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Australia Antigen and Lepromatous Leprosy Studies in South India and Elsewhere[1, 2]

Baruch S. Blumberg and Liisa Melartin[3]

Australia antigen (Au(1)) was first detected in the serum of an Australian aborigine using an antiserum which developed in a hemophilia patient who had received a large number of transfusions ([5]). Shortly after its discovery, it became apparent that Au(1) was closely related to a hepatitis virus and that it might be located directly on such a virus ([10]). Au(1) is a virus-like particle ([1]) which can apparently be transmitted to patients by transfusion and may then cause hepatitis ([4]). It has been identified in the nucleus of patients with viral hepatitis using direct fluorescent antibody technics ([12]). The evidence in favor of the virus-hepatitis hypothesis has been reviewed recently ([4, 14]).

Although Au(1) is rare in normal Americans, it is relatively common (i.e., 3-15%) in many individuals living in tropical countries and tends to be persistent in these persons ([4, 7]). These individuals who have Au(1) in their blood are usually asymptomatic and may be "carriers" of hepatitis virus. On the basis of studies conducted in Cebu, Philippines, from 1964 to 1967, it was established that there was a significantly higher frequency of Australia antigen in patients with lepromatous leprosy than in either patients with tuberculoid leprosy, or in nonleprosy patients from the same area ([6, 8]). The difference was due primarily to an increased frequency in young lepromatous males. Lepromatous leprosy patients with Au(1) appear to have slightly elevated SGPT levels and may have chronic anicteric hepatitis ([3]).

Family studies have been completed in two areas (Cebu, and Bougainville, Trust Territory of New Guinea) which are compatible with the explanation that susceptibility to chronic infection with "Au(1) virus" is inherited as a simple autosomal recessive trait ([7, 9]). Large numbers of people in the tropics who appear to bear those genes and who have Au(1) do not become clinically ill with hepatitis when infected. If these genetic studies can be confirmed, they may bear directly on the question of inherited susceptibility to leprosy infection and as such could be of importance to a general understanding of the nature of the disease.

In our first report on Au(1) and lepromatous leprosy we commented on the necessity of confirming the association by studies in places other than Cebu ([8]). In order to do this it is necessary to locate places where both Au(1) and lepromatous leprosy are relatively common, so that appropriate epidemiologic studies can be designed. Consequently, we conducted a survey of Au(1) in leprosy patients and controls in several locations in India, Singapore, Japan and Australia during a field trip undertaken in August 1967. In addition collections were made in Italy, Greece, Brazil and Hong Kong under separate arrangements. As a consequence of these studies, we identified several locations where useful projects could be planned and, in addition completed a preliminary study in South India, which is consistent with the Cebu study.

MATERIALS AND METHODS

The population studies (including the Cebu study described elsewhere ([6, 8])) are

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[3] B. S. Blumberg, M.D., Ph.D., Associate Director for Clinical Research, The Institute for Cancer Research, 7701 Burholme Avenue, Fox Chase, Philadelphia, Pennsylvania 19111; L. Melartin, M.D., Visiting Research Physician, The Institute for Cancer Research. *Dr. Melartin's present address*: State Serum Institute, Helsinki, Finland.

TABLE 1. *Summary of leprosy studies.*

Location	Lepromatous			Tuberculoid			Nonleprosy		
	Number		% Pos.	Number		% Pos.	Number		% Pos.
	Tested	Pos.		Tested	Pos.		Tested	Pos.	
Cebu, Philippines[8]	803	80	9.9	605	26	4.3	1,287	64	4.9
South India	556	35	6.3	385	5	1.3	253	7	2.8
Japan[4]	180	3	1.8	50	0	0	1,034	5	0.5
Singapore									
Chinese	143	4	2.8	33	0	0	112	1	0.9
Hong Kong									
Chinese	102	5	4.9	42	1	2.4	57	0	0
Derby, Australia									
Aborigines	170[a]	0	0	—	—	—	217	0	0
Darwin, Australia									
Aborigines	23	1	4.3	27	1	3.7	1,807	38	2.1
Greece	53[b]	2	3.8	—	—	—	857	15	1.8
Italy	50[b]	0	0	—	—	—	127	0	0
Florianopolis, Brazil									
White[13]	158	0	0	42	0	0	275[c]	0	0
Total	2,238	130	5.8	1,184	33	2.8	6,026	130	2.2

[a] Includes lepromatous and tuberculoid leprosy cases.
[b] Includes a small number of tuberculoid cases.
[c] Includes 166 nonleprosy cases from Porto Alegre.

shown in Table 1. This table shows areas where studies could profitably be pursued. The diagnosis, age, and sex breakdown for India, and the previously published Cebu data are shown in Table 2.

