Precipitin and Skin Tests in the Diagnosis of Mycetoma Due to *Nocardia brasiliensis.* (28270)

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A precise demonstration of the presence of circulating antibodies in patients with mycetoma due either to *Nocardia brasiliensis* or *Nocardia asteroides* is lacking. Laboratory animals immunized with these microorganisms show antibodies in their blood, as revealed by agglutination, precipitation or complement fixation reactions(1-3); however, all of these lacked the requisite specificity.

The literature also contains contradictory reports regarding skin hypersensitivity studies carried out with these microorganisms(4-8). The materials* used to elicit responses have been likewise, crude and non-quantitative.

This paper is concerned with the immunological response of patients with mycetoma due to *N. brasiliensis,* using purified *Nocardia* polysaccharides as antigens for serology, and purified *Nocardia* proteins to evoke skin reactions.

Materials and methods. Serological tests were carried out using Ouchterlony's(10) double-diffusion precipitin reaction in agar. Sera from 14 patients with mycetoma due to *N. brasiliensis* were tested and compared with a number of sera from tuberculous patients, healthy individuals, as well as with sera from patients with other infectious and non-infectious diseases.

As antigens for the precipitin reactions we used 2 polysaccharides isolated from *N. brasiliensis* (Poly I Nb and Poly II Nb) and 2 others from *N. asteroides* (Poly I Na and Poly II Na). Poly I Na and Poly I Nb are apparently the same and are regarded as group specific. Arabinose and galactose are the monosaccharide constituents of Poly I Nb and Poly I Na and their molar ratios are similar. They cross-react when tested with rabbit antisera prepared against either *Nocardia* species. Furthermore, either polysaccharide I absorbed serum antibodies against the other.

Poly II Nb and Poly II Na are species specific; they react with rabbit antisera prepared against the homologous *Nocardia* species only. They contain mannose in addition to arabinose and galactose, with the molar ratios of the last 2 sugars variable according to species. A full account on the purification and the immunological characterization of these polysaccharides is being reported elsewhere(11).

Sera obtained from the patients had been preserved at 0°C after addition of merthiolate to a final concentration of 1:10,000. Usually, 0.1 ml of serum was deposited in the center well of the agar plates, and 0.1 ml containing 50 μg of each of the purified polysaccharide antigens was placed in the peripheral wells. The plates were incubated at 21°C for up to one week in a moist chamber. All precipitin bands that appeared were visible between 24-72 hours of incubation.

Skin tests. For these tests we used purified protein derivatives from *N. brasiliensis* (RS-117) and from *N. asteroides* (RS-81). For comparison, a purified preparation of tuberculin (RT-23) was used. All substances used to elicit skin responses, including the tuberculin, were kindly provided by Dr. Mogens Magnusson of the Tuberculosis Department, Statens Seruminstitut, Copenhagen, Denmark, who has reported the methods used for preparation of these substances(12,13). He also tested their specificity in guinea pigs sensitized with various strains of *N. brasiliensis* and *N. asteroides*(9).

Following a preliminary evaluation of the skin test materials, all patients received an intradermal dose of 0.2 μg in 0.1 ml. Two hundredths of a μg in 0.1 ml was used for the

* The term "sensitin" has been suggested(9) for a nonantigenic substance prepared from a microorganism, capable of revealing sensitivity of the delayed type which is evoked by the organism. Tuberculin, histoplasmin and coccidioidin are regarded as examples of "sensitin".

