Serum Immunoglobulins in Leprosy Patients with Different Spectra of Clinical Manifestations[1] (36612)

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(Introduced by W. D. Sawyer)

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Leprosy is associated with altered immunological activity. Several lines of evidence demonstrate generalized impairment of the cell mediated immune response in patients with lepromatous leprosy, and, to a lesser degree, in those with tuberculoid disease (1–6). It was recently shown that the defect of the cell mediated response in these patients was contributed, in part, by a factor(s) present in some leprosy sera (7, 8). On the other hand, leprosy patients may have a normal or a slightly hyperactive humoral immune response (4, 9). Antibodies reactive with mycobacterial antigens have been detected in the sera of these patients (10), but the role of the antibodies in the disease process is not known. As the different classes of immunoglobulins differ in biological activities, knowledge of the proportion of the various classes of serum immunoglobulins in leprosy patients may contribute to the understanding of the mechanism(s) underlying the disease process. The present study deals with the determinations of IgG, IgA, IgM and IgD levels in the sera of patients with leprosy and an attempt to correlate the immunoglobulin levels with clinical data. Elevation of serum IgE in these patients was determined qualitatively by immunodiffusion and the result was compared with that from the normal population.

Materials and Methods. Selection of patients. One hundred twenty-five patients from a leprosarium near Bangkok were studied (Table I). All but 10 were 21 years old or older. Most patients were known to have had the disease for at least 10 years. The disease was diagnosed and classified as lepromatous, tuberculoid, or borderline (dimorphous) on the basis of dermatological and neurological examinations, bacteriological smear of the skin lesion, and histological examination of the skin biopsy. Although a lepromin skin test (2), using Mitsuda antigen containing an equivalent of 1.6×10^8 bacteria/ml, was performed on all patients, the result was used for prognosis of, but not for the diagnosis of the cases. Positive lepromin reactions occurred in 98% of patients with tuberculoid leprosy and in 50% of borderline cases, but in only 2% of the lepromatous group.

At the time this investigation was initiated, 97% of the patients had been under treatment with diaminodiphenyl sulfone for at least 1 year (Table I). No patients had received steroid or other immunosuppressive drugs during their stay at the leprosarium. Although the disease in most patients with tuberculoid leprosy was inactive at the time of study, half of the lepromatous and the 6 borderline cases had active disease, as evidenced by clinical and bacteriological examinations. One-third of the lepromatous patients had erythema nodosum leprosum.

Collection of samples. Blood was collected by venipuncture; the serum was separated by centrifugation and stored at −20° until analyzed.

Immunoglobulin determinations. Serum levels of IgG, IgA, IgM and IgD were measured quantitatively by the radial immunodiffusion method (11) using commercial immunoplates (Hyland Laboratories and Melpar, Inc.). The limit of sensitivity for the measurement of IgD, as performed in this

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TABLE I. Clinical Characteristics of Patients.

Type of leprosy	No. of patients	Sex		Age (yr)		Duration of disease (years)		Duration of treatment (years)		No. with active disease[a]
		Male	Female	Range	Mean	Range	Mean	Range	Mean	
Tuberculoid	40	25	15	19–70	40	2–44	18	1–33	12	6
Borderline	6	6	0	11–42	23	1–9	4	1–6	2	6
Lepromatous	79	39	40	18–69	37	4–46	17	1–46	12	40
Total	125	70	55	11–70	37	1–46	16	1–46	11	52

[a] See text for explanation.

laboratory, was 1.4 mg/100 ml. Those serum samples with IgD values below this limit were not considered in statistical analyses. The elevation of serum IgE was determined qualitatively by the Ouchterlony technique (12) using goat anti-human IgE.[2] Immunoelectrophoretic analysis of the serum was performed as originally described (13).

Leprosy card test. An agglutination test for leprosy, similar to the rapid plasma reagin (RPR) card test for syphilis, was performed on all serum specimens as described (14). Briefly, 0.03 ml of serum or its dilutions was mixed with 1/66 ml of cardiolipin–lecithin antigen suspension[3] on a "teardrop" surface and the card was then agitated by hand for 4 min before the results were read. The proportion of cardiolipin and lecithin in the suspension was reported to be such that it reacted to only a slight degree, if at all, with nonleprosy sera and there appeared to be no correlation of reactivity with syphilitic sera (14). This claim was substantiated in this laboratory, *i.e.*, none of the positive sera reacted with antigen suspension used in the RPR test for syphilis.

