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Stability of Individual Antimycobacterial Precipitation Patterns During Treatment for Lepromatous Leprosy [1]

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The formation of antimycobacterial antibodies is a characteristic feature of lepromatous leprosy ([1, 3, 9, 11, 16-18, 20-22]). Gel diffusion studies using cultivable mycobacteria as antigen have demonstrated a gradual increase both in the percentage of precipitin positive sera and in the number of demonstrable precipitins with increasing bacteriologic indices ([15]). Furthermore, a gradual decrease in antimycobacterial antibody titer following years of successful antileprosy treatment has been reported ([18]). However, information is still scanty concerning the early effects of antileprosy treatment on the titer and spectrum of antimycobacterial antibodies. Especially in view of the suggested role of immune complexes in the development of *erythema nodosum leprosum* (ENL) ([4, 6, 7, 10, 23, 24]), more knowledge of the serologic consequences of such treatment is clearly demanded.

In recent years the crossed immunoelectrophoresis (CIE) technic has been used successfully for studies on the antimycobacterial antibody response in leprosy ([2, 11]). The present investigation was designed as an attempt to establish the degree of individual variation, as well as the variation among different lepromatous patients with respect to the CIE pattern of antibody response during the first months of antileprosy treatment.

MATERIALS AND METHODS

Patients and sera. The study included 60 serum samples from 16 patients who were diagnosed clinically, histopathologically ([19]) and by bacteriologic skin smears as cases of lepromatous leprosy. The patients ranged in age between 13 and 41 (mean 22 years) and showed a male to female ratio of 5:1. All were outpatients attending the Addis Ababa Leprosy Hospital. Patient No. 13 had received antileprosy treatment for two years, eight years before the present trial. Otherwise none of the patients had received such treatment at the time of the first bleeding. From each of the patients one to six subsequent blood samples were collected during a period of four to eleven months following initiation of the DDS treatment (DDS was given at the dose of 100 mg/day to all except patient No. 1 who received 50 mg/day).

Reference serum. Precipitation lines produced in CIE by rabbit hyperimmune serum against sonicated *M. leprae* harvested from armadillos has been described previously ([8]). The designations chosen for those precipitation lines have been adopted for the corresponding lines obtained in the present investigation.

M. leprae antigen. Details on the preparation of soluble *M. leprae* antigen from infected armadillo tissue have been given previously ([12]). In short, the preparative steps included homogenization, dehydration, oil-chloroform extraction and finally ultrasonication. The protein content of the final product used in the CIE test was 4.6 mg/ml. This antigen preparation had been shown to produce seven precipitation lines in CIE against various serum pools from lepromatous patients. ([11]).

Crossed immunoelectrophoresis. The Laurell method ([13]) modified according to Axelsen ([2]) was used. In order to facilitate the comparison of lines obtained in tests on individual sera with those obtained with the reference, the intermediate gel method was employed.

RESULTS

As shown in Table 1, all patients tested in the present CIE system showed antibody activity against *M. leprae* antigen. Although the number of immune precipitates varied from one patient to the other, no serum produced less than two or more than seven such lines. In the individual patient there was no obvious correlation between the pretreat-

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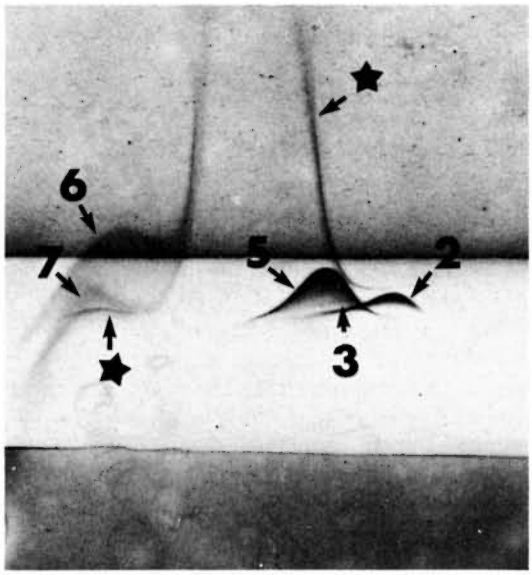

FIG. 1. Crossed immunoelectrophoresis of *M. leprae* antigen against lepromatous serum (patient No. 3). An identical pattern composed of 7 precipitation lines was observed in 7 consecutive serum samples from this patient over a period of eight months from initiation of the DDS treatment.

ment levels of Bacteriologic and Morphologic Indices, respectively, and the number of precipitates in the gel. Similarly, no correlation could be found between the clinical severity or the duration of the disease and the number of such lines. As judged by the stability of the individual precipitation pattern, no major shifts in the titer of the involved precipitins occurred during the period of observation.

The constellation of two, or more often three, precipitates in characteristic positions in the gel created a basic pattern that was easily recognized with all the sera tested. These lines corresponded to anti-2, 5 and 7, respectively, of the reference system ([8]). In addition, a precipitation line corresponding to the high peak in Figure 1 was produced by the sera of six patients, two of whom also showed antibody activity against antigens No. 1 and No. 6, respectively. Only one of the sixteen patients produced as much as seven precipitins (Fig. 1). However, two of these precipitins could not be identified by any of the seven lines obtained with the reference rabbit hyperimmune serum.

