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Crossreactivity Between *Mycobacterium leprae* and Various Actinomycetes and Related Organisms[1]

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It has been known for many years that mycobacteria are rich in both interspecies and intergenerical crossreacting antigens ([3, 5, 6, 11, 13, 14, 22, 24]). Years before the armadillo-grown *Mycobacterium leprae* cells became available, it was also postulated that *M. leprae* shared several antigens with other mycobacterial species ([1, 15, 17]). This assumption was based on the fact that antigen preparations from mycobacteria reacted with sera from patients with leprosy. Similar studies also demonstrated antibodies against antigens from nocardiae and rhodococci in leprosy sera ([2, 8, 21]). The present author and colleagues demonstrated, furthermore, that such sera contained antibodies against a ribosomal precipitinogen ([21]). This precipitinogen, designated β, had been shown in strains of mycobacteria and rhodococci and in some nocardiae ([15, 21, 22]) but not in any tested corynebacteria ([19]).

The discovery that *M. leprae* cells grow in armadillos facilitated the study of its antigens. Harboe and co-workers found, using immunoelectrophoresis, seven distinct antigens in an *M. leprae* preparation and demonstrated extensive crossreactivity between these antigens and antigens of *M. avium*, *M. bovis* var. BCG, *M. lepraemurium*, *M. smegmatis*, *Nocardia asteroides*, and *N. caviae* ([10, 28]). Furthermore, the above-mentioned β-precipitinogen has recently been demonstrated in *M. leprae* by means of armadillo-derived *M. leprae* material ([20]).

The aim of the present study was to elucidate the antigenic crossreactivity between *M. leprae* and strains representing different species of *Corynebacterium*, *Mycobacterium*, *Nocardia*, *Streptomyces*, and related taxa. The purpose was also to investigate sera from patients with leprosy concerning the presence of antibodies against these organisms. Special interest was focused on the relationship between *M. leprae* and strains of *Streptomyces*.

MATERIALS AND METHODS

Bacterial strains. Armadillo-derived *M. leprae* and 35 strains (here referred to as the comparison strains) representing 34 different species of *Corynebacterium*, *Mycobacterium*, *Nocardia*, *Rhodococcus*, *Streptomyces*, and related taxa were analyzed (Tables 1 and 3).

Antigen preparations. The *M. leprae* material was provided from IMMLEP by Dr. R. J. W. Rees. It consisted of a sonicate of *M. leprae* cells prepared according to the IMMLEP protocol 1/79. The extract obtained after centrifugation at 30,000 *g* for 25 min was employed. The comparison strains were cultivated on liquid or solid Sauton media or glucose nutrient broth, and the antigen preparations were made as earlier described ([22]).

Antisera. Sera from nine Ethiopian patients with lepromatous leprosy were employed. These sera were provided by Dr. M. Harboe. Antigen preparations from the 25 strains given in Table 1 were inoculated in rabbits for the production of antisera ([18]). A monospecific antiserum against the earlier-described precipitinogen β ([21]) was prepared according to a method developed by Harboe, *et al.* ([9]). The β precipitates were cut out of the gel, washed, sonified, and used for inoculation in a rabbit.

Precipitation systems. Twenty-five homologous precipitation systems were established. The principles for the establishment and the use of serological precipitation (reference) systems have been described previously ([18, 22]). In order to reveal the precipitinogen β, a special system was established—the β system. It was heterologous in order to exclude some irrelevant precipitates and consisted of the *M. fortuitum*

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TABLE 1. *Number of precipitinogens revealed when material from* M. leprae *was analyzed by precipitation systems representing 25 of the comparison strains.*

Precipitation system		No. of precipitinogens	
Lab no.	Species		
GB242	*Arthrobacter globiformis* NCIB 8907, ATCC 8010	—[a]	—[b]
GB246	*Corynebacterium bovis* NCTC 3224	1	—
GB243	*Corynebacterium glutamicum* NCIB 10025	1	—
GB252	*Corynebacterium ulcerans* NCTC 7910	1	—
GB244	*Kurthia zopfii* NCIB 11155, ATCC 10538	—	—
GA009	*Mycobacterium avium* G. Penso Ceppo Faisan IV	5	2
GA001	*Mycobacterium bovis* var. BCG A. Lind Swedish substrain	3	2
GA923	*Mycobacterium farcinogenes* NCTC 10955	4	2
GA023	*Mycobacterium fortuitum* G. Penso 456	3	3
GA120	*Mycobacterium kansasii* E. H. Runyon P16	4	2
GA010	*Mycobacterium phlei* NCTC 8151	2	1
GA924	*Mycobacterium senegalense* NCTC 10956	4	1
GA029	*Mycobacterium smegmatis* NCTC 8152	4	2
GA713	*Mycobacterium tuberculosis* H37Rv ATCC 27294	5	3
GA681	*Mycobacterium vaccae* ATCC 15483	4	2
GB144	*Nocardia amarae* M. Goodfellow N667	—	—
GA761	*Nocardia asteroides* I Juhlin M-ö 5006	3	1
GA875	*Nocardia asteroides* II ATCC 19247	2	1
GA873	*Nocardia otitidis-caviarum* ATCC 14629	2	—
GB245	*Rhodococcus bronchialis* NCTC 10667	—	—
GA785	*Rhodococcus corallinus* I Juhlin M-ö 5007	2	—
GA766	*Rhodococcus rubrus* I Juhlin 107	3	—
GB285	*Streptomyces diastaticus* S. T. Williams 496	1	—
GB202	*"Gordona" aurantiaca* NCTC 10741	1	—
GB205	*"Mycobacterium" album* ATCC 25969	2	—

