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Leprosy

IV. The Quantitation of Immune Globulins (IgG, IgA, and IgM) in Leprosy Sera[1, 2]

Soo Duk Lim and Ramon M. Fusaro[3]

Recent investigations of immunoglobulins have broadened our concept and understanding of their role in disease states ([3, 4, 5, 7, 14, 15]). In addition to IgG, the other immunoglobulins, IgA, IgM, and IgD are composed partly of antibodies that result from infectious processes. With the clarification of our understanding of gamma globulins and with technics such as agar plate diffusion and the Oudin capillary tube method, it is now possible to make reliable quantitative studies of these globulins in different disease states ([1, 6, 8, 16]). Previously we had measured IgA and IgM in the serum of patients with leprosy ([9, 10]). The technic used in that study was a semiquantitative variation of the immunoelectrophoretic method ([11]) and did not give the exacting quantitative data obtainable with agar plate diffusion or Oudin capillary tubes. The study here reported is an extension of our original study, with the newer methods.

MATERIALS AND METHODS

Subjects. The controls consisted of 153 healthy Koreans (63 men, 90 women) of whom 66 per cent were Mantoux-positive, without any detectable active clinical tuberculosis. The sex and age distribution of the group is shown in Table 1. The age range was from 11 to 50 years. The leprosy group consisted of 216 untreated patients with various types of leprosy, who were seen at the Dermatology Clinic, University Hospital, College of Medicine, Seoul National University, Seoul. The diagnosis and classification of each patient as to type of leprosy were made with the criteria noted in our previous paper ([10]). Table 2 shows the classification of patients by age, sex, and type of leprosy. The serum from the patients was collected before any treatment was instituted and handled as before ([9]).

Methods. Quantitative studies were made with specific anti-IgG, anti-IgA, and anti-IgM rabbit sera. We confirmed that each antiserum showed only one precipitation line (ppt.) against its respective antigen on an immunoelectrophoretic slide (Figs. 1, 2, 3). The capillary tubes (1.7 mm. x 100 mm., Kimax) were prepared by boiling them in weak detergent solution and rinsing them a minimum of five times in distilled water. The tubes were then rinsed with ethanol and acetone and allowed to dry, and then placed in a silicone solution for five minutes (1 part Silicad in 20 parts water, Clay Adams, Inc. 141 East 25th St., New York, N.Y. 10010). After distilled water rinses the excess water was drained away and the tubes were dried at 100°C for 10 minutes.

The agar antiserum capillary tubes were prepared in the following manner. With the use of a hot water bath (45°C) a 1 per cent agar solution was made in phosphate buffer (pH 7.35). The amount of antisera added to the agar depended upon the titer of the antisera; sufficient antisera was added, however, to give a final concentra-

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[3] S. D. Lim, M.D., Ph.D., Assistant Professor, Department of Dermatology, College of Medicine, Seoul National University, Seoul, Korea; R. M. Fusaro, M.D., Ph.D., Associate Professor, Division of Dermatology, University of Minnesota, Minneapolis, Minnesota 55455.

TABLE 1. *Sex and age distribution of control subjects.*

Age (yrs.)	Sex	No. of subjects	Average age (yrs.)
<20	M	24	13
	F	22	14
	M & F	46	14
20–39	M	44	28
	F	23	28
	M & F	67	28
>39	M	22	48
	F	18	51
	M & F	40	50

tion of agar of 0.3 per cent. Capillary tubes were filled (one-half of their length) by placing tubes in the heated agar-antiserum mixture. With the finger placed over the open end of the tube, to prevent emptying of contents, the opposite end of the tube was plugged by forcing modeling clay into it. The agar was allowed to solidify at room temperature for several minutes and the tubes were then stored in sealed containers at 4°C.

The antigen diffusion procedure was carried out in the following manner. A capillary tube (0.7 mm. ID x 75 mm.) was filled with serum to be tested. This tube was inserted into the open end of the agar-antiserum capillary tube. The test serum was carefully layered on the agar-antiserum surface without puncturing the interface and without permitting an air bubble to form. The large capillary tube was then placed upright on a capillary board (in holes slightly larger than the tubes). The tubes were incubated at 37°C for 24 hours.

