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Leprosy

II. IgA and IgM Immunoproteins in Leprosy Sera[1, 2]

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In a previous paper ([5]) we have reported immunoelectrophoretic changes in the IgA and IgM immunoglobulins in the sera of fifteen leprosy patients. IgA and IgM globulins are two of three major serum proteins (immunoproteins) that result from various disease processes in the body. In the first paper we noted that both immunoproteins were elevated in concentration in the serums of leprosy patients. The present report summarizes the serum protein changes in 232 leprosy patients studied by electrophoresis (EP) and microimmunoelectrophoresis (IEP).

MATERIALS AND METHODS

On 232 patients seen at the Dermatology Clinic, University Hospital, College of Medicine, Seoul National University, Seoul, Korea, the age, sex, diagnosis and classification of leprosy (Table 1) were established by the following criteria: (a) clinical findings, (b) histopathologic examination, (c) bacteriologic studies and (d) the lepromin reaction. The patient's serum was collected before he was treated and it was handled as previously reported ([6]).

The serum electrophoresis was done by the microslide agar gel method ([10]). The microimmunoelectrophoretic procedure and technic was the same as previously described ([5, 6]). The antiserums were anti-human serum horse serums No. 13477, 13464, 13459, 13482, 306 and 223 (Pasteur Institute).

During each immunoelectrophoretic "run" (a batch of six slides) serums from a normal subject and a patient with leprosy were present on each microslide and each slide was made and examined in triplicate. The serum's positions in the agar wells on microslide No. 2 were reversed from those on microslides No. 1 and No. 3.

Antigen dilutions provided a semiquantitative analysis of the concentration of the serum protein. The patient's serum was diluted 1:2 and 1:4 with normal saline and used in the agar wells. If the arcs of the leprosy serum were longer and thicker than comparable arcs of the normal serum, this observation indicated that the IgA and IgM globulin concentrations in the leprosy serum were higher. Normal serum controls showed IgA and IgM arcs when undiluted antigen (serum) was used; the IgA fraction was only faintly seen on 1:2 dilution and absent on 1:4 dilution, but the IgM fraction was not seen in either dilution. On IEP analysis of the 1:4 dilutions of the leprosy serum, a positive finding was indicated by the presence of IgA and IgM arcs and their absence in normal serum. The same criteria were applied to the IgM findings in the 1:2 dilution. In the case of IEP IgA analysis of the 1:2 dilutions of leprosy serum, a positive finding was indicated by a more prominent arc in this serum than in the corresponding IgA arc of the normal serum.

RESULTS

The serum electrophoresis (EP) findings (Table 2) were similar in some respects to those described in previous reports ([3, 4, 8, 9]). Although certain differences are present, these differences have limited value depending upon the use to which the data are put. The large standard deviations limit their clinical application.

Tables 3 and 4 demonstrate the number of leprosy patients whose IgA and IgM globulins were in greater concentration

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TABLE 1. *Sex and age distribution of 232 leprosy patients.*

Type of leprosy	Sex		Age in years			Total
	M	F	<21	21–40	>40	
Lepromatous (L)	65	21	16	56	14	86
Tuberculoid (T)	66	25	12	61	18	91
Indeterminate (I)	45	10	10	39	6	55
Total	176	56	38	156	38	232

TABLE 2. *Serum protein electrophoresis.*

Subjects	Protein concentration in % of total serum proteins $\bar{x} \pm$ S.D.				
	Albumin	$Alpha_1$	$Alpha_2$	Beta	Gamma
Normals (20)	62.9 ± 7.2	4.8 ± 1.4	9.7 ± 2.4	7.6 ± 2.1	18.2 ± 3.7
Type of leprosy					
Lepromatous (L)	58.3 ± 5.8	4.7 ± 1.4	9.7 ± 2.3	7.5 ± 1.5	19.4 ± 4.0
Tuberculoid (T)	59.8 ± 4.9	4.5 ± 1.1	9.9 ± 2.2	7.6 ± 1.5	17.7 ± 3.0
Indeterminate (I)	61.2 ± 6.2	4.7 ± 1.6	9.4 ± 2.1	7.6 ± 3.1	17.0 ± 2.7