Results. Immunoglobulin determination. The mean levels of IgG, IgA, IgM and IgD in 125 leprosy patients were significantly higher than the corresponding levels in healthy controls of comparable age distribution from the same area (Table II; $p < .05$ for all classes). Whereas the IgA and IgD levels in the sera of these patients were much higher than the normal mean values (15, 16), the other 2 immunoglobulins were less strikingly increased. The frequency distributions of the 4 immunoglobulins by concentration are presented in Fig. 1. Statistical analysis of the 4 immunoglobulin values in individual patients showed a significant correlation between the IgG and the IgA values (correlation coefficient $= 0.52$, $p < .05$). Qualitative determination of IgE in the sera of these patients suggested that there was some elevation in the IgE concentration, as evidenced from the finding that 44% of the patients had IgE detectable by the Ouchterlony technique as compared with none in the normal population of this area.

Immunoglobulin levels of patients with lepromatous and with tuberculoid leprosy were similar (Table II). Because of the limited number of borderline cases, statistical comparison of the results from this group of patients was not performed with respect to the other 2 groups. The mean IgG and IgA levels in the sera of the 6 borderline cases appeared to be lower than those of patients with the other 2 types of disease (Table II). The patients in the borderline group were, however, younger and had had the disease for a shorter period. When a comparison was made between the borderline group and the group of patients with lepromatous and tuberculoid diseases of comparable duration the differences became less obvious. Immunoelectrophoretic patterns of the sera from the 3

2 A gift from Dr. D. S. Rowe, WHO International Reference Center for Immunoglobulins, Lausanne, Switzerland.

3 Antigen suspensions (lot no. 2, 1-S and lot no. 5, 2-S, both of which gave identical results) were kindly supplied by Dr. J. Portnoy, Biological Research Laboratories, Hynson, Westcott and Dunning, Inc., Baltimore, MD.

TABLE II. Immunoglobulins in the Sera of Patients with Leprosy and in Normal Sera.

Persons	Immunoglobulin (mg/100 ml)				No. positive/ no. tested
	IgG	IgA	IgM	IgD	IgE
Tuberculoid leprosy					15/29
No.	40	40	40	30	
Mean	2048	541	171	8.7	
Range	700–4500	160–1200	44–600	2.0–29.0	
SD[a]	687	230	119	6.9	
Lepromatous leprosy					18/46
No.	79	79	79	60	
Mean	2268	547	137	10.5	
Range	875–3950	165–1525	41–475	1.4–45.0	
SD	626	271	74	9.1	
Borderline leprosy					ND[c]
No.	6	6	6	ND	
Mean	1720	419	142		
Range	1250–2000	245–518	115–165		
SD	305	104	19		
Leprosy (total)					33/75
No.	125	125	125	90	
Mean	2171	539	148	9.9	
Range	700–4500	160–1525	41–600	1.4–45.0	
SD	648	253	90	8.5	
Normal[b]					0/50
No.	93	93	93	38	
Range	800–2850	88–800	30–340	1.4–8.5	
Mean	1480	282	126	3.7	
SD	425	134	63	1.9	

[a] SD, standard deviation.
[b] Taken from Refs. (15 and 16).
[c] ND, not done.

groups of patients were similar when reacted against rabbit antiserum to normal human serum and were indistinguishable from the pattern of serum from normal controls.

Analysis of immunoglobulins with respect to the duration of the disease process indicated that, of the 4 immunoglobulin classes measured quantitatively, only the level of IgA showed a correlation with the duration of clinical disease (Table III). The mean IgA value of patients who had disease more than 15 years was higher than that in those who had disease less than 15 years, but the relationship was not clearly significant ($.1 > p > .05$). When the patients were grouped according to age, *i.e.*, groups with age below and above the mean age of 37 years, the mean IgA value in the older patients was significantly higher than in the younger patients (Table III, $p < .05$). The relation of the IgA value and the age of the 125 patients had a correlation coefficient of 0.28 ($p < .05$), as compared with 0.08 ($p > .05$) for the normal controls.

Although all lepromatous leprosy patients in this series had received treatment for at least 1 year and the average time that these patients had such treatment was 12 years (Table I), not all patients had responded, and 40 of 79 patients still had active disease. A comparison of the immunoglobulin G, A, M and D levels in the active and the inactive lepromatous groups was made; the mean IgA level in patients who had recovered from

