Clinical Science

A "New" Antigen in Leukemia Sera

The "Australia antigen" is found in the sera of some normal individuals from foreign populations. The total absence of the antigen from the sera of normal United States subjects and its relatively high frequency in acute leukemia suggests that the presence of the antigen may be of value in the diagnosis of early acute leukemia. Whether the antigen results from or precedes the leukemia process remains to be seen.

Baruch S. Blumberg, MD, Harvey J. Alter, MD, and Sam Visnich

Patients who receive large numbers of transfusions for anemia and other causes may develop precipitins in their blood. These precipitins may react in agar gel double diffusion experiments with specific human serum lipoprotein found in the blood of other individuals. Since these precipitins were found only in patients who had received transfusions they were thought to be antibodies against serum lipoproteins which developed in the patients as a result of the repeated transfusions. The precipitin is referred to as an isoprecipitin since it develops against a specificity found in an individual from the same species. The antilipoprotein isoprecipitin[1,2] developed in approximately 30% of 47 patients with thalassemia who had received transfusions. Isoprecipitins also developed in smaller number of transfused patients with other diseases. All precipitins stained with sudan black, a dye specific for lipid. Immunoelectrophoretic and ultracentrifugal studies showed that the protein with which the isoprecipitins reacted was a low density lipoprotein. The reactor specificity associated with the beta lipoprotein is inherited as an autosomal-dominant trait and several lipoprotein specificities have been found.[3-5]

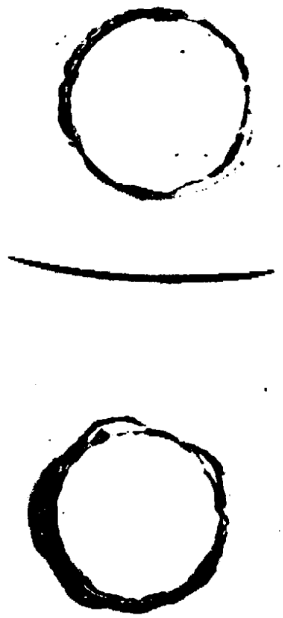
In 1963, sera from patients with hemophilia who had received transfusions were tested for the presence of isoprecipitins using a panel of 24 sera from normal individuals, including sera from foreign populations. Two of the hemophilia sera formed a clearly defined precipitin line with one of the panel sera (from an Australian aborigine), but with none of the others. In contrast to the usual findings the precipitin line stained only faintly with sudan black; it did, however, stain with azo carmine, a general protein stain (Fig 1). Subsequent studies have shown that this protein system differs from that detected with the antilipoprotein antisera. The serum protein with which the hemophilia isoprecipitin reacts has not been fully characterized and has been tentatively called the "Australia antigen." This paper will describe the epidemiologic and immunologic aspects of the Australia antigen-isoprecipitin system.

Materials and Methods

Double diffusion in agar gel was done using a micro-Ouchterlony technique on lantern slides.[5] Each of the two hemophilia sera containing the isoprecipitin was placed in the center well of a seven-hole micro-Ouchterlony pattern. The sera to be tested for presence of Australia antigen were placed in the peripheral wells. When a panel of antigen-containing sera were identified in this manner, they in turn were each placed in the center wells of similar seven-hole Ouchterlony patterns, and the sera of patients who had received transfusions, which were to be tested for the presence of isoprecipitins, were placed in the peripheral wells. In the final testing program two sera-containing Australia antigens which reacted with all the hemophilia antisera first discovered, were selected to screen for the remaining antisera. Two of the strongest hemophilia antisera were used in screening for the sera containing Australia antigen. In screening for antilipoprotein antisera, the sera from patients who had received transfusions were tested using a panel of 24 sera selected from four or more population groups as in previous studies.[2]

From the Institute for Cancer Research, Philadelphia (Dr. Blumberg), and the National Institutes of Health, Bethesda, Md (Dr. Alter and Mr. Visnich).

Reprint requests to 7701 Burholme Ave, Philadelphia 19111 (Dr. Blumberg).