TABLE 3. *Number of patient groups with prominent IgA and IgM precipitating arcs, by age.*

Type of leprosy	Age in years	Patients' serum					
		Undiluted		Diluted 1:2		Diluted 1:4	
		IgA +	IgM +	IgA +	IgM ++	IgA ++	IgM ++
Lepromatous (L)	<21	3	16	6	16	4	9
	21–40	30	54	34	51	36	33
	>40	11	14	11	14	8	9
Tuberculoid (T)	<21	10	7	10	5	8	1
	21–40	56	37	52	37	42	3
	>40	17	12	15	17	10	1
Indeterminate (I)	<21	7	6	9	6	7	1
	21–40	25	18	23	17	15	5
	>40	5	3	5	2	2	0

Percentage was calculated from the numbers of patients with prominent arcs (+) or with arcs present in patients' serum and absent in normals (++), using the total number in each type. (See Table 1 and Fig. 2).

TABLE 4. *Percentage of patients with prominent IgA and IgM precipitating arcs.*

Type of leprosy	Patient's serum						Total patients in each type
	Undiluted		Diluted 1:2		Diluted 1:4		
	IgA + %	IgM + %	IgA + %	IgM + %	IgA ++ %	IgM ++ %	
Lepromatous (L)	51	99	59	94	56	59	86
Tuberculoid (T)	91	62	85	54	66	6	91
Indeterminate (I)	67	49	67	45	44	10	55

Percentage was calculated from the numbers of patients with prominent arcs (+) or with arcs present in patient's serum and absent in normals (++), using the total number in each type. (See Table 3 and Figs. 1 and 3).

than normal. IgA globulins were elevated in the serums of the three types of leprosy patients; however, IgA globulins were more prominent in the tuberculoid serums. On serial dilution of serums, the IgA globulins were still demonstrable in approximately 50 per cent of all patients' serums. The IgM globulins were elevated in all patients' serums and were most prominently elevated in the lepromatous serums. On serial dilution of the serums of the three types of leprosy patients, the IgM fraction was still present in 59 per cent of lepromatous patients' serums and in 10 per cent or less of the serums of the others.

DISCUSSION

The sample of leprosy patients was similar to that of other published reports [1] with respect to sex and age. Leprosy is more frequent in males than females, and has a higher total prevalence in the third and fourth decades of life. Each of these phenomena (Table 1) has been related to the social activity of the various segments of the population [1].

Serum electrophoretic findings were similar to those of other investigators with respect to the elevated gamma globulins in the lepromatous type of leprosy and to the depressed albumin in both the lepromatous and tuberculoid forms of leprosy. Reported changes in α and β globulins have not been consistent. The changes in α and β globulins that we noted agreed with some reports but differed from others [3, 4, 8, 9]. Our EP findings did not correlate with the results of our IEP study. In some analyses, the serum proteins demonstrated by EP were normal, while the IgA and IgM fractions demonstrated by IEP were elevated. So far all EP studies have not clarified the immunoglobulin changes in leprosy serum.

Table 4 shows the IgA and IgM immunoglobulin results of our IEP study in the three types of leprosy patients (summation of Table 3). The percentage of patients with elevated IgA and IgM immunoglobulins (undiluted serum) indicated that the globulin responses were very different in lepromatous and tuberculoid types of leprosy (Fig. 1).

Figure 2 shows that IgA immunoglobulin responses in the lepromatous type of leprosy differed with the ages of the patients (undiluted serum, Table 3). The percentage of patients with prominent arcs in the different age groups was as follows: (a) $<$ 21 years, 19 per cent; (b) 21-40 years, 54 per cent, and (c) $>$ 40 years, 79 per cent. The IgA globulins in the other types of leprosy did not show any marked difference. The IgM globulin responses were similar in all age groups of the three types of leprosy (Table 3).

