds, Brushfield's spots, large interpupillary distance, short middle phalanx of the fifth finger, "mongoloid crease" of the palm, high palatal arch, etc. However, none of these had the typical Down's syndrome

chromosome pattern on examination of the peripheral blood. As noted below, mosaicism cannot be ruled out by chromosome analysis of a relatively small number of cells from a single source. The difference in frequency of Australia antigen between the Down's syndrome and the control group is highly significant ( $\chi^2 = 11.8$ ,  $P < 0.001$ ). The protein was not found in any of the normal individuals from the United States. Australia antigen has been found in patients with leukemia, hemophilia, and thalassemia as well as in those with Down's syndrome. Forty-eight sera from patients suspected of having either serum or infectious hepatitis were tested. Of these, five were found to have Australia antigen. The findings in hepatitis will be described in greater detail in a subsequent paper. In

addition, a single patient with Fanconi's anemia (hypoplasia of the marrow) and one patient (from Finland) with Hodgkin's disease have the antigen (Table 1).

The distribution of Australia antigen in leukemia and related disease groups is given in Table 2. The frequency is relatively high in acute granulocytic leukemia patients and to this date has not been found in any patient with chronic granulocytic leukemia. Australia antigen was found in a single case of the relatively rare chronic reticuloendotheliosis. None of the polycythemia vera patients had detectable Australia antigen.

We have recently concluded a study on Cebu Island, the Philippines, which included blood specimens from 584 patients with lepromatous leprosy, 377 patients with tuberculoid leprosy, and 764 controls without leprosy (19, 20). There was a significantly higher incidence of Au(1) in the lepromatous leprosy cases as compared with either the tuberculoid leprosy cases or the controls (9.4% lepromatous, 3.4% tuberculoid, 4.8% control). This difference is even more striking if only young men (under 20 years) are considered (26.7% lepromatous, 7.9% tuberculoid, 5.5% controls). As noted above, Au(1) is relatively common (about 5%) in nonhospitalized, apparently normal populations in several Asian and Oceanic countries including the Philippines (8).

TABLE 2. Distribution of Australia Antigen in Leukemia and Related Diseases

Disease	Total No. of Cases	Positive for Australia Antigen	
		no.	%
Acute granulocytic leukemia	38	7	18.4
Chronic granulocytic leukemia	41	0	0
Acute lymphocytic leukemia	58	2	3.5
Chronic lymphocytic leukemia	30	4	13.3
Other*	10	2†	—

\* Lymphosarcoma, 3, reticulum cell sarcoma, 3, chronic reticuloendotheliosis, 1, plasma cell leukemia, 1, acute stem cell leukemia, 2.

† Chronic reticuloendotheliosis, 1, acute stem cell leukemia, 1.

#### AGE

The age distribution by 20-year groups of the trait in the Down's syndrome patients is shown in Table 3, in which this distribution is compared with that in several populations of apparently normal people in whom Au(1) occurs and with that in the sera of leprosy patients and controls from Cebu. The normal and patient groups used in the comparison were selected because of the high frequency of Au(1) and the availability of relatively large numbers of samples. The frequency of Au(1) decreases with age in all of these populations. When each of the populations is divided into younger (0 to 39) and older (40+) age groups, the differences in frequency of Au(1)

TABLE 3. The Distribution of Australia Antigen by Age in Patients with Down's Syndrome and in Three Other Populations

Age Group	Down's Syndrome, New Jersey			Marshall Islands, U.S.T.T.P.I.*			Cebu, Philippines†			Manila, Philippines		
	Cases	Positive		Cases	Positive		Cases	Positive		Cases	Positive	
yr	no.	no.	%	no.	no.	%	no.	no.	%	no.	no.	%
0-19	34	12	35.3	196	16	9.5	348	30	8.6	22	2	9.1
20-39	30	6	20.0	148	12	8.1	948	62	6.5	132	6	4.5
40-59	11	1	9.1	85	4	4.7	378	16	4.2	36	1	2.8
60+	0	0	—	67	1	1.5	128	3	2.3	6	0	0
Total	75	19		496	33	6.2	1,802	111	6.2	196	9	4.6

\* U.S. Trust Territory of the Pacific Islands.

† The Cebu population includes persons with and without leprosy.