1. Formation of precipitin line between serum from leukemia patient (top well) and hemophilia serum (bottom well).

Immunoelectrophoresis was done by a modification of the method of Grabar and Williams.[6] The precipitin lines were first stained with sudan black and then with azo carmine.[7] Sera were fractionated by ultracentrifugation in high density salt medium.[8] Fractions with a specific gravity greater and less than 1.063 were prepared.

A total of 28 hemophilia sera were studied. These included samples from patients from Mt. Sinai Hospital, NY, New York Hospital, and the Clinical Center of the National Institutes of Health. They were all United States whites and had all received transfusions many times with fresh frozen plasma and whole blood obtained from the hospital blood banks as well as commercial sources. The racial makeup of the donors could not be known with certainty, but they probably included United States whites and Negroes. The hemophilia serum used in the immunoelectrophoresis experiments contained antibodies against both lipoproteins and the Australia antigen. It was obtained from a patient (1) who had received more than 900 transfusions.

The sources of some of the normal sera used in the studies are given in the Tables. The sera from patients were obtained at the Clinical Center, National Institutes of Health, New York Hospital, and elsewhere. The calculation of the probability value was done by using two by two tables and Fisher's χ-square method.

Results

Frequency of the Isoprecipitin.—The sera of 107 patients who had received approximately ten or more transfusions, and of 150 normal individuals who had not received transfusions, were tested for the presence of the Australia isoprecipitin (Table 1). Eleven isoprecipitin containing sera were found in the patient group; none was found in the normal population. Of these 11, eight were patients with hemophilia, one had plasma thromboplastin component deficiency disease (PTC), one thalassemia, and one aplastic anemia. The frequency is highest in the hemophilia and related PTC group, and the difference from the other groups combined is statistically significant ($P<0.001$). It had previously been found that the frequency of antilipoprotein isoprecipitin was very high in thalassemia as opposed to other patient groups.[2] Hemophilia sera containing the Australia isoprecipitin were tested for the presence of antibeta lipoprotein; they were found to have an incidence similar to other patients who did not have thalassemia. Only one of the patients in the latter group had an anti-Australia antigen isoprecipitin.

There were two sera which contained both antilipoprotein and anti-Australia antigen isoprecipitins. This is approximately the number expected by chance.

Frequency of Antigen.—A total of 1,704 sera from nonhospitalized and presumably normal subjects were tested for the presence of Australia antigen using at least two, and generally more antisera (Table 2). Reactors were found only in Oceanic, Oriental, and Mediterranean populations, and in one from the American Indian population. No reactors were found among the approximately 700 United States sera tested including cord serum from 18 newborns. The highest frequencies were

Table 1.—Isoprecipitins in Patients Who Had Received Transfusions Which React With the Australia Antigen and With Lipoproteins

	Antibody to Australia Antigen				Antibody to Lipoprotein			
Disease	Total	Present	Absent	% Present	Total	Present	Absent	% Present
Hemophilia	28	8	20	28.6	31	3	28	9.7
PTC*	2	1	1	....	2	0	2	....
Thalassemia	48	1	47	2.1	47	14	33	29.8
Other Diseases	29	1	28	3.4	33	3	30	9.1
Total	107	11	96	10.3	113	20	93	17.7
Normal controls	150	0	150	0	200	0	200	0

*Plasma thromboplastin component deficiency disease.

found in a small group of native Taiwanese and in Australian aborigines. All of these tests were done on stored sera.

Sera from a total of 659 patients with various illnesses were tested against one or more of the antisera as shown in Table 3. Of the ten reactors found, eight had leukemia (including one patient with a diagnosis of both acute lymphocytic leukemia and multiple myeloma, and one patient with a newly described preleukemia syndrome associated with a missing chromosome in marrow cells,[10]) and two had thalassemia. These patients with thalassemia were siblings whose parents were born in Greece. As noted in Table 2, the frequency of reactors in presumably normal Greeks is 4%. The difference between the frequency in leukemia patients and that in all the disease groups combined is statistically significant ($P<0.001$). All of the patients with the Australia antigen had received transfusions. However, approximately 300 of the patients who did not have the Australia antigen had also received transfusions.