Migration of the precipitating band from the agar-serum interface was measured in 0.1 mm. units in an illuminated viewing box with a dark background. The indirect light source provides maximum definition of the precipitating band. A standard curve for the estimation of the concentration of each immunoglobulin in the unknown leprosy sera was made by comparing the distance of the migration band. A standard curve for the estimation of the concentration of each immunoglobulin in the unknown leprosy sera was made by comparing the distance of the migration band of the unknown serum with the band of three different concentrations of a known standard serum (normal) on semilogarithmic paper (Fig. 4). This was done for each of the three immunoglobulins (IgG, IgA, and IgM).

In order to assure reproducibility, all of the test serums were measured in triplicate and the average of the values obtained was used in determination of the concentration of the immunoprotein. In addition, each test for immune globulin was made three times at intervals of three days. The results of the tests were reproducible under the above conditions. In any experiment only agar-antiserum capillary tubes from a single preparation of agar-antiserum mixture were used. Agar-antiserum tubes from different batches or preparations were never used at the same time in an experiment. A

TABLE 2. *Classification of leprosy patients by type of leprosy, sex, and age.*

Type	Sex		Age (yrs.)			Total
	M	F	<20	20–39	>39	
Lepromatous (L)	61	21	13	53	16	82
Tuberculoid (T)	62	21	7	60	16	83
Indeterminate (I)	42	9	7	37	7	51
Total	165	51	27	150	39	216