A total of 479 sera from patients with lepromatous leprosy were collected at the Central Leprosy Teaching and Research Institute (CLTRI) at Chingleput (near Madras), South India, in 1966. An additional 77 sera from lepromatous patients were collected in the same institution in 1968. A total of 272 sera from patients with tuberculoid leprosy were tested in 1966. These included 196 sera collected at the Silver Jubilee Children's Clinic (SJCC), near Madras, in 1966, 34 sera collected at the same place the previous year and stored at 4°C, and 42 sera collected by the CLTRI mobile unit in 1965 and stored at the same temperature. An additional 117 sera from tuberculoid leprosy patients were collected in 1968 and tested at that time. To our knowledge none of the tuberculoid patients were institutionalized. The nonleprosy controls included 31 employees of the Chingleput Medical College Hospital and 98 nonleprosy outpatients at the Polambakkam Leprosy Center (which is not far from Chingleput) collected in 1966. Sera from an additional 126 nonleprosy patients were collected in 1968.

The study conducted in Cebu is described in detail in other publications already referred to. Briefly, it comprises sera from 803 patients with lepromatous leprosy, 605 with tuberculoid leprosy and 1,287 individuals without leprosy. The lepromatous cases were mostly patients at the Eversley Childs Sanitarium (for leprosy) and the tuberculoid leprosy patients were mostly patients seen at the Cebu Skin Clinic and the Cebu Traveling Skin Clinic. Some of the lepromatous patients were outpatients and some of the tuberculoid patients were tested while in the sanitarium, and this is described in detail in the original publication. (No significant difference in frequency was found between the lepromatous leprosy inpatients and outpatients ([6]).)

The sera from the Japanese leprosy patients were collected at the National Institute for Leprosy Research, Kitatama, Tokyo. They included 188 patients with lepromatous leprosy, and 55 patients with tuberculoid leprosy. There were 1,034 sera from nonleprosy controls. These were collected from blood donors in the Tokyo area and from other nonleprosy individuals from Tokyo, Osaka, and Fukuoka.

The Singapore sera were nearly all from Chinese residents of that city. The leprosy cases were collected at the Trafalgar Home and at the outpatients facilities associated with that institution. The nonleprosy sera were from blood donors and other individuals who did not have leprosy.

Two collections were made in Australia; one at the East Arm Leprosarium which is near Darwin, Northern Territory, and the second at Derby on the western coast of Western Australia. All the patients are Australian aborigines. A detailed analysis of the type of leprosy was not available from these institutions. Control sera included sera from aborigines of various western and northern bands. In addition, sera collected from bands resident in the Derby area were also available and are recorded separately. The frequency of Au(1) varies widely in different bands of Australian aborigines.

The sera from Greek leprosy patients were collected at Agia Varuara near Athens and were nearly all from patients with the lepromatous form of the disease. The nonleprosy controls were collected from individuals without leprosy in various parts of continental Greece and the adjacent islands. These nonleprosy sera are described in another publication([11]).

The sera from Italian leprosy patients were collected by Dr. L. Bonomo of the University of Bari. Nearly all had the lepromatous form of the disease. The nonleprosy control sera were from patients and normal individuals from several locations in North Italy.

The sera from Brazilian patients were collected by Dr. F. M. Salzano, primarily in Florianopolis and are described in detail elsewhere ([13]).

The sera from Hong Kong were from Chinese patients. They were collected by Professor J. B. Gibson and Dr. Janie C. Y. Shang of the University of Hong Kong (O.D.M. grant No. R-1873), and were as-

sembled as part of their larger study on leprosy now in progress. These preliminary results are presented here with the permission of Professor Gibson.

The presence of Au(1) is detected by immunodiffusion technics in agar gel using a micro-Ouchterlony pattern. The anti-Au(1) antiserum is placed in the center well of the pattern and the sera to be tested in the peripheral wells. Human and rabbit antisera were used in all the studies. A clear precipitin band forms between the center well and the peripheral wells containing sera with the antigen. Observations are made on the precipitin band and the slides are then dried and stained with azo carmine to increase the sensitivity.

RESULTS

Only the Indian studies (and the previously published Cebu studies) are sufficiently large to allow comparisons between the disease and control groups. (As noted above, the other populations are included to indicate areas where studies could subsequently be pursued.) The MN chi program ([8]), which gives a probability value corrected for variation with age, was used to test the differences between the several disease and control categories. In the Indian studies, the frequency of Au(1) in the lepromatous leprosy group is higher than in either the tuberculoid leprosy ($p = .0214$) or nonleprosy ($p = .0472$) control groups. There is no significant difference between the tuberculoid and nonleprosy groups ($p = .9828$). The frequency is higher in males than in females ($p = .005$) and in younger males than in older males ($p = .02$). These age, sex and diagnosis differences are the same as those previously reported from the larger Cebu study. The ratios of frequencies of the lepromatous:tuberculoid, and lepromatous:nonleprosy for males and females are about the same in both of the studies (Tables 1, 2) although the absolute values are lower in the Indian population.