The leukemia sera were studied in greater detail. The antigen was found in all the larger subclassifications with the exception of chronic myelogenous leukemia. The patients with reacting sera were of both sexes, and their ages at the time of the diagnoses varied from 6 to 55 years. Of the ten patients in whom the antigen was found, eight are now dead, including all but one of the eight patients with leukemia, and one of the patients with thalassemia.

A total of 54 patients with acute leukemia were studied. Of these, six had the Australia antigen and were all dead. Of the 49 who did not have the Australia antigen, 19 were dead. The sample size is too small to permit adequate corrections for age, duration and intensity of disease, and other factors, but there does appear to be a gross difference with respect to mortality between these two groups.

Characteristics of Antibody and Antigen.—The original isoprecipitin-containing sera and the reacting sera had been found using specimens which had been collected and stored at −20 C for various lengths of time up to three years. Several specimens collected at different times were subsequently obtained from patients who had received transfusions and whose sera contained the isoprecipitins, and from patients with leukemia and normal subjects whose sera contained the Australia antigen. These studies showed that the isoprecipitin was present in sera or plasmas which had been stored at −20 C for up to 27 months. The Australia antigen was present in fresh sera as well as in sera or plasmas stored for up to six years. These studies also indicate that within the limits of observations, the presence of the Australia antigen is essentially an invariant characteristic of the individual; that is, if it is present at one point in time, it is present when tested subsequently.

The isoprecipitin appears to be a 7S γ-globulin.[9] Australia antigen migrates in the beta and slow α-globulin region on immunoelectrophoresis, but can readily be distinguished from the antigen which reacts with the antilipoprotein isoprecipitin found in the serum of patient 2[1] as shown in Fig 2. (The top basin contains the antilipoprotein antiserum from patient 2,[4] and the second basin serum from hemophilia patient 1. The latter has isoprecipitins directed against both the Australia antigen and lipoprotein. The electrophoresis well contains the serum of a patient with leukemia which reacts with serum from patient 2 and with both of the isoprecipitins in serum from patient 1. Two distinct lines of slightly different mobility were seen between the sera of patients 1 and 3. The lower one, which stains blue with sudan black, corresponds to the patient 2 lipoprotein line. The upper one, which stains only faintly or not at all with sudan black,

Table 2.—Australia Antigen in Normal Populations

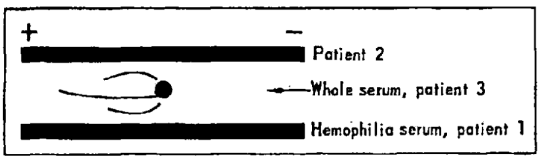
Population	No. Tested	Australia Antigen Present	
		No.	%
Aborigines, Australia	208	12	6
Chinese, USA and Taiwan	65	0	0
Eskimo, Alaska[13]	24	0	0
Greeks, Greece[14]	179	8	4
Indians, Canada[15]	78	0	0
Indians, Mexico	100	1	1
Israelis	96	2	2
Japanese, USA	48	0	0
Koreans	1	1	
Micronesians, Rongelap[16]	193	7	4
Negro, USA[17]	241	0	0
Newborn children, white	18	0	0
Polynesians, Bora Bora	24	1	4
Samaritans, Israel[18]	125	2	2
Taiwanese	23	3	13
Tristan da Cunha Islanders	42	0	0
Vietnamese	24	1	4
White, USA (NIH* employees)	215	0	0
Total	1,704	38	

*National Institutes of Health.