TABLE 4. Blood Factors Studied in Australia Antigen-positive (Au(1)) and -negative (Au(0)) Sera\*

Study†	Positive, Au(1)		Negative, Au(0)		Total Au(1) + Au(0)	
	No. Studied	Mean Value	No. Studied	Mean Value	No. Studied	Mean Value
Hemoglobin, g/100 ml	19	15.2	26	15.5	45	15.4
RBC count, million/mm <sup>3</sup>	19	4.48	26	4.65	45	4.58
Hematocrit reading, %	19	43.0	26	43.8	45	43.5
MCV, $\mu^3$	19	96.2	26	94.5	45	95.2
MCH, $\mu\mu\text{g}$	19	33.9	26	33.5	45	33.7
MCHC, %	19	35.3	26	35.5	45	35.4
WBC count, /mm <sup>3</sup>	19	7,153	26	7,725	45	7,483
Polymorphonuclear cells, %	19	56.3	23	54.0	42	55.0
Stab cells, %	19	5.9	23	4.4	42	4.2
Lymphocytes, %	19	34.1	23	36.9	42	35.7
Monocytes, %	19	2.3	23	2.3	42	2.3
Eosinophils, %	19	2.8	23	2.0	42	2.4
Basophils, %	19	0.4	23	0.5	42	0.5
WBC G-6-PD, $\mu\text{moles TPN}$ reduced/ $10^6$ WBC/min	9	17.1	9	15.6	18	16.4
WBC G-1-PUT, $\mu\text{moles UDPG/hr}/10^6$ WBC	9	29.1	10	26.3	19	27.6
Platelets, /mm <sup>3</sup>	19	191,158	26	213,461	45	204,044
Uric acid, mg/100 ml	18	5.2	26	5.3	44	5.2
Vitamin B <sub>12</sub> , $\mu\mu\text{g/ml}$	17	698.6	23	347.1	40	496.5

\* None of the differences were significant with the exception of vitamin B<sub>12</sub>, where  $t = 2.926$  and  $P < 0.01$ .

† MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, G-6-PD = glucose-6-phosphate dehydrogenase, TPN = triphosphopyridine nucleotide, G-1-PUT = galactose-1-phosphate uridylyl transferase, UDPG = uridine diphosphate glucose.

are statistically significant for the Cebu population ( $0.02 > P > 0.01$ ) and nearly so for the Marshall Islands ( $0.1 > P > 0.05$ ) but not for the others.

#### CHROMOSOME STUDIES

Seventeen patients of the 75 Down's syndrome patients included in this study were selected for cytogenetic examination. Criteria used in the selection were low maternal age at birth or other indication of possible familial transmission of the syndrome, relatively high intelligence, and, in one case (385H), the relatively advanced age of the patient (60 years). One case was chosen at random. The selection was made without prior knowledge of the antigen phenotype. A minimum number of 10 metaphase chromosome counts and 1 karyotype analysis was made on each individual (leu-

kocyte cultures); in those cases in which the modal chromosome number was not 47, additional counts and analyses were made. Fourteen of the 17 patients were found to be trisomic for chromosome 21, the classical finding in Down's syndrome (21). Of the remaining 3, 1 (389H) was a mosaic of normal and trisomy-21 cells, the second (384H) was the carrier of a 13-15/21 translocation and effectively trisomic for 21, and the third (385) had only chromosomes that were entirely normal in number and morphology in the single cell type studied. More extensive cytogenetic analysis might have revealed additional mosaicism in which cells of subordinate clones were present in low frequency, but because it is possible to study the chromosomes of relatively few cell types from a limited number of sites, it is impossible to rule out mosaicism

completely in any human. None of these three had detectable Au(1).

Of the 14 typical Down's syndrome patients (that is, with 47 chromosomes), 3 had Australia antigen. Chromosome studies were done on 4 of the control patients. None of these had the typical Down's syndrome trisomy pattern.

The blood factors studies are shown in Table 4. With the exception of the vitamin B<sub>12</sub> values, there was no significant difference between the patients with and those without Australia antigen. The mean of the B<sub>12</sub> values for the positive group (Australia antigen present) was 698.6 and for the negative group (Australia antigen absent), 341.3. This gives a *t* value of 2.926 and  $0.01 > P > 0.001$  and indicates that it is highly unlikely that this difference between the means is due to chance. The levels of white blood cell galactose-1-phosphate uridyl transferase and glucose-6-phosphate dehydrogenase are higher on the average in the Au(1) than the Au(0) patients, but this difference is not significant. They are considerably higher than the values given for normal white blood cells in the table published by Mellman and colleagues (13). The hematocrit reading, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration for all the Down's syndrome patients combined are slightly higher than those for normals, confirming the findings of Naiman, Oski, and Mellman (22). The average of the uric acid levels for all patients combined is about the same as that found for male Down's syndrome patients by Mertz, Fuller, and Concon (23) (who used a different technique for measuring uric acid). They noted that young mongoloids had higher levels than did the control patients.