[a] Total number of revealed precipitinogens.
[b] Number of precipitinogens identified by the precipitation system.

(GA023) antigen preparation and the anti-*M. avium* (GA009) serum.

Immunodiffusion analyses. The analyses were carried out by means of a microplate modification ([27]) of the immunodiffusion technique according to Ouchterlony.

RESULTS

Table 1 gives the result obtained when the *M. leprae* antigen preparation was analyzed by immunodiffusion, using the 25 precipitation systems representing various actinomycetes and related organisms. The total number of precipitates obtained at the reaction between the *M. leprae* antigen and the reference antisera is given, as well as the number of precipitinogens identified by the precipitation system. *M. leprae* shared one antigen or more with all but four of the strains included. One precipitinogen was common to *M. leprae* and the three corynebacteria, and between two and five precipitinogens were common to *M. leprae* and the ten mycobacteria. None of the precipitinogens shared with the corynebacteria were identified, while many of the precipitinogens shared with the mycobacteria were identified by means of the precipitation systems. In many cases the precipitinogen β was identified by means of the monospecific anti-β serum and/or the heterologous reference system. The *M. leprae* also shared precipitinogens (one, two, or three) with the nocardia, rhodococcus, and streptomycetes strains, and with the strains designated *"Gordona" aurantiaca* and *"Mycobacterium" album.*

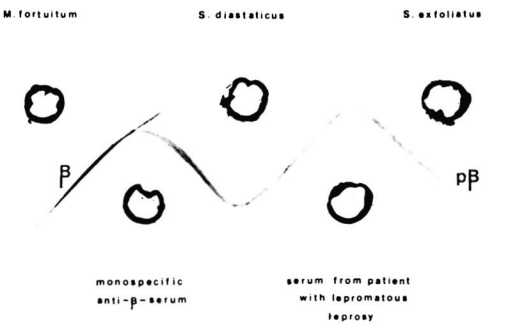
Sera from patients with lepromatous leprosy and antigen preparations from 33 of the comparison strains were analyzed by immunodiffusion (Table 2). The data showed that five or more of the nine leprosy sera reacted, forming one or more precipitates with one of the three corynebacterial strains, with six of the nine mycobacterial strains, and with six of the 11 streptomycetes strains.

Analyses by means of the β-system and

TABLE 2. *Number of sera—among nine tested—forming one or more precipitates when analyzed by antigen preparations from 33 of the comparison strains.*

Species	No. of reacting strains
Arthrobacter globiformis	0
Corynebacterium bovis	1
Corynebacterium glutamicum	7
Corynebacterium ulcerans	1
Kurthia zopfii	0
Mycobacterium avium	5
Mycobacterium bovis var. BCG	5
Mycobacterium fortuitum	7
Mycobacterium kansasii	3
Mycobacterium phlei	7
Mycobacterium senegalense	5
Mycobacterium smegmatis	3
Mycobacterium tuberculosis	6
Mycobacterium vaccae	0
Nocardia amarae	0
Nocardia asteroides I	3
Nocardia asteroides II	2
Nocardia otitidis-caviarum	1
Rhodococcus bronchialis	1
Rhodococcus rubrus	1
Streptomyces albus	5
Streptomyces diastaticus	7
Streptomyces exfoliatus	6
Streptomyces fulvissimus	0
Streptomyces goshikiensis	1
Streptomyces globisporus	6
Streptomyces griseinus	7
Streptomyces griseoruber	5
Streptomyces lavendulae	0
Streptomyces olivaceus	2
Streptomyces platensis	1
"Gordona" aurantiaca	0
"Mycobacterium" album	0

the monospecific anti-β-serum showed that the β-precipitate was found in many cases at the reaction between the leprosy sera and the mycobacteria. A precipitate fusing with β was also found when the leprosy sera reacted with the streptomycetes. A spur was registered, however, indicating that the β precipitinogen of streptomycetes is only partially identical with the β precipitinogen of mycobacteria (The Figure). This precipitinogen was designated pβ (partial β). Table 3 demonstrates that neither β nor pβ was found in any of the three corynebacterial strains tested, while β was found in all five of the mycobacteria tested. In addition, pβ was found in eight of the 11 streptomycetes.



