STUDIES ON HUMORAL IMMUNE RESPONSE IN LEPROSY

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Our study was undertaken to determine if the humoral immune status of leprosy patients varied with either the type of the disease or the geographic location of the disease. Another variable investigated was whether or not treatment versus non-treatment had any selected effect on the humoral immune status. Serum immunoglobulin levels were determined by single radial immunodiffusion and other immunoserologic profile testing was performed whenever possible. Immunoglobulin levels did show a difference between the different disease types of leprosy and the authors also feel that environmental and genetic factors may play a more significant role than previously thought.

INTRODUCTION

A method to study the various ways in which individuals respond to infections is to use an actual disease as a model to determine the nature of these reactions. Leprosy is an excellent example for the above type of study since two major forms are well established and patients often present a third transitional form as well. The most common responses to the infectious agent of this disease are lesions abounding with organisms as in the lepromatous patient or lesions where it is extremely difficult to detect the causative organism as in the tuberculoid patient. Since the infectious agent itself does not appear to differ in the two forms, it is more likely that the host response is the major determining factor as to

which form will be apparent clinically in the individual. Studies which include an evaluation of immunoglobulin (Ig) levels in leprosy (Lim and Fusaro, 1964, 1968a, 1968b, 1968c, 1976 ; Sheagren, 1969) have been directed at this problem. In the last decade some of the newer thoughts and investigative techniques regarding immune systems in man have enhanced our ability to study leprosy. Recent studies have shown that patients with lepromatous leprosy have an immunological deficit and this abnormality is featured by a deficiency of cell-mediated immunity which selectively involves T cells (Bullock, 1968, 1971 ; Dierks and Shepherd, 1968 ; Dwyer et al, 1973 ; Godal et al, 1971, 1972 ; Katz et al, 1971 ; Saha and Mittal, 1971 ; Sharma and Shrivastav, 1958 ; Shepard, 1968 ; Talwar, 1972 ; Turk and Waters, 1969,

1970; Waldorf et al, 1969).

The present study was undertaken to review whether humoral immune status of patients with leprosy differs in patients with different clinicohistological types of the disease and from different geographic areas of the world. We were also interested to know whether or not serum immunoglobulin patterns varied in patients with or without an appropriate treatment. When sufficient material was available, other immunoserologic profile testing was performed to find any correlative disease pattern within the leprosy population.

MATERIALS AND METHODS

Venous blood was obtained from 83 patients at Hale Mahalu Hospital*, Oahu, Hawaii, and serum was separated from clotted blood. Other serum samples, in dry ice, were sent by air mail from the National Leprosy Center, India (20 samples), the National Institute for Leprosy Research, Tokyo (49 samples), and the National Leprosy Colony, Okinawa (30 samples). All sera were stored at -20°C prior to use. Control sera consisting of 10 samples from donors of each sex with ages ranging from 20 to 65 were also obtained from the same geographic areas.

Serum immunoglobulin (IgG, IgA, IgM and IgD) levels were measured by a single radial immunodiffusion test using commercial kits from Kallstad Laboratories (Cheska, Minnesota). IgE values were determined by radioimmunoassay using a kit (Phadebas^R) from Pharmacia Diagnostic (Piscataway, New Jersey). Screening for rheumatoid factor (RF) was carried out by a latex slide test using the method described

by the manufacturer Hyland Laboratories (Costa Mesa, California). Antinuclear antibody (ANA) assays were done by immunofluorescent antibody techniques using the kits of Meloy Laboratories (Springfield, Virginia). Antithyroglobulin antibody (ATA) test was carried out based upon passive hemagglutination method using thyroglobulin-coated red blood cells Wellcome Reagents (Research Triangle Park, North Carolina). Antibodies to gangliosides (A-Ggs) were detected by a passive hemagglutination technique described by one of the authors (Gajl-Pecgalska et al, 1973; Nelson, 1973) which was modified by using a microtiter plate. Sera were also tested for hepatitis B antigen (HBsAg) and its antibody by RIA (Austria II) method using a kit from Abbott Laboratories (North Chicago, Illinois).

