A Study of Antibody Response in Leprosy[1, 2]

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Serologic reactions in leprosy infection have not been studied with as much interest as other aspects of the infection, for various reasons. Inability to cultivate the causative agent *in vitro* has made preparation of even relatively pure antigens difficult. Positive serologic tests are not characterized by an absolute specificity for leprosy, in part because no antigen specific for the leprosy bacillus has been isolated. Finally, serologic reactions are of little use to the clinician, because of the lack of specificity mentioned and because the presence of antibodies is in no way correlated with the state of resistance of the patient.

In spite of these factors, there are a growing number of noteworthy observations concerning antibody production in leprosy. Reports by Rees and co-workers ([7]), Sushida and Hirano ([8]), Navalkar *et al.* ([5]), and others indicate that antibodies reactive in precipitin tests with a wide variety of mycobacterial antigens are produced by most patients with lepromatous leprosy and many patients with borderline leprosy. In contrast, very few patients with tuberculoid leprosy have detectable circulating antibody against mycobacterial antigens.

Nonspecific serologic activities also are pronounced in lepromatous leprosy. Sera from these patients often give false positive tests for syphilis, and more recently Bonomo *et al.* ([1]) and Matthews and Trautman ([4]), among others, have reported the presence of auto-immune antibodies.

The purpose of the present study has been to study a number of the serologic features of leprosy in some detail, using sera from patients with the more important clinical manifestations of the disease. The aspects studied include the following: (1) frequency of circulating antimycobacterial antibody in various forms of leprosy, using soluble antigens from 10 strains of mycobacteria in precipitin tests, (2) frequency of occurrence of cryoprotein in various manifestations of leprosy, (3) levels of circulating precipitins during reactional phases and subsequent periods of quiescence, and (4) characteristics of the antibodies present in lepromatous sera.

MATERIALS AND METHODS

Precipitin tests. Soluble antigens were prepared from the following strains of mycobacteria by ultrasonic disruption of washed bacilli and extraction in buffered physiologic saline: **Noncultivable.** *Mycobacterium leprae* (bacilli separated from lepromas); *M. lepramurium;* bacilli separated from the tissues of hamsters inoculated with material from human leprosy. **Cultivable.** *M. tuberculosis* (H37Rv, Ravenel, and BCG); two anonymous strains isolated from human pulmonary lesions; and two cultivable mycobacteria isolated from the lesions of persons with leprosy.

The protein concentration of each antigen was determined by the method of Lowry and adjusted to about 10 mgm. per ml. These antigens were then used in double diffusion tests in agar according to the method of Ouchterlony.

Cryoprotein determination. The method of Matthews and Trautman ([4]) was used. Blood samples were taken with syringes warmed to about 40°C, and immediately placed in warmed tubes and held at 37°C for two hours. The sera were collected by centrifugation in a warmed centrifuge,

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placed at 4°C for 48 hours, and then examined for the presence of a precipitate. The presence or absence of antimycobacterial precipitins in these precipitates was studied by repeatedly washing them in a refrigerated centrifuge, then redissolving them in saline at 37°C, and preparing double diffusion slides, which were kept at 37°C.

Effect of thalidomide treatment on precipitin levels. Blood samples were taken at one-month intervals for six to eight months from patients who were being treated with thalidomide in order to control their reactional outbreaks. These sera were tested for the presence of precipitins against the 10 mycobacterial antigens listed above. Twofold serial dilutions of the sera were also tested against several antigens to determine if there were any appreciable quantitative differences in antibody levels during the course of treatment.

Antibody characteristics. Sera with high levels of antibody were separated on columns of G-200 Sephadex, using phosphate-buffered saline as the eluant. Fractions from the first two peaks, corresponding to macroglobulins and 7S gamma globulins, were tested for the presence of precipitating antibody.