THE FIGURE. Immunodiffusion analyses demonstrating that streptomycetes share an antigen (precipitinogen β) with mycobacteria, and that a patient with lepromatous leprosy produces antibodies against this antigen. The spur indicates that the β precipitinogen of the two streptomycetes strains is only partially identical with that of *M. fortuitum*.

DISCUSSION

The present study demonstrates that *M. leprae* shares antigens with various species of *Mycobacterium* and also with other species of related genera, such as *Corynebacterium, Nocardia, Rhodococcus,* and *Streptomyces.* The data show, furthermore, that *M. leprae* shares more antigens with the mycobacteria than with the other genera tested. The number of antigens shared between *M. leprae* and the mycobacterial species corresponds to the number of common antigens revealed in earlier studies of mycobacterial species by the same standardized immunodiffusion technique ([14, 18, 22]). The results are thus in accordance with the view that the leprosy organism belongs to the genus *Mycobacterium,* contradicting the results recently published by Imaeda, *et al.* ([12]). They demonstrated a closer DNA homology between *M. leprae* and corynebacteria than between *M. leprae* and mycobacteria.

The largest number of precipitinogens (five) was revealed when *M. leprae* was studied by means of the *M. avium* and the *M. tuberculosis* system, and the largest number of identified precipitinogens (three) was revealed by the *M. fortuitum* and the *M. tuberculosis* systems (Table 1). The *M. phlei,* in contrast, had only two antigens in common with *M. leprae,* one of which was

TABLE 3. *Presence of the precipitinogen β or a precipitinogen partially identical with β (pβ) in strains of* Corynebacterium, Mycobacterium, *and* Streptomyces[a].

Precipitation system		Presence of β
Lab no.	Species	
GB246	*Corynebacterium bovis* NCTC 3224	–
GB243	*Corynebacterium glutamicum* NCIB 10025	–
GB252	*Corynebacterium ulcerans* NCTC 7910	–
GA001	*Mycobacterium bovis* var. BCG Swedish substrain	β
GA023	*Mycobacterium fortuitum* G. Penso 456	β
–	*Mycobacterium leprae*	β
GA010	*Mycobacterium phlei* NCTC 8151	β
GA029	*Mycobacterium smegmatis* NCTC 8152	β
GB279	*Streptomyces albus* S. T. Williams 313	–
GB285	*Streptomyces diastaticus* S. T. Williams 496	pβ
GB282	*Streptomyces exfoliatus* S. T. Williams 060	pβ
GB283	*Streptomyces fulvissimus* S. T. Williams 593	pβ
GB290	*Streptomyces goshikiensis* S. T. Williams 190	pβ
GB278	*Streptomyces globisporus* S. T. Williams 199	pβ
GB277	*Streptomyces griseinus* S. T. Williams 047	pβ
GB276	*Streptomyces griseobrunnens* S. T. Williams 066	pβ
GB289	*Streptomyces lavendulae* S. T. Williams 069	–
GB281	*Streptomyces olivaceus* S. T. Williams 072	pβ
GB287	*Streptomyces platensis* S. T. Williams 041	–

[a] The results for *C. bovis, C. ulcerans,* and the five mycobacteria have been given earlier ([19, 20, 21]).

identified with this system. These results indicate that *M. leprae* is more closely related to *M. tuberculosis* than to the other mycobacterial species tested. Other factors than taxonomical linkage might, however, influence the results, and no solid conclusions concerning the taxonomical position of *M. leprae* within the genus *Mycobacterium* can therefore be drawn from the present data. The results published by Widebäck, *et al.* ([28]) also indicate that *M. leprae* is more closely related to *M. tuberculosis* than to other mycobacteria. Stanford and Rook ([25]) have suggested that *M. leprae* might be more closely related to *M. vaccae* than to other mycobacteria. Navalkar and colleagues ([16]), however, did not find any particularly close relationship between *M. vaccae* and *M. leprae,* and the present results agree with the latter findings. It is of interest to note that none of the nine patient sera tested reacted with the *M. vaccae* antigen preparation.