Table 3.—Australia Antigen in Patients

Disease	No. Tested	Australia Antigen Present	
		No.	%
Abetalipoproteinemia	4	0	0
Amyotrophic lateral 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
Hypercholesterolemia	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[10]	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	

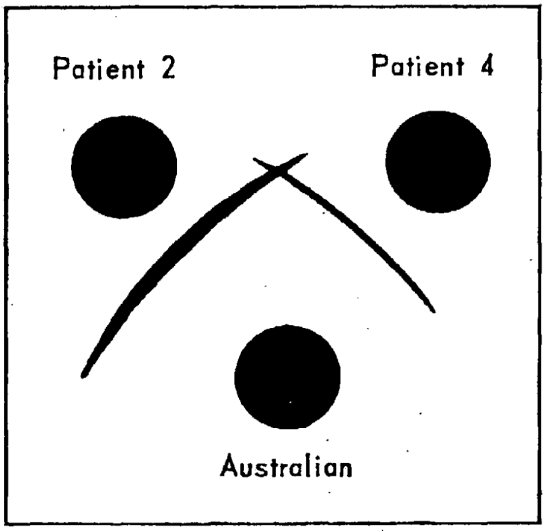
*Includes eight patients with rheumatoid arthritis, three with psoriatic arthritis, and four with Sjögren's disease.
†Other than lupus erythematosis.
‡One patient had both chronic lymphocytic leukemia and multiple myeloma, and is included in both categories.



2. Diagram of immunoelectrophoresis experiment: antilipoprotein (patient 2), leukemia (patient 3), and anti-Australia antigen (patient 1).

does stain with azo carmine.) Using the hemophilia serum from patient 1 (see above), two distinct lines with slightly different mobilities can be seen. One of these stains blue with sudan black and corresponds to the lipoprotein antigen which reacts with patient 2. The other, which has a slightly different mobility and a different curvature, does not stain with sudan black, but does stain with azo carmine. In addition, a reaction of nonidentity can be demonstrated by immunodiffusion when the Australia isoprecipitin system is compared with the typical lipoprotein precipitin as shown in Fig 3. (The serum of patient 2 contains an antilipoprotein isoprecipitin. The serum of a patient with thalassemia with an isoprecipitin against Australia antigen is in the top right hand well. The serum of the Australian aborigines has both the specific lipoprotein and the Australia antigen. The patient-2 line stains blue with sudan black and the patient-4 line stains red with azo carmine.) Additional characteristics of the Australia antigen and a method for its partial isolation are described in another paper.[9]

Specificity.—To determine if the antisera had different specificities, they were all tested against the same antigen placed in the center well of a seven-hole-Ouchterlony pattern. No crossing of lines were seen between the antisera. Similar experiments were used to compare the Australia antigen found in the presumably normal subjects with that found in the leukemia patients and no differences could be detected. On the basis of these initial experiments, we have not been able to detect any specific differences between the various antisera, nor between the Australia antigen found in normal persons, and in leukemia patients.



3. Diagram of Ouchterlony experiment showing crossing of antilipoprotein (patient 2) and anti-Australia antigen (patient 4) lines.