In the other locations (Table 1) if Au(1) is detected in the population at all, then it invariably occurs in higher frequency in the lepromatous group than in either the tuberculoid or in the nonleprosy controls. If Au(1) is not found in the general population then it is not found in the leprosy group.

DISCUSSION

There are inadequacies in the sample selection for the India study. The patients in the leprosy group originated from various places in South India, primarily the Madras area. No attempt was made to match the patients with nonleprosy controls from the same location and caste; they were collected from several locations in the general area from which the patients were recruited. The lepromatous cases were mostly institutionalized and the tuberculoid cases were mostly outpatients. If institutionalization were a factor in determining the frequency of Au(1), this could explain the observed difference. However, in the Cebu study institutionalization did not appear to be a factor in determining the frequency of Au(1) in leprosy patients. ([6])

The lepromatous sample is representative of the cases in the leprosarium at the time the study was done, since nearly all the inpatients were tested. The tuberculoid group represents only a fraction of all the cases in the region and we do not know if it is representative. For these epidemiologic reasons, the present study should be considered as preliminary until more extensive investigations can be done in this area. Despite this, the findings in the India study are very similar to those in Cebu and can be construed as a preliminary confirmation of our original findings.

We have identified several locations where additional studies could be done. It also appears that the frequency of Au(1) in the lepromatous cases is dependent on the frequency in the general population, but the ratio of lepromatous to control and lepromatous to tuberculoid remains about the same.

The interpretations of the Cebu studies, have been strengthened by finding the same Au(1) to lepromatous leprosy association in the preliminary India study. Also, on the basis of two independent family studies, we now have additional support for the genetic interpretations ([7, 9]).

TABLE 2. *Australia antigen in lepromatous leprosy, tuberculoid leprosy, and nonleprosy controls, in South India and Cebu*[6,8].

Age (yrs.)	Male			Female			Total		
	No. tested	No. Au +	% Au +	No. tested	No. Au +	% Au +	No. tested	No. Au +	% Au +
A. South India									
Lepromatous									
0–19	140	15	10.7	27	0	0.0	167	15	9.0
20–39	240	16	6.7	69	2	2.9	309	18	5.8
40–59	68	1	1.5	5	0	0.0	73	1	1.4
60+	3	0	0.0	0	—	—	3	0	0.0
Total	451	32	7.1	101	2	2.0	552	34	6.2
Tuberculoid									
0–19	56	5	8.9	23	0	0.0	79	5	6.3
20–39	157	2	1.3	43	0	0.0	200	2	1.0
40–59	63	1	1.6	35	0	0.0	98	1	1.0
60+	5	0	0.0	2	0	0.0	7	0	0.0
Total	281	8	2.8	103	0	0.0	384	8	2.1
Nonleprosy									
0–19	17	3	17.6	21	0	0.0	38	3	7.9
20–39	81	1	1.2	90	0	0.0	171	1	0.6
40–59	17	1	5.9	21	0	0.0	38	1	2.6
60+	1	0	0.0	3	0	0.0	4	0	0.0
Total	116	5	4.3	135	0	0.0	251	5	2.0
Total									
0–19	213	23	10.8	71	0	0.0	284	23	8.1
20–39	478	19	4.0	202	2	1.0	680	21	3.1
40–59	148	3	2.0	61	0	0.0	209	3	1.4
60+	9	0	0.0	5	0	0.0	14	0	0.0
Total	848	45	5.3	339	2	0.6	1187	47	4.0