It has been known for many years that mycobacteria of various species crossreact with other actinomycetes and corynebacteria ([3, 4, 19, 22]). The present study demonstrates that *M. leprae,* too, is rich in intergenerically crossreacting antigens. One antigen shared by *M. leprae* and other organisms is β, which has earlier been shown in ribosomal material from mycobacteria and which also exists in rhodococci and nocardiae ([21]). The present study demonstrates that the β-precipitinogen also exists in strains of *Streptomyces.* Their β is, however, not entirely identical with the mycobacterial β (The Figure). No β was found in the three tested corynebacteria, which is in accordance with earlier studies ([19]).

The β-precipitinogen is also one of the antigens responsible for the reaction between leprosy sera and various mycobacteria, and pβ is responsible for the reaction between such sera and streptomycetes. One of the *Streptomyces* species (*S. albus*) reacted with five of the sera but no pβ was revealed in this species. Another one, *S. fulvissimus,* contained pβ but did not react with any of the leprosy sera (compare Tables 2 and 3). These results can be explained by the presence in the sera of antibodies against other streptomycetes antigens than β, or by the lack of correspondence between antigen and antibody concentrations.

The presence of antibodies in leprosy sera against antigens of various species of *Corynebacterium, Mycobacterium, Nocardia, Rhodococcus,* and *Streptomyces* is probably due to the fact that these organisms share

antigens with the *M. leprae* bacillus. It may also be that the patients have been infected, or have encountered some of these organisms, and therefore have antibodies against their antigens. Further studies, including more patient sera as well as control sera, are needed to elucidate these questions.

Stanford and others ([7, 23, 26]) have postulated that the immune response of tuberculosis and leprosy might be altered by prior exposure to environmental mycobacteria. The species in the present study are environmental organisms isolated from soil, animals, etc. Only very few of them, such as *M. tuberculosis, M. avium, M. kansasii,* and *N. asteroides* for example, are known to be pathogens to man. Most of them, such as the streptomycetes, do not cause disease in man, nor do they belong to the normal human flora. Humans encounter them nevertheless, since they are common in the environment. It may be asked whether contact with these bacteria, which crossreact with *M. leprae,* can influence the immune response of the patients making them more—or less—resistant to leprosy.

SUMMARY

Serological crossreactivity was analyzed between *M. leprae* and strains of various species of *Corynebacterium, Mycobacterium, Nocardia, Rhodococcus, Streptomyces,* and related organisms. *M. leprae* shares antigens with most of these organisms, and sera from patients with lepromatous leprosy contain antibodies against them. The results demonstrate that *M. leprae* shares more antigens with the mycobacteria than with strains of the other tested genera, thus supporting the view that the leprosy organism belongs to the genus *Mycobacterium.* One precipitinogen (designated pβ) was found to be common to *M. leprae* and the streptomycetes, and sera from patients with lepromatous leprosy contain antibodies against this antigen.

RESUMEN

Se analizó la reactividad serológica cruzada que existe entre el *M. leprae* y cepas de varias especies de los géneros *Corynebacterium, Mycobacterium, Nocardia, Rhodococcus, Streptomyces,* y otros organismos relacionados. Se encontró que el *M. leprae* comparte antígenos con la mayoría de estos organismos y que los sueros de los pacientes con lepra lepromatosa contienen anticuerpos contra ellos. Los resultados también demostraron que el *M. leprae* comparte más antígenos con las micobacterias que con cualquiera de los otros géneros probados demostrando así que el organismo de la lepra pertenece al género *Mycobacterium.* Además se encontró que un precipitógeno (designado pβ) estuvo presente tanto en *M. leprae* como en los estreptomicetos y que los sueros de los pacientes con lepra lepromatosa contuvieron anticuerpos contra ese antígeno.

RÉSUMÉ

La réactivité croisée du serum a été étudiée chez *M. leprae* et chez les souches de diverses espèces de *Corynebactérie,* de *Mycobactérie,* de *Nocardia,* de *Rhodococcus,* de *Streptomyces* et d'organismes apparentés. *M. leprae* partage des antigènes avec la plupart de ces organismes, et les échantillons de sérum provenant de malades atteints de lèpre lépromateuse contiennent des anticorps correspondants. Ces résultats montrent que *M. leprae* partage davantage l'antigène avec les mycobactéries qu'avec les souches des autres genres qui ont été étudiées. Ceci renforce l'opinion que le microorganisme de la lèpre appartient au genre *Mycobacterium.* On a observé qu'un précipitinogène (que l'on a désigné par le terme pβ) était commun à *M. leprae* et aux streptomycetes; les échantillons de sérum provenant de malades atteints de lèpre lépromateuse contenaient des anticorps contre cet antigène.

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