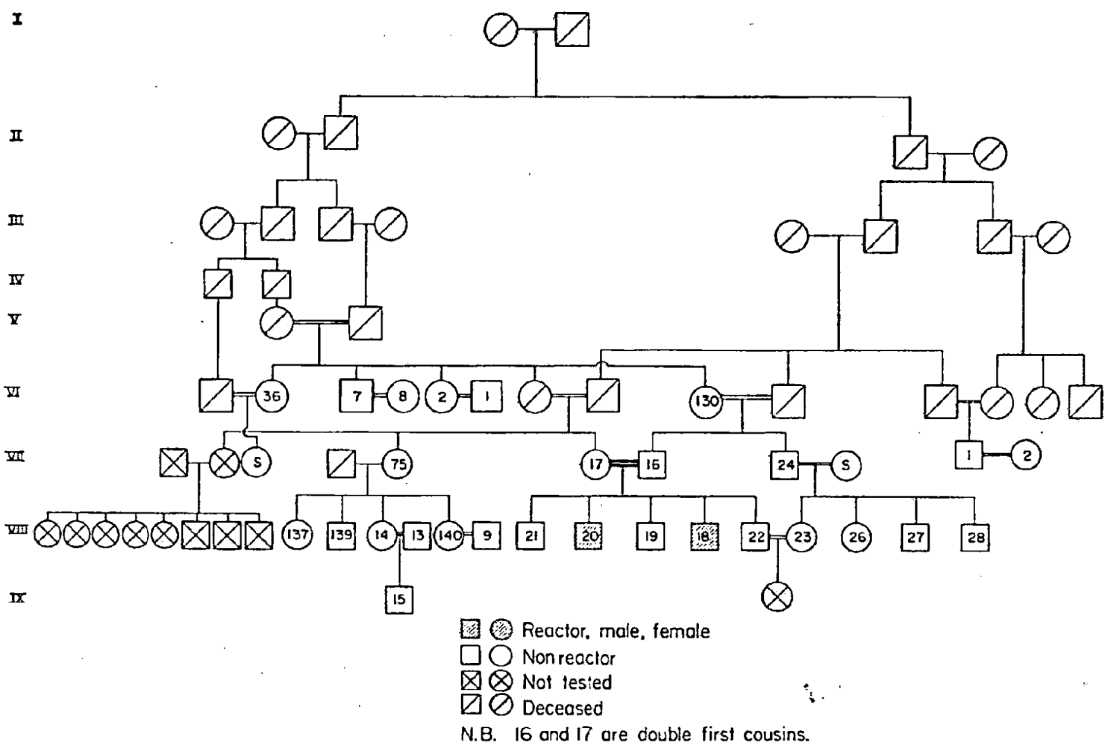
Family Studies.—The studies on normal populations were done on sera collected on field trips for other purposes and stored in the Institutes serum bank. In some cases, family sera were available, and the results were analyzed to determine if there was a family aggregation of Australia antigen. Batsheva Bonné examined 125 sera from Samaritans living near Tel Aviv, Israel[18] (Fig 4). This represented nearly all the members of this highly inbred community. Of these, two siblings who were the offsprings of a consanguineous marriage (both of whose parents were double cousins) were the only individuals with detectable antigen. In the Micronesian population, there was one father and son affected, but in the five other cases the individuals were only remotely related or unrelated. The reactors in the Greek study were not apparently closely related. Of the 47 patients with thalassemia studied, only two were positive, and they were siblings. Their parents and one aunt were not reactors. The two brothers of one of the patients with leukemia with the Australia antigen did not react with the antisera.

Comment

A second kind of isoprecipitin system has been revealed by this study. The Australia antigen with which the isoprecipitin reacts differs from the lipoprotein antigens previously described and the distribution of isoprecipitins and reactors in patients and in normal populations is very different from that found for the lipoprotein system.

It has not been firmly established that the isoprecipitin in the hemophilia sera is an antibody nor that the protein with which it reacts is an antigen. However, by analogy with the lipoprotein system, it is probable that this is the case and these terms have been used in describing the system.

In our discussions it has been assumed that the "antibody" is present in the sera of patients with hemophilia and other patients, and the "antigen" in the leukemia and normal sera. This appears likely since the patients with hemophilia and other patients had all received transfusions whereas some of the individuals in whose sera the Australia antigen was found had not. The possibility still remains, however, that the rare normal sera and leukemia sera actually contain an antibody against an antigen present in hemophilia patients. It is hoped that further studies with patients with hemophilia may help resolve this point.



4. Portion of a pedigree from the Samaritan community, representative of nearly all members, showing the presence of the Australia antigen and the close interrelation of the parents.

The high incidence of this isoprecipitin in the hemophilia-patient group suggests that either hemophilia per se predisposes to the formation of the isoprecipitin or that the administration of fresh frozen plasma (which distinguishes the treatment of hemophilia and PTC patients from the other individuals who had received transfusions) particularly predisposes to the formation of the antibody. Preliminary evidence suggests that the Australia antigenic sites may be revealed by a freezing process which leads to the denaturation of lipoprotein. This possibility will be discussed in another paper.[9]

The high incidence of the Australia antigen in the leukemia population as compared with normals or other patient populations suggests either that; (1) persons with the Australia antigen have an increased susceptibility to the development of leukemia, or (2) the antigen itself is a manifestation of the disease process, perhaps secondary to an alteration in some normal serum constituent with a resultant change in antigenic configuration, or (3) the Australia antigen is related to the virus which has been suggested as the cause of leukemia.