TABLE 1. *Number of immune precipitates at different time intervals (months) from start of treatment (0 = pretreatment serum, +time of ENL).*

Patient no.	Age/ sex	Histol. class.	Initial %		Time in months									
			BI	MI	0	½	1	2	4	6	8	10	12	
		early												
1	13M	LL	4.0	4.3	2	2	2	2		2			2	
2	30M	BL	4.5	14.5	3	3	3	3			3	3		
3	19F	LI	3.5	4.0	7	7	7	7	7	7		7		
4	17F	BL	3.3	4.0	4	4								
5	22F	BL	4.2	6.0	3	3	3	3	3				3	
6	23M	LI	4.8	8.0	3	3	3		3			3		
7	20M	LI	3.7	5.0	5		5	5		5				
8	30M	L	4.7	7.0	5+			5	5			5		
9	21M	LL	3.5	0	3		3	3		3		3+	3+	
10	21M	BL	3.3	1.5	4		4		4			4		
11	20M	LL	2.5	3.0	3	3								
12	29M	BL	3.3	3.0	2	2	2	2		2				
13	21M	LL	3.0	4.1	3	3	3+		3		3			
14	21M	BL	4.0	7.3	3				3					
15	15M	LL	3.7	2.0	4				4					
16	26M	LL	3.6	5.6	3		3+		3					

Sera that primarily produced two to three precipitates against the *M. leprae* sonicate would frequently reveal one or two more such lines in typical locations when for reference the rabbit hyperimmune serum was added according to the intermediate gel method. Thus, the otherwise very weak anti-3 line could frequently be clearly demonstrated by this technic.

Patient No. 10 had biopsy verified ENL at the time of the first bleeding; patients Nos. 9, 13 and 16 developed typical signs of florid ENL after one to ten months of treatment. No convincing changes in the antibody pattern of these patients were observed at the time of this complication (Table 1).

DISCUSSION

All patients included in the present study showed antibody activity in CIE against a soluble antigen preparation of *M. leprae*. However, the individual response varied considerably. Thus, some patients produced precipitation lines against two antigen components only, in the majority of cases three to five such lines were found whereas no serum produced more than seven precipitation lines in the present experimental system. These results are in agreement with the current concept of *M. leprae* as a species with surprisingly few antigenic components as compared to other mycobacteria ([8, 11, 12, 15]). However, as two of the seven precipitation lines produced by one of our patients could not be identified according to the present reference system, the number of soluble antigenic components of *M. leprae* must at least be nine. Further components are likely to be detected with modifications of the experimental system.

Experimental studies on the antigens of *M. leprae* utilizing sera from lepromatous patients have been found to compare favorably with those employing hyperimmune sera produced in rabbits as a source of antimycobacterial antibody ([11]). One would expect, therefore, that possible drug induced alterations in the presentation of *M. leprae* antigen in such patients should be reflected by changes in specificities and titer of their antibody response.

However, in spite of an adequate drug effect as judged by a falling mean BI and the reduction of all MI's to 0%, the individual antibody pattern remained remarkably stable throughout the period of study. This also applies to the four patients who developed typical episodes of ENL. Similar results were recently obtained by Melsom *et al* ([14]) using a highly sensitive and specific radioimmune assay technic. Based in part on the same serum material as described above, including sera from various stages of ENL, these authors reported only very moderate variations in the individual antibody titers against antigen No. 7 during the first year of DDS treatment. These observations are of considerable interest, especially in view of the common concept of ENL as an immune complex complication of lepromatous leprosy, frequently induced by drug treatment ([4, 7, 24]).

The stability of the antibody pattern observed in our patients may be explained by the enormous bacillary load of lepromatous patients, the mere bacteriostatic effect of DDS on these bacilli and their extremely slow disintegration in the tissues of such patients. Thus, a maximal antigenic stimulus probably persists for years in spite of efficient bacteriostatic treatment ([5]).

Obviously, the failure to demonstrate serologic changes in connection with ENL may be due to an inadequate method for the demonstration of the relevant type of antibody. Our results may also be an indication that the antibodies involved in ENL should be looked for in the tissues rather than in the circulation ([4, 24]).

SUMMARY

Sixty serum specimens obtained from 16 lepromatous patients at intervals during the first year of DDS treatment were studied in crossed immunoelectrophoresis against an *M. leprae* sonicate for possible variations of specificities and titers of antimycobacterial antibodies. All sera tested showed antibody activity against *M. leprae*, the number of precipitation lines produced varying between two and seven. In individual patients the numbers and positions of the precipitation lines remained remarkably constant throughout the period of study.

RESUMEN

Se estudiaron 60 muestras de suero obtenidas, de 16 pacientes con lepra lepromatosa, a diferentes intervalos del primer año de tratamiento con DDS. El estudio se hizo por la técnica de la

inmunoelectroforésis cruzada usando como antígeno un sonicado de *M. leprae*, con la idea de buscar las posibles variaciones en las especificidades y niveles de los anticuerpos antimicobacterianos. Todos los sueros probados mostraron actividad de anticuerpo anti-*M. leprae* y el número de lineas de precipitación varió entre 2 y 7. Individualmente, el número y la posición de las lineas de precipitación permanecieron constantes durante todo el tiempo del estudio.

RÉSUMÉ

On a étudié par des méthodes d'immunoélectrophorèse croisées avec un sonicat de *M. leprae*, 60 échantillons de sérum chez 16 malades de le lèpre à des intervalles divers au cours de la première année de traitement par la DDS, en vue de mettre en évidence des variations éventuelles de la spécificité et des titres d'anticorps anti-mycobactériens. Tous les échantillons de sérum qui ont été étudiés présentaient des anticorps contre *M. leprae*, le nombre de lignes de précipitation variant entre deux et sept. Chez les malades individuels, le nombre et la position des lignes de précipitation restait remarquablement constant pendant toute la période d'étude.

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