Ten sera were treated with 0.1 M 2-mercaptoethanol for 18 hours at room temperature, and then dialyzed against repeated changes of buffered saline at 4°C. Simultaneously, equal volumes of serum were treated with buffered saline. The treated and control sera were tested in double diffusion slides against two of the strongest mycobacterial antigens.

Passive cutaneous anaphylaxis in guinea-pigs was studied with a number of these sera. One-tenth ml. of serum was injected intradermally; after four to six hours, 0.5 ml. of mycobacterial antigen mixed with 0.5 ml. of one per cent Evans blue was injected intravenously. The guinea-pigs were observed for 30-60 minutes for the development of reactions at the sites of injection.

RESULTS

Precipitin tests. Table 1 presents the results obtained when sera from patients with distinct clinical manifestations of leprosy were tested for the presence of precipitins against 10 strains of mycobacteria. This table indicates a high frequency of precipitins in the sera from patients with lepromatous leprosy (75% of the 76 tested) and a very low frequency in sera from tuberculoid and indeterminate cases. The group with reactional borderline leprosy is small,

TABLE 1. *Frequency of antimycobacterial antibody in leprosy sera.*

Clinical form of leprosy	No. sera tested	No. positive sera
Lepromatous		
Reactional	41	33
Nonreactional	35	24
Tuberculoid	8	1
Indeterminate	10	1
Borderline		
Reactional	5	3
Nonreactional	9	1

TABLE 2. *Number of sera from each clinical type of leprosy that reacted with each of the 10 mycobacterial antigens used in the method of double diffusion and precipitation in agar.*

Strain of mycobacterium	Number of sera			
	L (76)	T (8)	B (14)	I (10)
Cultivable				
M. tuberculosis				
BCG	9		2	
H37Rv	22	1	2	1
Ravenel	24	1	2	1
Photochromogen 440	39	1	3	
Valero-anonymous	53	1	4	1
24-human leproma	49	1	4	1
129-human leproma	54	1	3	1
Noncultivable				
M. leprae	11	1	1	
M. lepraemurium	35	1	3	1
Mycobacterium from hamsters injected with lepromatous material	37	1	4	1

but the frequency of precipitins in this group is high.

Many of these positive sera gave precipitin lines with eight, nine, or all 10 of the antigens used. Table 2 indicates the number of sera that reacted with each of the antigens used. The weakest antigen of the cultivable mycobacteria was that prepared from BCG; the weakest antigen from noncultivable mycobacteria was that of *M. leprae*. No consistent pattern of reaction with certain antigens that might constitute subgroups of the 10 strains used has emerged from these data; so they are not presented in more detail.

Some sera gave two or three lines against some of the antigens used, particularly those sera from patients with reactional lepromatous leprosy of long duration. Sera that reacted against all or nearly all of the antigens used usually gave a line of identity with all of the antigens, as well as additional nonidentity lines with the stronger antigens. Mycobacteria isolated from lepromas did not give any lines not shared by any of the other mycobacteria.

Cryoprotein determination. Thirty-four samples of blood were taken as described earlier from patients in a local leprosarium and examined for the presence of cryoproteins. The results of this test are presented in Table 3. The frequency of occurrence of cryoproteins in lepromatous leprosy is obviously high (23 of the 25 sera tested), as has been reported by Matthews and Trautman ([4]). Seven positive sera among the other nine tested suggest that cryoprotein may be frequent in all forms of long duration.

TABLE 3. *Frequency of cryoproteins in sera from leprosy patients.*

Clinical form of leprosy	No. sera tested	No. positive sera
Lepromatous		
Reactional	10	8
Nonreactional	15	15
Indeterminate	2	2
Tuberculoid	5	4
Borderline		
Reactional	1	0
Nonreactional	1	1

We began these studies with sera from a group of patients with leprosy from the Vargas Hospital in Caracas; of the 10 sera studied, only one contained cryoprotein. This low frequency may have been due to lack of experience with the technic, but it seems more likely that this alteration in the serum may become apparent only as a result of relatively long and severe infection, which was more characteristic of the group of patients from the leprosarium.