B. Cebu									
Lepromatous									
0–19	85	24	28.2	46	3	6.5	131	27	20.6
20–39	304	34	11.2	100	4	4.0	404	38	9.4
40–59	147	11	7.5	67	5	7.5	214	16	7.5
60+	31	0	0.0	23	0	0.0	54	0	0.0
Total	567	69	12.2	236	12	5.1	803	81	10.1
Tuberculoid									
0–19	58	5	8.6	59	3	5.1	117	8	6.8
20–39	173	8	4.6	123	4	3.2	296	12	4.0
40–59	76	2	2.6	47	0	0.0	123	2	1.6
60+	36	4	11.1	33	0	0.0	69	4	5.8
Total	343	19	5.5	262	7	2.7	605	26	4.3
Nonleprosy									
0–19	183	20	10.9	184	6	3.3	367	26	7.1
20–39	321	21	6.5	278	10	3.6	599	31	5.2
40–59	114	6	5.3	117	0	0.0	231	6	2.6
60+	60	0	0.0	30	1	3.3	90	1	1.1
Total	678	47	6.9	609	17	2.8	1287	64	5.0
Total									
0–19	326	49	15.0	289	12	4.2	615	61	9.9
20–39	798	63	7.9	501	18	3.6	1299	81	6.2
40–59	337	19	5.6	231	5	2.2	568	24	4.2
60+	127	4	3.1	86	1	1.2	213	5	2.3
Total	1588	135	8.5	1107	36	3.2	2695	171	6.3

We have made some conjectures relative to these findings which are published elsewhere ([6, 9]), but will be summarized briefly here. We have interpreted the genetic studies to mean that patients who are homozygous for a gene designated Au^1 (i.e., genotype Au^1/Au^1) are more susceptible to chronic infection with "Au(1) virus" than are individuals with alternate phenotypes (i. e., Au^1/Au, Au/Au) and as a consequence have detectable Australia antigen in their blood. When these patients become infected, they do not become obviously ill and appear to be carriers of hepatitis. It should be emphasized that this finding does not mitigate against the results which support the view that Australia antigen is a virus. That is, both the virus and genetic hypothesis are tenable.

What connection does this have to our findings in leprosy? We would like to propose a hypothesis which, although conjectural, has the advantage of being testable and in some respects provocative. This has been discussed in an earlier editorial ([2]). The postulated gene (Au^1) which in double dose confers increased susceptibility to chronic infection with hepatitis virus may also confer susceptibility to chronic infection with other organisms. If this were the case, in areas where both the gene and the organisms related to the susceptibility factor are common, there would be a correlation between chronic infection with hepatitis (i.e., Australia antigen) and the other organism. This appears to be the case for lepromatous leprosy in Cebu and India. If this hypothesis is correct then it would be expected that in areas of high leprosy incidence, lepromatous leprosy would follow a pattern of autosomal recessive inheritance; and this can be tested.

The susceptible individuals may be at some advantage when they become infected with hepatitis in that they do not appear to have any clinical evidence of the disease. This could constitute a form of protection against organisms, i. e., to become infected but not develop any disease as a consequence of the infection. However, bearers of the susceptibility genes suffer a compensatory disadvantage in that when chronically infected with other organisms they may develop serious disease. This appears to be the case when infection occurs with Hansen's bacillus. According to this hypothesis, when the genetically susceptible individuals are infected they are more likely to develop the lepromatous form of the disease than individuals with other genotypes. It is also immediately obvious that this susceptibility is not an absolute one, and other factors such as age, sex and possibly other genes are also involved. Susceptible individuals not exposed to the organism could not, of course, develop the disease despite their susceptibility.

Family studies in areas of high leprosy endemicity combined with Australia antigen and lepromin testing could show whether Au(1) and susceptibility to lepromatous leprosy are associated and whether the traits are inherited in a related manner.

SUMMARY

The frequency of Australia antigen in lepromatous leprosy, tuberculoid leprosy, and nonleprosy controls has been determined in South India. The frequency is higher in lepromatous leprosy than in any other group and in this group the frequency is highest in young males. Surveys were also conducted in Japan, Singapore, Hong Kong, Australia, Greece, Italy, and Brazil to identify other areas where additional studies may be done.

RESUMEN

En India meridional se determinó la frecuencia del antígeno Australia en lepra lepromatosa, lepra tuberculoide y controles no-leprosos La frecuencia es mayor en la lepra lepromatosa que en ningún otro grupo y, dentro de este grupo, es mayor en varones jóvenes. También se hicieron estudios en Japón, Singapore, Australia, Grecia, Italia y Brasil, para identificar otras áreas donde se pueden desarrollar estudios adicionales.

RÉSUMÉ

On a déterminé dans le sud de l'Inde la fréquence de l'antigène australien dans la lèpre lépromateuse, dans la lèpre tuberculoide, et chez des témoins ne souffrant pas de lèpre. La

fréquence s'est révélée plus élevée dans la lèpre lépromateuse que dans n'importe quel autre groupe; dans cette forme de lèpre, c'est chez les jeunes adultes de sexe masculin que cette fréquence était la plus élevée. Des enquêtes ont été également menées au Japon, à Singapour, à Hong Kong, en Australie, en Grèce, en Italie et au Brésil, en vue d'identifier d'autres régions où des études complémentaires pourraient être poursuivies.

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