RESULTS

The patients from Hawaii were the most extensively studied because of the availability of more detailed historical and clinical information and the availability of fresh and adequate blood samples for the present analyses. The overall patient population was classified as to sex, age, race, geographic origin with references to the type of disease, either lepromatous (Lep), tuberculoid (Tub), or indeterminate (Ind), as shown in Table 1. Lepromatous cases were found overall to be 3 times more common than cases of the tuberculoid type. Racial distribution with types of the disease in the 83 Hawaiian cases were classified as follows as shown in Table 2. Twenty-two Hawaiian (19 lepromatous, 1 tuberculoid, and 3 indeterminate), and 8 Samoan, 5 Japanese, 4

* This Hospital was closed subsequent to this study.

TABLE 1
*Number of samples obtained from different geographic areas
distributed according to sex and type of leprosy*

Area	No. of Cases	Lepromatous		Tuberculoid		Indeterminate	
		Male	Female	Male	Female	Male	Female
Hawaii	83	48	25	1	1	3	5
India	20	12	0	7	1	0	0
Tokyo	49	18	9	11	11	0	0
Okinawa	30	13	7	10	0	0	0

TABLE 2
Racial distribution and types of leprosy in Hawaiian cases

Race	No. of Cases	Lepromatous	Tuberculoid	Indeterminate
Hawaiian	22	19	0	3
Mixed	16	13	1	2
Filipino	24	20	1	3
Samoan	8	8	0	0
Japanese	5	5	0	0
Portugese	4	4	0	0
Chinese	2	2	0	0
Micronesian	1	1	0	0
Korean	1	1	0	0
Total	83	73	2	8

Portuguese, 2 Chinese, 1 Micronesian, and 1 Korean (all lepromatous). In addition to the racial background of the Hawaiian patients, it was noted that 31 patients were immigrants who had migrated from the Philippines (23 patients) or from Samoa (8 patients). Among the immigrants, a six month residence in Hawaii was the shortest, with most of the patients residing over 5 years and with 48 years the longest duration of residence. The shortest disease onset after immigration was found at 2 months and the longest at 48 years. The shortest period of time for treatment was 2 months while the longest was 23 years. Most of the patients had been treated with diaminodiphenylsulfone (DDS) and a

few cases with Diasone.

The breakdown of the age and type of the disease for all patients is given in Table 3. The total age distribution of the cases in different geographic locations was similar except in India cases where leprosy was found more frequently in younger age groups.

The results of IgG, IgA, and IgM levels for the patients in the four different geographic areas are given in Table 4 and the results are further broken down into sex groups in Table 5.

In general, the IgG level of the patients was shown to be higher than the normal group and in particular, IgG in the sera of lepromatous patients from India and Okinawa was found to

TABLE 3
Distribution of patient's age and type of leprosy

Area	Type	Age							Total
		11-20	21-30	31-40	41-50	51-60	61-70	<77	
Hawaii	Lepromatous	2	11	19	14	18	9	0	73
	Tuberculoid	0	0	1	0	1	0	0	2
	Indeterminate	0	1	1	3	0	1	2	8
India	Lepromatous	6	1	1	3	1	0	0	12
	Tuberculoid	5	2	0	1	0	0	0	8
	Indeterminate	0	0	0	0	0	0	0	0
Tokyo	Lepromatous	1	2	4	12	7	1	0	27
	Tuberculoid	1	1	4	1	7	4	4	22
	Indeterminate	0	0	0	0	0	0	0	0
Okinawa	Lepromatous	1	2	1	5	5	3	1	18
	Tuberculoid	0	6	2	1	0	1	0	10
	Indeterminate	0	0	0	1	0	1	0	2
Total		16	26	33	41	40	20	7	182

TABLE 4
Distribution of immunoglobulin levels (mg %) in different types of leprosy in different geographic areas

Area	Type	No. tested	Ig G	Ig A	Ig M
Hawaii	Lepromatous	73	1628±390	424±198	112± 61
	Tuberculoid	2	1385±215	490±100	72± 15
	Indeterminate	8	1570±586	548±189	112± 28
	Control	20	1140±328	198± 85	107± 47
India	Lepromatous	12	2628±608	391±183	345±176
	Tuberculoid	8	1618±249	343±112	120± 82
	Indeterminate	0	— —	— —	— —
	Control	20	1247±314	224± 92	152± 78
Tokyo	Lepromatous	27	1677±342	441±201	145± 82
	Tuberculoid	22	1569±543	321±188	90± 58
	Indeterminate	0	— —	— —	— —
	Control	20	1278±151	256± 69	134± 45
Okinawa	Lepromatous	18	2357±712	568±233	249±180
	Tuberculoid	10	1678±500	446±159	143± 47
	Indeterminate	0	— —	— —	— —
	Control	20	1314±188	243± 12	138± 6