The washed cryoprotein precipitates, when redissolved in saline at 37°C and used in double diffusion tests, did not give precipitin lines with any of the mycobacterial antigens.

The effect of thalidomide on precipitin levels. Serum samples were taken at one-month intervals for six to eight months from patients who were being treated with thalidomide in order to control their reactional symptoms. In spite of the very rapid disappearance of erythema nodosum leprosum and other symptoms after the initiation of this treatment, no diminution in the antibody content of the serum samples was observed. The criteria used, viz., relative position, intensity, and number of lines present, and the dilution of serum that still produced a visible precipitin line, are not strictly quantitative, but permit a gross evaluation of antibody concentration.

Antibody characteristics. Both separation on G-200 Sephadex and treatment with 0.1 M 2-mercaptoethanol indicate that the antimycobacterial antibodies present in sera from leprosy patients are not confined to either the 19S macroglobulin or 7S globulin fractions of serum. Upon treatment of sera with 2-mercaptoethanol, it was observed that some lines were completely eliminated; others diminished in intensity, and others seemed to be unaffected by this treatment.

Several efforts were made to devise a more sensitive or simpler method for detection of antimycobacterial antibodies, using leproma-derived antigens. We have used standard lepromin (160 x 10^6 bacilli per ml., prepared by the Mitsuda-Hayashi-Wade modification) both before and after

ultrasonication, and after concentration four-to six-fold, as the antigen in double diffusion tests. This antigen in our hands gives lines only with the sera that have unusually large amounts of antibody as measured with the other mycobacterial antigens that we have used; of 108 sera tested, only four gave a precipitin line with the concentrated antigen. The sensitization of red blood cells with lepromin, determined by the method of Middlebrook and Dubos, also gave very few positive reactions, with low titers. Finally, the technic of passive cutaneous anaphylaxis was used to study some of these sera. Of 20 sera tested, all with precipitating antibody against mycobacteria, only one gave a positive reaction, very atypical of the PCA reactions we obtain with other antigen-antibody systems. The reaction developed slowly, becoming evident only after about 20 minutes, and produced only a pale, diffuse area of bluing, about 12 mm. in diameter, at the site of the injection.

DISCUSSION

This work confirms the observations of a number of authors indicating that the frequency of circulating antimycobacterial antibodies is high in persons with lepromatous leprosy ([5, 6, 7]). The frequency is intermediate in borderline leprosy, and low in the tuberculoid and indeterminate forms. These antibodies are not specific for *M. leprae*, but rather for antigens shared by all of the 10 strains of mycobacteria that we have used.

This production of circulating antibodies in leprosy undoubtedly requires the persistence of relatively large numbers of bacilli for a long period of time. Our efforts to produce antisera in rabbits by immunization with bacilli separated from lepromatous tissue have not been successful, in spite of biweekly injections for nearly a year.

The presence of antimycobacterial antibodies in leprosy patients is clearly not related to the state of resistance of the patient, since the incidence is highest in those individuals whose disease is most severe. Undoubtedly the predominantly intracellular environment of the bacilli, as well as such structural features as the lipid coat, prevent effective combination of antibody in any sort of protective mechanism.

Reference has been made to the fact that some of the mycobacterial antigens used in this study are much stronger than others, in spite of similar protein content. This same variation has been observed, though to less extent, in separate preparations of antigen from a given strain of mycobacteria. Since at least some of the important soluble antigens of mycobacteria are polysaccharides ([2]), quantitation of the concentration of antigen in terms of protein content undoubtedly reflects quite inadequately the actual composition of these extracts. Whether all of the antigens that give precipitin lines with antimycobacterial antibodies are polysaccharides or not has not been established.