The first hypothesis implies that the Australia antigen is present in a patient before he develops signs and symptoms of leukemia. The long-term observations necessary to support this possibility have not yet been made. The Micronesian, Samaritan, and Australian aborigine populations would be suitable for such a study. In the case of the "preleukemic"[10] (Table 4) patient the presence of the Australia antigen predated the development of frank leukemia.

Changes in red blood cell antigens during the course of leukemia lend indirect support to the hypothesis that the Australia antigen results from, rather than precedes the leukemia process. Many authors have demonstrated alterations in red blood cell ABO specificities during the course of leukemia.[11] More recently, it has been shown that there is a loss of I specificity on the red blood cells of certain patients with leukemia.[12] The I antigen is almost universally present in normals and patients with other diseases.

The third hypothesis is being tested in collaboration with workers at the National Institutes of Health.

The total absence of the Australia antigen from normal United States subjects studied and its relatively high frequency in acute leukemia suggests that the presence of the antigen may be of value in the diagnosis of early acute leukemia.

The available data are too few to support a genetic hypothesis, but none of the family studies are inconsistent with simple recessive inheritance of the specificity.

Summary

An isoprecipitin is present in the sera of many patients with hemophilia who have received transfusions. It reacts with a protein (the "Australia antigen") that is found in the sera of some normal individuals from foreign populations but is absent in sera of the United States populations studied. It is found in approximately 10% of patients with leukemia.

R. Rosenfield, MD, and E. Smith, MD, of Mt. Sinai Hospital provided the hemophilia antisera. P. Carbone, MD, E. Cohen, MD, E. J. Freireich, MD, and A. B. Rey, MD, provided the leukemia sera. Other valuable sera used in the experiments were provided by T. D. Dublin, MD, Marion Erlandson, MD, J. Fahey, MD, H. H. Fudenberg, MD, R. Kirk, PhD, L. Laster, MD, L. Rosen, MD, C. Sheba, MD, N. R. Shulman, MD, and A. G. Steinberg, PhD.

Samples of sera on patient 1 were provided by J. M. Hill, MD, of the Wadley Research Institute and Blood Bank, Dallas.

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THE IMPARTIAL NEGATIVE.—So far as I know there are only a few medical journals in the world whose proprietors employ a full-time editor, among them the *Journal of the American Medical Association* and its counterpart in the United Kingdom, the *British Medical Journal*, which I have the honour to edit. I use the word "honour" deliberately, though in the context it has a very commonplace and pedestrian ring about it. To edit the *British Medical Journal* is regarded as an honour, and I have often wondered why. It is a harassing, exacting, worrying, and ungrateful kind of job. Much of one's time is spent in saying "No!" to writers of monographs, articles, books, and letters, and to advertisers—self-advertisers and the legitimate trade. Each year I have to reject solely on grounds of space some two thirds of the articles submitted for publication in the *British Medical Journal*. These disappointed authors cannot feel otherwise than a bit cast down, and however polite the formula of rejection they will consider the editor an obstacle in the way of their progress. As a full-time editor who no longer practices medicine or works in the laboratory, I often feel acutely uncomfortable when returning to an author a paper which is the result of weeks, maybe months, of patient observation by the bedside and experiment in the laboratory—work summed up in voluminous tables, histograms, and the like, work, one has sadly to reflect at times, which had much better be left undone or done in a different way. At least, I feel, that is some justification for the existence of full-time medical editors who do not practice their original profession. By looking on and seeing all sides of the game, they do stand a chance of achieving impartiality. They can sum up the opinions of two or three expert referees on a paper, and come to the final decision which every editor has to make: "Yes" or "No." It is in the exercise of this judicial capacity that a medical editor is taxed most severely. He needs a fund of that rare commodity, common sense; and ability to assess evidence and a keen nose for the humbug, the self-advertiser, for the man who wants to sell something.—Clegg, H.: An Editor's Prejudices, *Int Rec Med*, vol 168, Oct, 1955.