be markedly elevated when compared to the sera from other areas. The sera of the patients from Hawaii and Tokyo did not show significant differences in the IgG level among the three leprosy types and between the sexes. However, in the lepromatous types in both sexes from Okinawa and in the male cases

of India, the IgG levels were higher than for the other leprosy types.

IgA values were significantly higher in leprosy patients than in normal groups and varied with different types of the disease, with the sexes, and with the different geographic areas.

IgM levels were found to vary with

TABLE 5

Distribution of immunoglobulin in levels (mg%) in different types of leprosy, sexes, and in geographic areas

Area	Type	Sex	No. tested	Ig G	Ig A	Ig M
Hawaii	Lepromatous	M	48	1651±401	427±202	106± 65
		F	25	1584±343	419±196	124± 51
	Tuberculoid	M	1	1600	509	56
		F	1	1170	390	87
	Indeterminate	M	3	1430±553	520±200	103± 29
		F	5	1654±252	565±180	117± 25
	Control	M	10	1250±280	210± 68	112± 37
		F	10	1330±320	276± 72	132± 32
India	Lepromatous	M	12	2628±608	391±183	345±176
		F	0	— —	— —	— —
	Tuberculoid	M	7	1616±268	332±117	113± 86
		F	1	1630	420	170
	Indeterminate	M	0	— —	— —	— —
		F	0	— —	— —	— —
	Control	M	10	1680±580	280± 78	127± 69
		F	10	1840±620	320± 82	134± 80
Tokyo	Lepromatous	M	18	1493±310	552±155	142± 85
		F	9	1942±193	558±170	322± 75
	Tuberculoid	M	10	1703±422	428±219	112± 51
		F	12	1518±124	327± 82	89± 30
	Indeterminate	M	0	— —	— —	— —
		F	0	— —	— —	— —
	Control	M	10	1235±180	243± 75	143± 53
		F	10	1307±261	228± 58	177± 60
Okinawa	Lepromatous	M	13	2522±634	635±244	241±213
		F	7	2049±796	442±158	263±108
	Tuberculoid	M	10	1678±500	446±158	143± 47
		F	0	— —	— —	— —
	Indeterminate	M	0	— —	— —	— —
		F	0	— —	— —	— —
	Control	M	10	1329±147	265± 73	111± 26
		F	10	1300±330	221± 97	167± 36

the type of disease, sex and geographic areas. In sera from all geographic locations the IgM levels of the lepromatous groups were higher than those of the tuberculoid groups. IgM levels of patients in the indeterminate group were also higher than those of the tuberculoid group.

Most of the serum samples demonstrated no detectable IgD by the method utilized which detects levels of 3 mg% or more. A few samples showed detectable level but there was no pattern of significant elevation based on the type of leprosy, geographic area, sex or age.

TABLE 6
*IgE levels (u/ml) in sera of lepromatous and
 tuberculoid leprosy patients from Tokyo and Okinawa*

Area	Type	No. tested	IgE level Mean S.D.
Tokyo	Lepromatous	27	508.9±456.3
	Tuberculoid	22	245.0±234.0
	Control	20	
Okinawa	Lepromatous	20	707.5±645.1
	Tuberculoid	10	417.0±405.1
	Control	20	

TABLE 7
*Comparison of immunoglobulin levels between twelve Indian lepromatous
 and tuberculoid patients with and without treatment*

Type	Treatment	Ig G	Ig A	Ig M
Lepromatous	non-treatment	2100	240	275
		1630	260	350
		3200	450	440
	(Mean±S.D.)	(2310±805.8)	(316.7±115.9)	(355±82.6)
	treatment	2800	560	220
		3600	510	215
		2500	295	210
	(Mean±S.D.)	(2666±568.6)	(455±140.8)	(215±5)
Tuberculoid	non-treatment	1850	240	170
		1630	420	170
		1875	350	109
	(Mean±S.D.)	(1785±134.8)	(336.7±90.7)	(149.7±35.2)
	treatment	1250	260	62
		1850	205	155
		1700	270	235
	(Mean±S.D.)	(1600±312.2)	(245±35)	(150.7±86.6)

IgE values were measured only on the Tokyo and Okinawa samples and the results are shown in Table 6. Lepromatous cases showed a higher mean value than the tuberculoid cases.