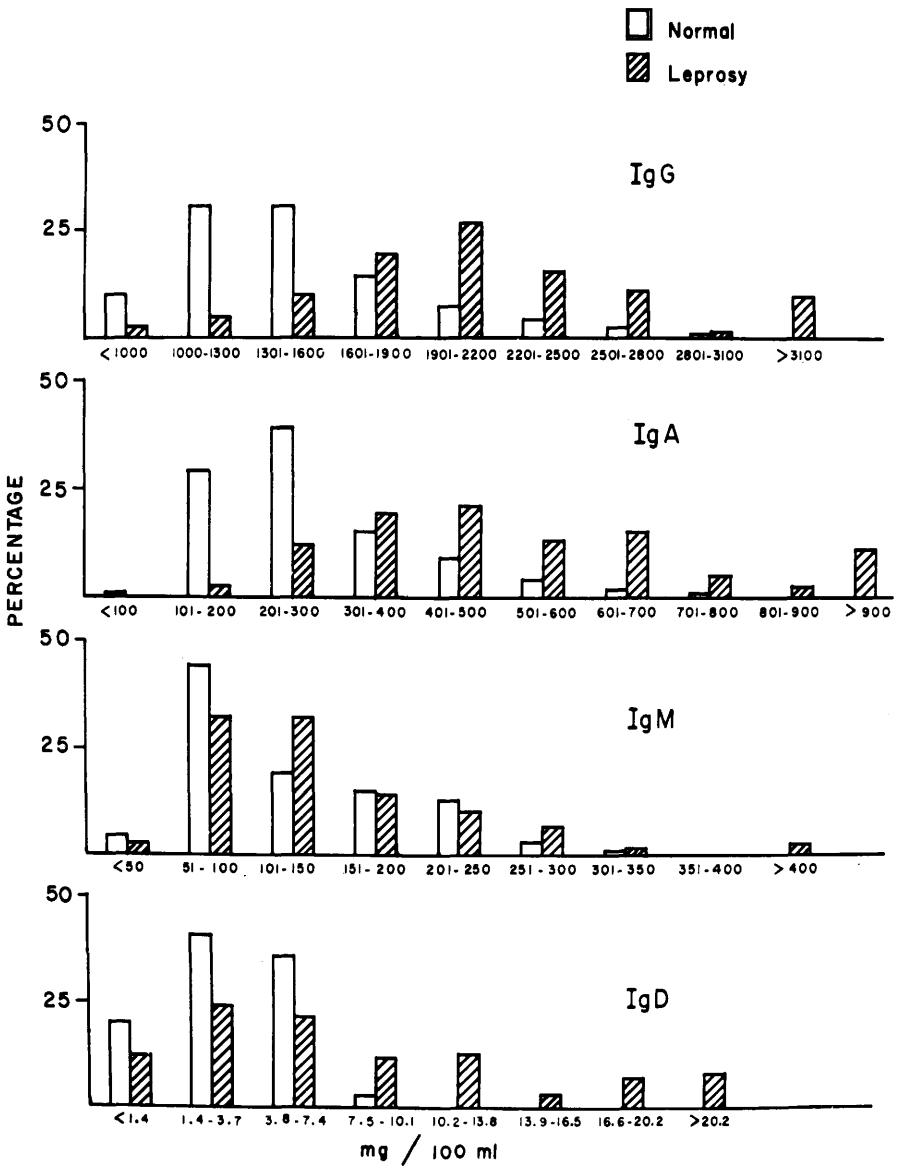

FIG. 1. Distribution of immunoglobulin G, A, M and D levels in normal sera and in the sera of patients with leprosy.

the disease was higher than in patients who still had active disease (Table III). In contrast, patients with active disease had significantly higher serum IgM level than those with inactive disease (163 vs 112 mg/100 ml, $p < .01$). Too few active tuberculoid cases were available for statistical analysis. The limited data suggested, however, that the patients with active disease had a slightly lower IgA value than those with inactive disease (507 vs 547 mg/100 ml). The immunoglobulin profile, both qualitatively and quantitatively, of the lepromatous groups with and without erythema nodosum leprosum was similar.

Card test for leprosy. The results of the card test are summarized in Table IV. Only 24% of the lepromatous cases had a positive

TABLE III. Serum IgA Levels in Leprosy Patients of Different Clinical Categories.

Classification	No. of patients	Serum IgA (mg/100 ml) Range	Mean	SD	p
Disease more than 15 yr	62	220–1525	584	273	$.1 > p > .05$
Disease less than 15 yr	63	160–1225	495	224	
Active lepromatous disease	40	197–1300	503	240	$.2 > p > .1$
Inactive lepromatous disease	39	165–1525	592	295	
≧37 years old	63	165–1525	594	277	$.05 > p > .02$
<37 years old	62	160–1300	483	214	
Lepromatous with ENL[a]	23	220–1300	582	305	$p > .1$
Lepromatous without ENL	47	165–1525	571	271	

[a] Information concerning erythema nodosum leprosum (ENL) was not available for all lepromatous cases used for immunoglobulin analyses.

reaction; 50% of the positive cases had a titer of 1:5 or greater. No significant correlation was observed between a positive card test and the progress of disease; a positive reaction was found in 32% of active lepromatous cases. On the other hand, only 3 of the 23 cases with erythema nodosum leprosum were card test positive. Only 1 of the 40 tuberculoid cases was positive by this test, and in that patient, the result was equivocal. The immunoglobulin levels of the positive sera were similar to those of the negative sera.