The immunologic state of patients with leprosy is defined by the response to the lepromin test. A positive lepromin test is seen in tuberculoid leprosy, presumably as a reflection of the "immune status" of these patients. It is interpreted as a state of par-

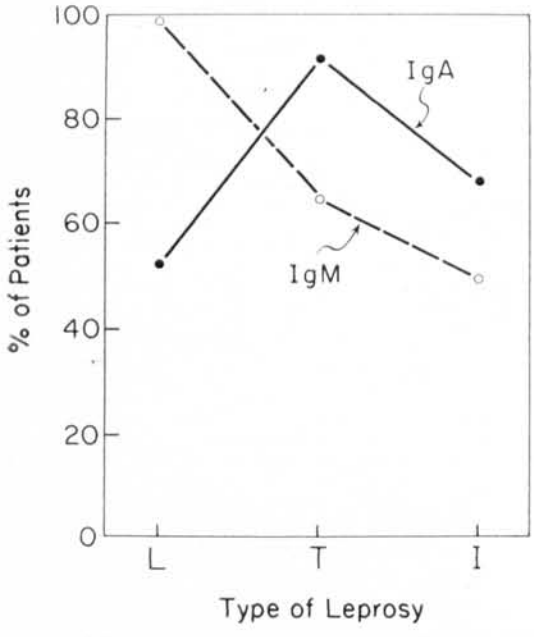

FIG. 1. Percentage of the three types of leprosy patients (based on use of undiluted patient's serum) with prominent IgA and IgM precipitating arcs.

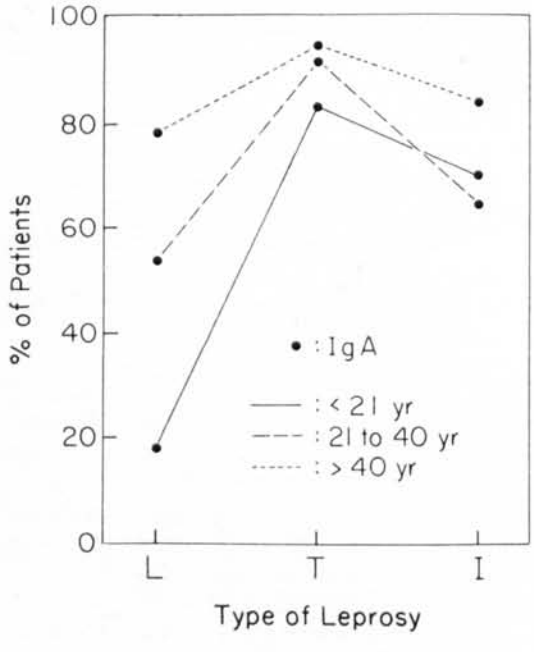

FIG. 2. Percentage of the three types of leprosy patients (based on use of undiluted patient's serum, and in relation to the patient's age) showing prominent IgA precipitating arcs.

tial protection. The question whether the IgA and IgM responses in the various types of leprosy have any relationship to the lepromin reaction and the immune status of the leprosy patient cannot be answered by our results.

A semiquantitative analysis of the amount of IgA and IgM globulins was obtained by diluting the serum 1:2 and 1:4 (Tables 3 and 4). We realize that this method is not so accurate as the Oudin capillary tube or plate quantitative method; however, our study was begun several years ago before these new technics were generally available. In spite of the reservation with regard to the quantitative use of the semiquantitative IEP method, certain indications are evident in the data. Figure 3 illustrates the data from Table 4. The more prominent IgA fraction was found in a larger percentage of the patients with the tuberculoid type of leprosy. When the serums (antigens) were diluted, the percentage of patients with prominent arcs was reduced by less than a half in the T and I types of leprosy. The lepromatous type serum did not show a similar reduction. However, the IgA fraction was still demonstrable in approximately 50 per cent of all patients' serums when the serum was diluted 1:4 (Fig. 3).