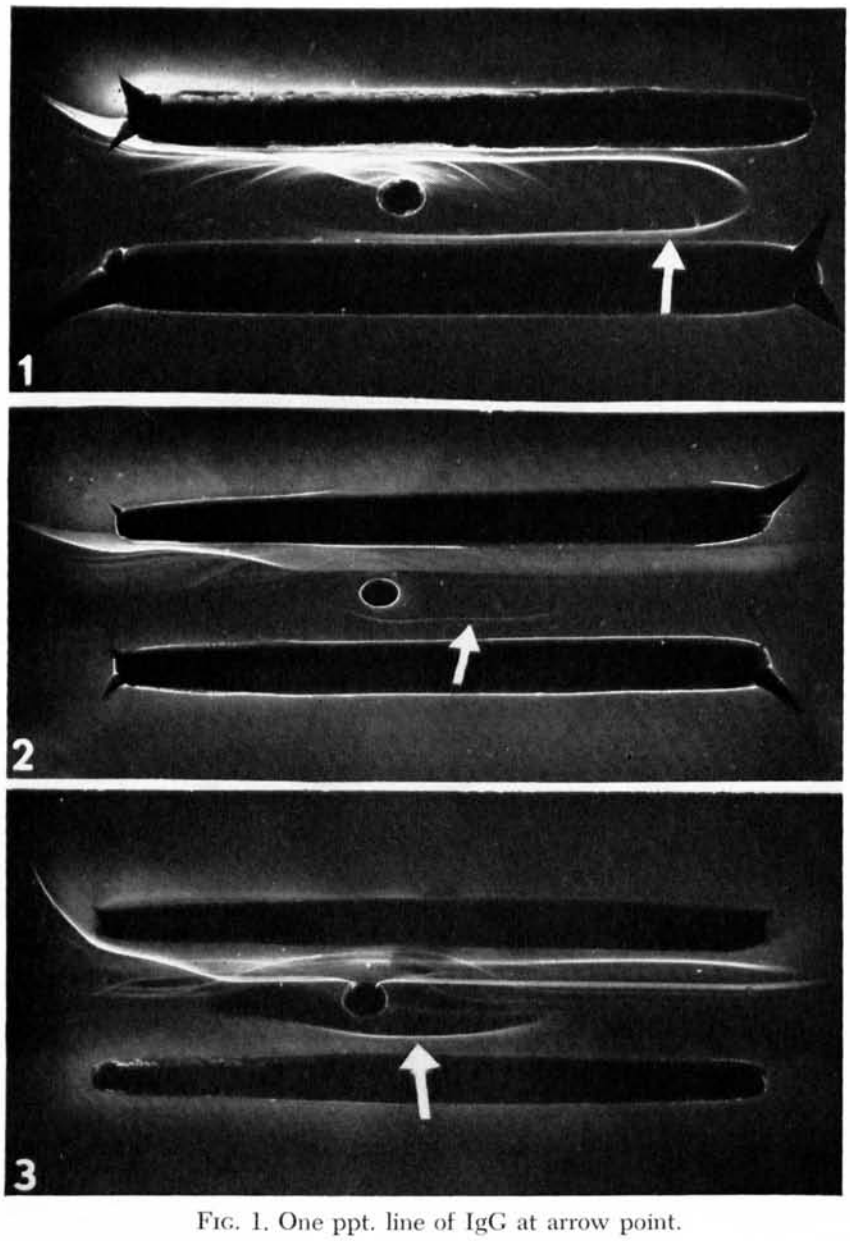

FIG. 1. One ppt. line of IgG at arrow point.

FIG. 2. One ppt. line of IgM at arrow point.

FIG. 3. One ppt. line of IgA at arrow point.

standard serum was used in every experimental run.

The sensitivity of the technic varies, depending on the antiserum titer of the rabbit antiserum used in the experiment, but in our experiments the lowest detectable concentrations of immune globulin for these experiments were 50 mgm./100 ml. for IgG, 10 mgm./100 ml. for IgM, and 20 mgm./100 ml. for IgA. The maximum value of reading error due to the scale of the viewer box in measuring the migration distance of the precipitation band is 25 mgm./100 ml. for IgG, 0.5 mgm./100 ml. for IgM, and 5 mgm./100 ml. for IgA.

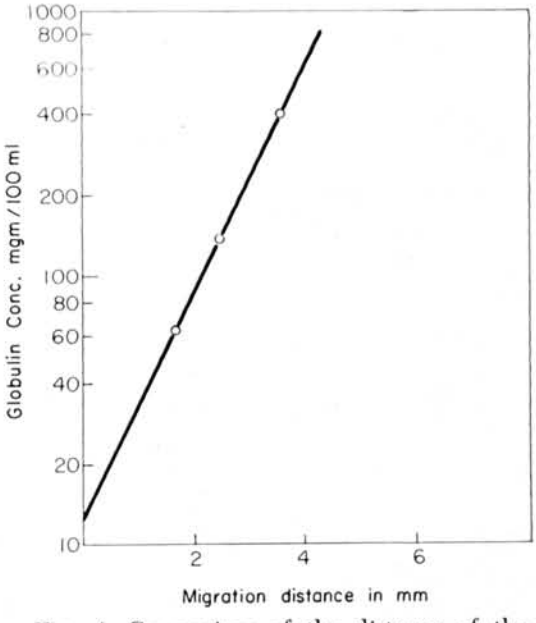

FIG. 4. Comparison of the distance of the migration band of the unknown serum and the three immunoglobulins IgG, IgA, and IgM.

RESULTS

The concentration of the immunoglobulins in the control subjects is summarized in Table 3. As there is no significant difference in levels of immune globulins in each group with respect to sex, the values were grouped according to age of subject (Table 4). The concentrations of immunoglobulins in the sera of 216 patients with leprosy are summarized in Tables 5, 6, 7 and 8. Table 9 compares the immunoglobulin levels of the control subjects with those of the patients with leprosy. There are significant differences in certain values, as noted in the table. The patterns of IgG, IgA, and IgM changes are distinctively different in the three types of leprosy (Table 10).