Discussion. Hypergammaglobulinemia is associated with chronic infections (17–19). The present data show that the sera from both lepromatous and tuberculoid leprosy patients contained significantly more IgG, IgA, IgM and IgD than the sera of normal adults in the same area. The proportions of serum samples from leprosy patients with immunoglobulin values higher than the upper normal limit (mean + 2 SD) were 33% for IgG, 38% for IgA and 47% for IgD. The increase in IgM was not as striking as in the other 3 classes of immunoglobulin and only 13% of the patients had an IgM value higher than the upper normal limit (15). Although the analysis of IgE in the serum of these patients was done by a qualitative method, it is nevertheless clear that, like the other 4 classes of immunoglobulins, IgE in these patients was higher than in the normal population of comparable age group.

TABLE IV. Results of "Leprosy Card Test."

Type of leprosy	Card test results: No. positive/no. of patients	% Positive	No. with titer ≧5/no. positive
Tuberculoid	1/40	2.5	0/1
Borderline	3/6	50	ND[a]
Lepromatous	19/79	24	8/19
Total	23/125	18	9/23

[a] ND, not determined.

With few exceptions, the hypergammaglobulinemia of chronic infections is associated with an increase in the IgG component (18). Although on a weight basis the hypergammaglobulinemia observed here in patients with leprosy was due to IgG, and there was a significant correlation between IgG and IgA, the increase in IgA was more striking relative to the normal mean value, *i.e.*, almost double the normal mean. Previous observations on a few leprosy cases (4, 19, 20) are consistent with the present results. The serum from patients with lepromatous leprosy, especially from those having erythema nodosum leprosum, amyloid, or both, were found to have a much higher IgA level than in normal controls (4). In 3 cases with amyloid, the mean IgA level was 1030 mg/100 ml compared with only 360 mg/100 ml for those without the complication (4). Although in the present series no difference between the groups with and without erythe-

ma nodosum leprosum was observed, 11% of all patients had IgA level higher than 1000 mg/100 ml. One additional case of lepromatous leprosy not included in this series showed serum immunoglobulin pattern suggestive of paraproteinemia, *e.g.*, the levels of IgG, IgA, IgM and IgD were 1680, 3750, 24 and 3.0 mg/100 ml, respectively. The significance of these observations is not known; it is possible that the increased IgA in the serum may be associated with protective antibody and that this antibody may play a role in recovery from infection. The finding that lepromatous patients who had recovered from infection had a higher IgA level than those with active disease is consistent with the notion above. Other chronic conditions associated with elevated serum IgA are tuberculosis (21), liver diseases (22), rheumatoid arthritis (23), thalassemia (24), and malnutrition (unpublished data). More investigation is needed to define the role of IgA in these diseases. A marked increase of the IgD level in leprosy patients was also interesting and deserves further investigation because only a few conditions have been associated with elevated serum IgD (20, 25, 26).

It is apparent that the card test for leprosy as employed in the present study needs to be improved if it is to have any significant diagnostic value. While a positive reaction may be reliable enough in the diagnosis of lepromatous leprosy, a high percentage of negative reactions in the lepromatous cases make it less useful as a diagnostic tool. The test may nevertheless be used in conjunction with other methods in the diagnosis of the disease.

Summary. The concentrations of serum immunoglobulins G, A, M and D from 125 patients with leprosy (40 tuberculoid, 79 lepromatous, and 6 borderline cases) were quantitated by the radial immunodiffusion technique; elevation of IgE in these sera was qualitatively detected by the Ouchterlony technique. The results were analyzed with respect to the type, the duration, and the progress of the disease. The mean levels of IgG, IgA, IgM and IgD were 2171, 539, 148, and 9.9 mg/100 ml, respectively (the corresponding levels in the normal controls were 1480, 282, 126, and 3.7 mg/100 ml). The immunoglobulin alterations were similar in patients with lepromatous and tuberculoid types of disease. Only the increase of IgA seemed to be correlated with the duration of infection, *i.e.*, higher IgA levels were associated with longer duration of infection. Patients who had apparently recovered after drug therapy had higher IgA level, but less IgM than patients with active disease.

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