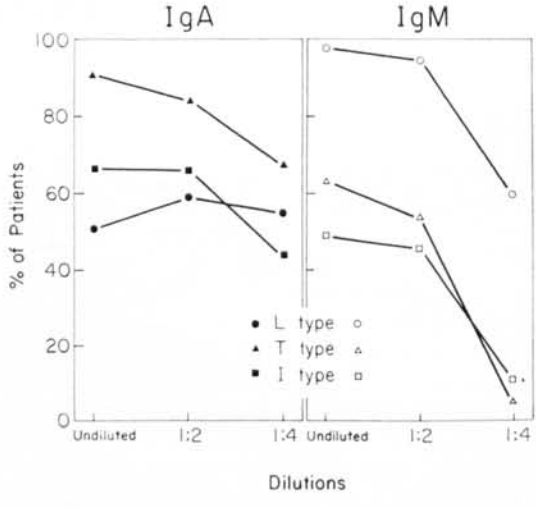

FIG. 3. Semiquantitative analysis of the concentration of IgA and IgM serum proteins in the three types of leprosy patients.

The more prominent IgM fraction was found in undiluted serum in practically all lepromatous patients (99%) and in the tuberculoid and indeterminate types in only 62 per cent and 49 per cent respectively (Table 4). The IgM globulins were demonstrable in a smaller percentage of the patients' serums diluted 1:2 and 1:4 (Fig. 3). In the IEP study of the T and I type serums with 1:4 dilutions approximately 10 per cent demonstrated IgM fractions, while 59 per cent of the L type serums showed a precipitating arc (Fig. 3), a difference suggesting that the IgM globulin concentration was elevated in the serum of the lepromatous patients.

Our findings do not agree with those of Bergot *et al.* [2] who reported moderate elevation of β_2M (IgM) in the serums from lepromatous and indeterminate forms of leprosy. They stated that the β_2M "reaction is more heterogenous" and that there is a "massive increase in β_2M" in the tuberculoid forms. Our findings with respect to IgM showed a higher percentage of patients with elevated IgM fractions in lepromatous types of leprosy, and the dilution studies did not indicate a massive response in the tuberculoid type.

With quantitative analysis of IgA, IgM and IgG globulins, by the Oudin capillary tube technic, preliminary results [7] have confirmed our observations with respect to IgA and IgM globulins changes as demonstrated by the semiquantitative IEP method.

SUMMARY

Immunoelectrophoretic studies indicated that the response of IgA globulins in the three types of leprosy was most prominent in the tuberculoid (91%) and least in the lepromatous type (51%). When considered with respect to the ages of the lepromatous patients, a wide difference in IgA globulin responses was noted. IgM globulins were observed in almost all patients with lepromatous leprosy (99%) and were more prominent than in the other forms: tuberculoid type (62%) and indeterminate (49%). These findings are different from those of other investigators.

RESUMEN

Estudios immunoelectrophoréticos indicaron que la respuesta de IgA globulina en los tres tipos de lepra fué mas prominente en la forma tuberculoide (91%) y menos en tipos lepromatosos (51%). Considerado con respecto a la edad de los pacientes lepromatosos, mayor diferencia en la respuesta con IgA globulina fué notada. IgM globulina fué observada en casi todos los pacientes con lepra lepromatosa (99%) y fué mas prominente que en las otras formas: tipo tuberculoide (62%) e indeterminado (49%). Estos resultados son diferentes de aquellos hechos por otros investigadores

RÉSUMÉ

Des études immuno-électrophorétiques menées chez les malades atteints des trois formes de lepre ont montré que la réponse des globulines IgA était la plus marquée dans la forme tuberculoïde (91%) et la moins marquée dans la forme lépromateuse (51%). Une grande variation dans les réponses de la globuline IgA a été observé lorsque l'âge des malades lépromateux a été pris en considération. Les globulines IgM ont été trouvées chez presque tous les malades atteints de lèpre lépromateuse (99%); elles étaient plus fréquentes que dans les autres formes, n'ayont été observées que chez 62% des malades du type tuberculoïde et chez 49% des sujets atteints de la forme indéterminée. Ces résultats sont différents de ceux trouvés par d'autres chercheurs.

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