Previously we reported ([10]) that the immune response of IgA in lepromatous leprosy serum appeared to vary with respect to the age of the patient. Tables 11, 12, and 13 show the values for IgG, IgM,

TABLE 3. *Concentration of immunoglobulins in control subjects (immunoglobulins, mgm./100 ml. serum).*

Age (yrs.)	Male				Female			
	Mean	No.	±S.D.	±S.E.	Mean	No.	±S.D.	±S.E.
				IgG				
<20	2002	22	262	56	2151	24	275	26
20–39	2061	23	310	65	1934	44	268	41
>39	2035	18	240	57	2115	22	262	56
All subjects	2033	63	269	34	2036	90	284	30
				IgM				
<20	100	22	15	3	89	24	21	4
20–39	95	23	20	4	94	44	23	3
>39	95	18	21	5	94	22	24	5
All subjects	96	63	25	3	93	90	20	2
				IgA				
<20	264	22	70	15	257	24	61	12
20–39	278	23	57	12	274	44	59	9
>39	255	18	63	16	269	22	68	15
All subjects	267	63	62	8	268	90	62	7

S.D. = Standard Deviation.
S.E. = Standard Error.

and IgA concentration in the sera of the three types of leprosy patients subclassified in three age groups. The IgG and IgM levels differ from the normal values; however, our previous findings with respect to IgA were not confirmed.

DISCUSSION

The increase noted in serum IgG concentration is to be expected in leprosy, as the antibodies to bacterial infections are present in IgG immunoproteins ([3, 12]). It is generally stated that IgM is the first

TABLE 4. *Concentration of immunoglobulins in control subjects by age.*

Immunoglobulins (mgm./100 ml. serum)	Age (yrs.)	Mean	No.	±S.D.	±S.E.
IgG	<20	2080	46	262	39
	20–39	1978	67	287	35
	>39	2079	40	253	40
	All subjects	2035	153	279	23
IgM	<20	94	46	19	3
	20–39	94	67	21	3
	>39	94	40	22	4
	All subjects	94	153	21	2
IgA	<20	260	46	65	10
	20–39	275	67	58	7
	>39	263	40	68	11
	All subjects	267	153	63	5

TABLE 5. *Concentration of immunoglobulins in sera of leprosy patients by age.*

Immunoglobulins (mgm./100 ml. serum)	Age (yrs.)	Mean	No.	±S.D.	±S.E.
IgG	<20	2535	27	644	126
	20–39	2428	150	581	48
	>39	2485	39	687	110
	All patients	2498	216	379	26
IgM	<20	129	27	53	10
	20–39	108	150	38	3
	>39	104	39	27	4
	All patients	110	216	39	3
IgA	<20	265	27	81	16
	20–39	279	150	78	6
	>39	261	39	72	12
	All patients	274	216	78	5

immunoprotein to become significantly elevated in an acute infection ([12]). Since it is elevated in leprosy, which is a chronic insidious disease, the levels may be due to stimulation by the various antigenic substances from the bacillus and the reaction of the tissue to the bacillus ([2, 12, 13]). Another possible explanation for the elevated IgM levels, especially in the "L" type of leprosy, is the continuous multiplication of *Mycobacterium leprae* (new bacilli are always present) and the resulting reaction of the host tissue to this large population of bacteria.

TABLE 6. *Concentration of immunoglobulins in sera of patients with lepromatous type leprosy.*

Immunoglobulins (mgm./100 ml. serum)	Age (yrs.)	Mean	No.	±S.D.	±S.E.
IgG	<20	2826	13	758	211
	20–39	2505	53	490	67
	>39	2722	16	954	239
	All patients	2598	82	637	70
IgM	<20	162	13	63	18
	20–39	127	53	48	7
	>39	120	16	39	10
	All patients	131	82	50	6
IgA	<20	241	13	73	20
	20–39	263	53	85	12
	>39	273	16	63	16
	All patients	261	82	79	9

TABLE 7. *Concentration of immunoglobulins in sera of patients with tuberculoid type leprosy.*