#### DISCUSSION

There is a significantly higher frequency of Au(1) in the Down's syndrome patients

than in the controls. From this, we may conclude that the high frequency is associated with Down's syndrome and that it is not due to institutionalization alone. The frequency appears to decrease with age in the Down's syndrome patients as it does in other populations. There is a higher average vitamin B<sub>12</sub> level in the Au(1) individuals compared with the Au(0), but this finding should be confirmed before further comment is warranted. All the Down's syndrome patients with Au(1) and whose chromosome complement is known have the usual 21 trisomy.

The present state of knowledge concerning Australia antigen has been summarized in the introduction. The most striking feature of our data is the discovery of Australia antigen in high frequency in patients with Down's syndrome, leukemia (particularly acute granulocytic leukemia and chronic lymphocytic leukemia), and viral hepatitis. These diseases have been linked for other reasons: Patients with Down's syndrome have an increased risk of developing leukemia, particularly acute myelogenous leukemia (11); an association has been found between epidemics of hepatitis and the birth 9 months later of children with Down's syndrome (24); furthermore, it has been reported that sera of patients convalescing from hepatitis cause chromosome aberrations in tissue cultures (25).

Most of the disease associations could be explained by the association of Au(1) with a virus, as suggested in our previous publications (2, 6, 8). The discovery of the frequent occurrence of Au(1) in patients with virus hepatitis raises the possibility that the agent present in some cases of this disease may be Australia antigen or be responsible for its presence. The presence of Australia antigen in the thalassemia and hemophilia patients could be due to virus introduced by transfusions. This, however, could not be the only explanation, since many transfused patients neither have the antigen nor an antibody against it. To our knowledge,

none of the Down's patients with Australia antigen have received transfusions, and its presence in these patients may be the result of very early or maternal infection. Associations between Down's syndrome and thyroiditis (26) and rubella (27) have also been suggested, but these have not been studied in respect to Au(1).

As noted in the introduction, a striking family clustering of Au(1) has been found in Filipino and other populations (8). This could be explained on a simple infection hypothesis if the attack rate were higher in children than it is in adults. (See Table 3 of this paper and Table 5 of reference 8.) However, the segregation in the families is consistent with simple autosomal recessive inheritance. If Au(1) is inherited, as these results suggest, this would require an explanation consistent with the infection theory. It is possible that the trait is inherited in some groups such as the Filipinos but not in others. Or, the presence of the appropriate genes may confer an added susceptibility to infection with the virus. In areas where infection is very common, the trait could follow a pattern of mendelian segregation as it appeared to do in the Philippine studies.

Studies on the biochemical and immunologic nature of Au(1) are in progress, as are additional studies on the association with hepatitis. When the results of these are available, a more explicit interpretation may be possible.

#### SUMMARY

Australia antigen (Au(1)) found in patients with leukemia (particularly those with acute granulocytic leukemia) has now been found in high frequency in patients with Down's syndrome and virus hepatitis. Down's syndrome patients with Australia antigen have, on the average, a higher level of vitamin B<sub>12</sub> than do the patients without this trait. Several hypotheses are discussed in an attempt to explain the associations.

#### ACKNOWLEDGMENTS

This study was made possible as a result of the enthusiastic support and encouragement of Mr. F. W. Russell, Superintendent, and Dr. J. D. Nelson, Medical Director, of the State Colony at New Lisbon, New Lisbon, N. J. We are also indebted to the residents of the colony who participated in this study and to their parents, who have shown a continuing interest in the progress of our work.

Many of the valuable sera of patients used in this work were presented to us by physicians from the Philadelphia area and elsewhere to whom we are grateful. These include Drs. Erlandson, Erslev, Freireich, Frost, Joseph, Lawrence, Lee, McElfresh, Naiman, Osgood, Ultman, Whereat, Winchell, Woldow, and Wolman. We are particularly indebted to Dr. James E. Prier, Director, Division of Laboratories, Pennsylvania Department of Health, for supplying serum specimens from patients suspected of having viral hepatitis.

#### SUMMARIO IN INTERLINGUA

Antigeno Australia, trovate in patientes con leucemia (particularmente in casos de acute leucemia granulocytic) ha essite incontrate con un alte frequentia in patientes con syndrome de Down e hepatitis viral. Patientes con syndrome de Down e antigeno Australia ha, al media, un plus alte nivello de vitamina B<sub>12</sub> que patientes sin iste tracto. Es commentate plure hypotheses presentate pro explicar le mentionate associationes.

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