The presence of abnormal cryoproteins in the sera of persons with lepromatous leprosy is only one of a number of serologic alterations that have been reported. The studies reported here suggest that abnormal cryoprotein appears after prolonged infection, not only in lepromatous, but also in other forms of leprosy. It is not surprising that chronic infection of this type, often accompanied by visceral involvement and involving prolonged treatment, should lead to serum abnormalities. The well-known adjuvant activity of lipids may play a role in the eventual development of some of these abnormalities, but whether they contribute to the severity of the disease is still a matter of conjecture.

It has been suggested that antibodies may play a role in the reactional symptoms seen in some patients ([3]), in part because there is no doubt that antigen-antibody complexes of even innocuous substances may be toxic. The presence of antimycobacterial antibody alone in the serum of patients with lepromatous leprosy is not sufficient to initiate reactions, since many patients with high levels of these antibodies do not have reactional symptoms. Also, the symptoms disappear quickly with thalidomide treatment, although there is no apparent change in levels of precipitating antimycobacterial antibody during the course of six to eight months. Here again,

the intracellular locale and the lipid coat of the bacilli may inhibit effective combination, leading to the production of toxic complexes only when some unknown factor causes either the death and disintegration of large numbers of bacilli or the liberation of soluble antigenic substances.

The extraordinary dichotomy between the antibody-forming and delayed hypersensitivity mechanisms in a given disease is very well illustrated in leprosy. Adequate explanation for the concurrent anergy toward the Mitsuda reaction and abundance of antimycobacterial antibodies in the lepromatous patient will only be provided by detailed analysis both of the distinct antigens involved and of the host's response at the subcellular level to these antigens.

SUMMARY

This study confirms the high frequency of antimycobacterial antibodies in the sera of patients with lepromatous leprosy and the low frequency in sera from patients with tuberculoid and indeterminate leprosy. Ten strains of mycobacteria, three of which were noncultivable and seven cultivable, were studied; they all contained at least one common antigen.

Cryoproteins were found also in the sera of a high proportion of patients with all manifestations of leprosy of long duration.

Treatment effective in the elimination of reactional symptoms did not appreciably alter the levels of antibodies in the sera of these patients.

The antimycobacterial antibodies in these sera were found in both the 19S and 7S classes.

RESUMEN

Este estudio confirma la alta frecuencia de anticuerpos antimicobacterianos en el suero de pacientes con lepra lepromatosa y la baja frecuencia en sueros de pacientes con lepra tuberculoide e indeterminada. Diez cepas de micobacterias, tres de las cuales fueron no cultivables y siete cultivables, fueron estudiadas; todos ellos contienen a lo menos un antígeno común.

Cryoproteínas fueron encontradas también en el suero de una alta proporción de pacientes con todas manifestaciones de lepra de larga duración.

El tratamiento efectivo en la eliminación de síntomas reaccionales no alteraron apreciablemente los niveles de anticuerpos en el suero de estos pacientes.

Los anticuerpos antimicobacterianos en estos sueros fueron encontrados en ambas clases 19S y 7S.

RÉSUMÉ

Cette étude confirme le frequence élevée d'anticorps antimycobactériens dans le sérum de malades atteints de lèpre lépromateuse et la faible incidence de ces anticorps dans le sérum de malades atteints de lèpre tuberculoide ou de lèpre indéterminée. On a étudié 10 souches de mycobactéries, dont 3 ne pouvaient être cultivées; 7 pouvaient être cultivées. Toutes ces souches contenaient au moins un antigène en commun.

On a également trouvé des cryoprotéines dans le sérum d'une haute proportion de malades ayant présenté pour une longue période les manifestations de la lèpre.

Un traitement actif pour éliminer les symptomes réactionnels n'a pas modifié de manière appréciable le niveau de ces anticorps dans le sérum de ces malades.

Les anticorps antimycobactériens observés dans ces échantillons de sérum appartenaient tant à la classe 19 S qu'à la classe 7S.

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