Immunoglobulins (mgm./100 ml. serum)	Age (yrs.)	Mean	No.	±S.D.	±S.E.
IgG	<20	2468	7	418	161
	20–39	2411	60	573	74
	>39	2310	16	392	98
	All patients	2410	83	525	58
IgM	<20	96	7	12	5
	20–39	97	60	26	3
	>39	88	16	15	4
	All patients	95	83	24	3
IgA	<20	276	7	30	12
	20–39	294	60	73	9
	>39	264	16	42	10
	All patients	286	83	66	7

IgG levels in each age group for all leprosy patients (Table 11) are significantly higher than the control values. The altered IgG values are due to the elevated levels in all age groups in the "L" and "T" types of leprosy and in the 20-39 years age group in the "I" type. The IgM levels are significantly elevated in the age group under 20 and the age group from 20-39 years of age (see *all leprosy patients*, Table 12); however, the levels of IgM are elevated only in the "L" type and are significantly higher in all three age groups. In the younger leprosy patients the IgG and IgM responses are marked (Table 11, *all patients*: IgG 2535 ± 126 mgm./100 ml., and Table 12, *all patients*: IgM 129 ± 10 mgm./100 ml.). However, when one considers the data not by age but by type of leprosy, the most prominently elevated levels of IgG and IgM are in the "L" type of leprosy (Table 9). The values are found to be higher when one examines the data by age group (Table 11, IgG "L" types, 2826 ± 211 mgm./100 ml., and Table 12, IgM "L" type, 162± 18 mgm./100 ml.). In addition, the IgM level in the "L" type in the group less than 20 years of age (Table 12) is significantly elevated ($p < 0.05$) over the IgM levels in the older group 39 years of age or more. It appears that the major immune response of the younger leprosy

TABLE 8. *Concentration of immunoglobulins in sera of patients with indeterminate type leprosy.*

Immunoglobulins (mgm./100 ml. serum)	Age (yrs.)	Mean	No.	±S.D.	±S.E.
IgG	<20	2061	7	304	117
	20–39	2345	37	599	98
	>39	2345	7	395	152
	All patients	2306	51	546	77
IgM	<20	100	7	16	6
	20–39	97	37	30	5
	>39	102	7	21	8
	All patients	98	51	27	4
IgA	<20	298	7	123	47
	20–39	277	37	82	13
	>39	230	7	131	50
	All patients	247	51	95	13

TABLE 9. *Comparison of concentration of immunoglobulins in sera of control subjects and leprosy patients.*

Immunoglobulins (mgm./100 ml. serum) (mean ± S.E.)	Normal	Leprosy			
		All patients	L type	T type	I type
IgG	2035 ± 23	2498 ± 26[a]	2598 ± 70[a]	2410 ± 58[a]	2306 ± 77[a]
IgM	94 ± 2	110 ± 3[a]	131 ± 6[a]	95 ± 3	98 ± 4
IgA	267 ± 5	274 ± 5	261 ± 9[a]	286 ± 7[a]	274 ± 13

[a] Level of significant difference from normal subjects $P < 0.01$.

patient is in the IgG and IgM immunoglobulins. It is especially evident in the IgM response in the younger lepromatous patient, in whom the prognosis of the disease is poor.

TABLE 10. *Statistically significant changes in Table 9.*

Leprosy	Immunoglobulins		
	IgG	IgM	IgA
All patients	+	+	0
L type	++	+	0
T type	+	0	+
I type	+	0	0

The symbol + (elevated) or − (decreased) indicates significant difference from normal values.
Double (++) indicates highest significant response.
0 indicates no significant difference.

With respect to IgA levels it is to be noted that the highest value (Table 9, 286 ± 7 mgm./100 ml., which is significantly different from the normal value) is in the "T" type of leprosy. There are no definite patterns with respect to age groups in various types of leprosy. However, we reported previously that in lepromatous patients the IgA response appeared more prominent in the older patients ([10]). This is suggested by the more elevated values of IgA in the group more than 39 years of age, and the low levels in the group less than 20 years of age (Table 13).

The only immunoglobulin changes seen in the "I" type of leprosy serum occur in the IgG globulins. The pattern of immunoglobulin changes in IgG, IgA, and IgM in the "I" type differs from the changes in the "T" and "L" type of leprosy (Table 10).