The IgG, A and M levels of 3 lepromatous and 3 tuberculoid cases which were non-treated are shown in Table 7 and compared with similar treated cases. All of these cases were from India and their ages ranges from 11 to

20 years. Three non-treated cases of the lepromatous type showed a significantly lower value of IgG and IgA with an increased IgM level when compared to the treated cases. In contrast, in the tuberculoid type IgG and IgA levels in untreated cases were moderately increased while IgM was in normal ranges in comparison to the treated cases. In all cases, however, whether lepromatous or tuberculoid and whether

TABLE 8
*Various antibodies found in sera of patients
 with lepromatous leprosy*

Area	Sex	No. tested	RF	ANA	ATA	A-Ggs
Hawaii	M	48	11	4	8	13
	F	25	6	2	6	5
India	M	12	2	0	0	3
	F	0	0	0	0	0
Tokyo	M	18	1	0	0	2
	F	9	1	0	0	0
Okinawa	M	3	1	1	1	1
	F	7	1	1	0	0

treated or non-treated all immunoglobulin values are elevated when compared to control values.

Tests were done for RF, ANA, ATA and A-Ggs. The results are shown in Table 8. In the sera tested, positive reactions to these antigens were found only in the lepromatous group and in general, were more commonly found in the Hawaiian patients. In the testing for HBsAg, 1 male out of 83 Hawaiian samples, 2 males out of 20 Indian samples, and none of 49 Tokyo and 30 Okinawa samples were positive. Only one serum, from Okinawa, of the total samples, demonstrated antibody to HBsAg. and Tokyo fell within normal values. This data lends support to the findings of Sheagren et al. (1969). The results obtained by Lim and Fusaro (Lim and Fusaro, 1964, 1968a, 1968b, 1968c, 1976), who reported there is a significant elevation of IgG and IgM in lepromatous patients while IgA values are normal, is in obvious contradiction. Tuberculoid case revealed elevated IgG and IgA levels while IgM fell within normal limits.

Comparisons of Ig levels between lepromatous and tuberculoid patients with and without treatment revealed no definite pattern due to treatment or non-treatment. Fusaro (1970) stated

the reason for the discrepancy with the report of Sheagren et al. (1969) is based upon the treatment or non-treatment of patients at the time of their Ig evaluation. However, the present authors do not feel the treatment with various medications influence such selected effects on the Ig levels of patients since our results show no such pattern. A conclusive determination cannot be made due to the small number of cases available and our analysis is based only upon the Indian population.

It is possible that the method of Ig quantitation may have been responsible for the difference in the two previous investigations. Lim and Fusaro (Lim and Fusaro, 1964, 1968a, 1968b, 1968c, 1976) used a capillary tube method while Sheagren et al. (1969) used a single radial immunodiffusion test. However, our present results suggest that genetic and environmental factors may influence Ig levels in immune response against agent (s) or leprosy, in particular, lepromatous type.

The high incidence of autoimmune antibodies such as rheumatoid factor and antinuclear antibody were only found in the lepromatous group. There was not a significant difference seen in the presence of autoantibodies be-

tween male and female groups. The number of patients from the different geographic areas tested is not comparable, but the Hawaiian group seemed to develop autoantibodies which were not related to age group differences as might be seen in the general population. It could be assumed that environmental factors may influence the humoral immunity in the production of autoantibodies and it could also be anticipated that the alteration of serum protein could occur in chronic diseases which might lead to the formation of autoantibodies.

It is interesting to note that anti-ganglioside antibody was found predominantly in male groups of lepromatous patients. Yokoyama et al. (1963) have reported that the serum of patients with central nervous system disorders such as multiple sclerosis and amyotrophic lateral sclerosis showed antibody activities against gangliosides although the titer was rather low. Since leprosy cases have been found to have developed some central nervous system involvement as a chronic type of manifestation, one may wonder if it reflects the same neurological mechanism as these other disorders.

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