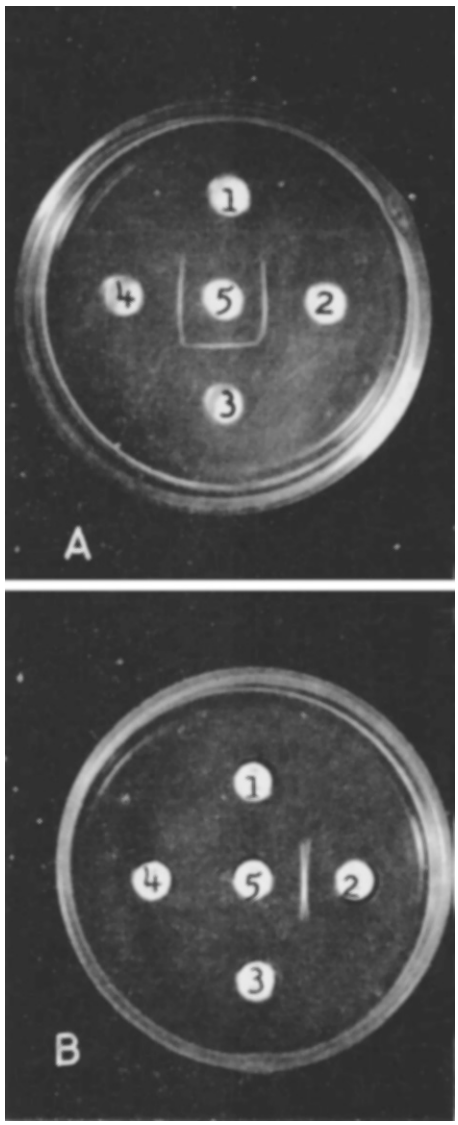

FIG. 1. Double diffusion precipitin reactions of antisera and polysaccharide antigens of *Nocardia brasiliensis*. The center wells (5) contain the antigens; in A, polysaccharide I and in B, polysaccharide II, both from *N. brasiliensis*. Peripheral wells of both plates contain antisera as follows: 1, serum from a healthy person; 2, serum from a patient with mycetoma caused by *N. brasiliensis*; 3, serum from a tuberculous patient; 4, serum from a patient with leprosy.

tuberculin tests. A skin reaction was considered positive if 6 mm or more of induration was present at the site of injection within 24 to 48 hours. Reactions of less than 6 mm were considered negative.

Results and discussion. All the sera from patients with mycetoma due to *N. brasiliensis* gave one band of precipitation per serum with either Poly I Nb or Poly I Na, and 2 bands with Poly II Nb (Fig. 1). This pattern of precipitation is the same as that previously observed with rabbit antisera(11).

That Poly I Nb and Poly I Na are similar, if not identical, is supported by previous observations with rabbit antisera and also by the findings presented here which indicate that both of the polysaccharides precipitated serum antibodies from patients with mycetoma due to *N. brasiliensis*. Furthermore, either Polysaccharide I absorbed serum antibodies against the other. The fact that a high percentage of tuberculous patients as well as leprosy patients showed circulating antibodies against Polysaccharide I (Table I) strongly suggests that these pathogenic mycobacteria may possess a polysaccharide antigen similar to the Polysaccharide I from *Nocardia*. These data alone could also be interpreted as indicating a closer relationship between *Nocardia* and the mycobacteria than between *Nocardia* and *Actinomyces* or *Streptomyces*.

Polysaccharides II by contrast, appear to be species specific. Poly II Nb precipitated serum antibodies only from patients with mycetoma by *N. brasiliensis* (Fig. 1). Although we were not able to obtain sera from humans with diseases caused by *N. asteroides*, sera from rabbits infected with *N. asteroides* precipitated Poly II Na but did not react with Poly II Nb(11).

Three of the patients with mycetoma due to *N. brasiliensis* who had been treated with a daily oral dose of 500 mg of Sulfadimethoxine (Madribon, Hoffmann-LaRoche) for periods of 6 months to one year, showed serum antibodies only against Polysaccharides I.

All patients with mycetoma due to *N. brasiliensis* reacted positively with the homologous protein preparation (RS-117) at doses of 0.2 μg/0.1 ml, showing an area of induration of 20 to 25 ml of diameter. In contrast, the comparable protein preparation from *N. asteroides* (RS-81) elicited positive reactions in only 2 of the cases (one case of mycetoma by *N. brasiliensis* and one of tuberculosis)

TABLE I. Precipitin Reactions and Skin Tests in Patients with Mycetoma and Other Diseases, Using Purified *Nocardia* Polysaccharides and Proteins.

No. of cases	Clinical and laboratory diagnosis	Precipitin reaction with *Nocardia* polysaccharides			Skin hypersensitivity tests with *Nocardia* proteins	
		I	II			
		N. brasiliensis and *N. asteroides*	*N. brasiliensis*	*N. asteroides*	*N. brasiliensis*	*N. asteroides*
14	Active mycetoma due to *N. brasiliensis**	+†	+‡	—	+	—§
3	Mycetoma by *N. brasiliensis* arrested by chemotherapy	+	—	—	+	—
1	Mycetoma due to *Streptomyces madurae*	—	—	—	—	—
1	Actinomycosis *(Actinomyces bovis)*	—	—	—	—	—
2	Lepromatous leprosy	+	—	—	non tested	non tested
58	Tuberculosis	+	—	—	—	—‖
13	Tuberculosis¶	—	—	—	—	—
63	Hepatic or renal diseases	—	—	—	—	—
51	Healthy adults	—	—	—	—	—

* In all cases of mycetoma *(N. brasiliensis* and *S. madurae)*, actinomycosis *(A. bovis)* and of tuberculosis *(M. tuberculosis)*, the microorganisms were cultured and further identified.