We find it difficult to correlate these immunoglobulin changes with the various

TABLE 11. *Comparison of concentration of IgG in sera of control subjects and leprosy patients by age.*

Age (yrs.)	Control subjects	IgG immunoglobulins (mgm./100 ml. serum) (mean ± S.E.)			
		Leprosy			
		All patients	L type	T type	I type
<20	2080 ± 39	2535 ± 126[a]	2826 ± 211[a]	2468 ± 161[a]	2061 ± 117
20–39	1978 ± 35	2428 ± 48[a]	2505 ± 67[a]	2411 ± 74[a]	2345 ± 98[a]
>39	2079 ± 40	2485 ± 110[a]	2722 ± 239[a]	2310 ± 98[b]	2345 ± 152

[a] Level of significance $P < 0.01$.
[b] Level of significance $P < 0.05$.

TABLE 12. *Comparison of concentration of IgM in sera of control subjects and leprosy patients by age.*

Age (yrs.)	Control subjects	IgM immunoglobulins (mgm./100 ml. serum) (mean ± S.E.)			
		Leprosy			
		All patients	L type	T type	I type
<20	94 ± 3	129 ± 10[a]	162 ± 18[a]	96 ± 5	100 ± 6
20–39	94 ± 3	108 ± 3[a]	127 ± 7[a]	97 ± 3	97 ± 5
>39	94 ± 4	104 ± 4	120 ± 10[a]	88 ± 4	102 ± 8

[a] Level of significance $P < 0.01$.

TABLE 13. *Comparison of concentration of IgA in sera of control subjects and leprosy patients by age.*

Age (yrs.)	Control subjects	IgA immunoglobulins (mgm./100 ml. serum) (mean ± S.E.)			
		Leprosy			
		All patients	L type	T type	I type
<20	260 ± 10	265 ± 16	241 ± 20	276 ± 12	298 ± 47
20–39	275 ± 7	297 ± 6	263 ± 12	294 ± 9	277 ± 13
>39.	263 ± 11	261 ± 12	273 ± 16	264 ± 11	230 ± 50

concepts and facts known about leprosy, such as (a) "malignant disease"—lepromatous type *vs* "benign disease"—tuberculoid type, (b) "protective immunity" in which the lepromin reaction is positive in the "T" type and negative in the "L" type, (c) presence or absence of *M. leprae* in the various types of leprosy, and (d) the various clinical manifestations, reactions and prognoses in the various types of leprosy.

At present the criteria for classification of leprosy are (1) clinical manifestations, (2) the presence or absence of *M. leprae* in lesions, (3) characteristic histopathology, and (4) reaction to lepromin testing. We suggest that a fifth criterion can be useful; that is, the immunoglobulin concentrations in the sera of patients with the various types of leprosy. Although there are limits to the applicability of this criterion because of the variability noted, the usefulness is no less than with the other four criteria, whose variability at times is equally disconcerting.

SUMMARY

Serums of leprosy patients show significant elevations in the concentrations of IgG, IgM, and IgA immunoglobulins with distinctive patterns for lepromatous, tuberculoid, and indeterminate types of leprosy. Young patients with lepromatous leprosy have very prominent elevations of their IgG and IgM immunoglobulins.

RESUMEN

El suero en los enfermos de lepra muestra alza significativa en las concentraciones de immunoglobulinas IgG, IgM, e IgA con distintos caracteres para la lepra lepromatosa tuberculoide e indeterminada. Enfermos jóvenes con lepra lepromatosa tienen alzas muy notorias de sus immunoglobulinas IgG e IgM.

RÉSUMÉ

Dans le sérum des malades de la lèpre, on observe des augmentations significatives dans la concentration des immunoglobulines IgG, IgM et IgA, avec des profils distincts pour les types lépromateux, tuberculoïde, et indéterminé de la maladie. Des malades jeunes, atteints de lèpre lépromateuse, présentent des élévations fort notables de leurs immunoglobulines IgG et IgM.

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