† All positive reactions with polysaccharides I *(N. brasiliensis* or *N. asteroides)* showed only one band of precipitation.

‡ All positive reactions with polysaccharides II from *N. brasiliensis* showed 2 bands of precipitation.

§ In one case, a 9 mm area of induration was noticed.

‖ In one case, an 8 mm area of induration was noticed.

¶ Most of the patients which gave negative reactions with Poly I had received long-term chemotherapy and surgery.

and these reactions were quite weak (8 to 9 mm of diameter).

None of the *Nocardia* protein preparations elicited positive skin reactions at the low doses used (0.2 μg/0.1 ml) in patients with either tuberculosis, actinomycosis, mycetoma due to *S. madurae,* or in healthy individuals. Preliminary tests showed, however, that almost every individual with a positive tuberculin reaction reacted positively with high doses of the *N. asteroides* protein preparation (2 μg/0.1 ml). It was of interest that even in high doses, the *N. brasiliensis* protein preparation elicited cross-reactions in only a few tuberculin-positive individuals.

It is concluded that both the precipitin reaction with Poly II Nb and the skin test with RS-117, are useful for diagnosis of mycetoma due to *N. brasiliensis*. It is possible that Poly II Na and the protein preparation RS-81 (from *N. asteroides*) may prove their value in diagnosis of diseases produced by *N. asteroides*.

Summary. By using a purified polysaccharide from *Nocardia brasiliensis* (Poly II Nb), precipitins were detected in sera of patients with mycetoma caused by this microorganism. Poly II Nb appears to be highly specific in the agar double-diffusion precipitin technique, since no cross-reactions were obtained with a number of sera from patients with other diseases, including tuberculosis, leprosy, actinomycosis, and mycetoma due to *Streptomyces madurae*. There are other polysaccharides in *N. brasiliensis* and *N. asteroides* (Poly I Nb and Poly I Na, respectively), which are very similar in composition, which cross-react and have lesser diagnostic specificity. Besides precipitating serum antibodies from patients with mycetoma due to *N. brasiliensis*, they also precipitate with sera from tuberculous and leprous patients, but not with sera from patients with certain other diseases or from healthy individuals. Purified proteins from *N. brasiliensis* (preparation RS-117) were used for skin

tests. Positive reactions were obtained in all patients with mycetoma due to *N. brasiliensis* but not in patients with other diseases or in healthy individuals. It is concluded that these tests may be of value in diagnosis of mycetoma due to *N. brasiliensis*.

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Quality and Yield of Vaccinia Virus from L Cell Cultures.* (28271)

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Technical difficulties have delayed progress in the measurement of the quality of viruses other than those of the influenza group for which the I/HA ratio has been so successfully applied, but direct particle counting has now advanced to fill this need. The quality of the vaccinia virus reported here will be expressed as the ratio of plaque forming units (pfu) to the number of particles as seen and counted in the electron microscope. Certain changes in virus quality have been observed, particularly in the early hours of rapid new virus production in infected L cells. These may be related to the von Magnus effect(1) with influenza viruses but there are essential differences.

Materials and methods. The WR (mouse neurotropic) strain of vaccinia virus, used in this work, has been kept in continuous passage in screw-capped bottle cultures of L cells. The last 280 passages have been made with 48- to 72-hour virus at multiplicity M = 20. Earle's L cells, in screw-capped tube cultures, were used for experiments on virus growth. These were incubated slantwise at 37°C until time for titration and virus particle count. Monolayers of L cells were used for plaque titration. Growth medium and complete details of the growth experiment have been published(2).

We wanted the increase in virus to be an index of particle production in all cells rather than of the fraction of cells producing. One requirement for this is synchrony in inoculation. This was provided by the sedimentation inoculation procedure(3) which accomplishes this purpose in less than 7 minutes. This short time has been shown to be adequate(4). The multiplicity of inoculation was 10 in all growth experiments. This number seems to have been adequate to infect all the cells.

Plaque titrations were made at the times when the samples were ready. None were frozen or otherwise stored to await titration. Samples were thoroughly dispersed with 9 kc sonic waves just prior to titration. Aggregation analyses, made on the electron micrographs used for particle counting, have shown the suspensions to contain over 80% active units(5).

Counting of virus particles was done by the agar sedimentation method of Sharp(6). Re-

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