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Isolation of a Group-specific Polysaccharide from Tissues Infected with *Mycobacterium leprae*[1]

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Investigation of the immunologically active substance in *Mycobacterium leprae* has been hampered because of the unavailability of pure cultures. However, this difficulty can now be circumvented in part by taking advantage of the identification of the group-specific antigen, Polysaccharide I (PolyINb), present in *Nocardia* and *Mycobacteria* ([1, 3, 5, 8]). This has been chemically characterized as a polymer of D-arabofuranose and D-galactopyranose in a molar ratio 3:1 ([4]).

The present paper deals with the identification of the group-specific polysaccharide (PolyINb) in extracts of human leproma, after physical removal and enzymatic destruction of all other components that might have been immunologically active.

MATERIALS AND METHODS

Isolation of polysaccharides. A fresh leproma weighing 3.6 gm. was incised for preparation of a bacterial smear, and after a high content of *M. leprae* had been confirmed, was cut into pieces and washed with sterile saline solution. The washed tissue was homogenized in a Virtis homogenizer at 0°C, suspended in saline, and adjusted to pH 2 with *M* HCl. Pepsin (Difco, 1:10,000) was added to give a final concentration of 2.5 per cent, and the mixture was incubated at 37°C for three days, the pH of 2 being maintained with *M* HCl. Toluene was added to prevent bacterial growth.

The pepsin-digested suspension was neutralized with *M* NaOH and centrifuged at 0°C at 3,000 r.p.m. for one hour; the supernatant was collected (8 ml. of crude extract).

One-half of the crude extract was treated five times with an equal volume of chloroform-butanol 9:1 v/v ([7]) to precipitate and thus remove the proteins. B-ribonuclease (2 mgm.) and deoxyribonuclease I (2 mgm.), dissolved in 0.1 *M* Tris buffer at pH 7.2 containing 0.02 *M* $Mg(Cl)_2$ were then added under toluene. After this treatment the material was dialyzed five times against one liter of distilled water at 4°C. The deproteinization procedure was then repeated as many times as necessary to remove all protein. The resultant solution was concentrated under reduced pressure in a rotatory evaporator (purified fraction).

Determination of sugars. Sugars were estimated by the Molisch and the phenol-sulfuric acid method ([2]).

Immunologic procedures. Agar precipitation was carried out in 1 per cent agar (ion agar, Oxoid) containing 0.85 per cent NaCl and merthiolate (final concentration 100 μg./ml.) at pH 5.8-6.2 The plates were incubated for 24-72 hours either at room temperature or at 4°C ([6]).

Precipitation in capillary tubes was performed by mixing equal volumes of serum and antigen and incubating as before.

RESULTS

In an agar Ouchterlony plate the crude extract gave five precipitation bands with the serum of a lepromatous patient, one

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FIG. 1. **1.** Polysaccharides from leproma purified extract. **2.** Serum from a patient with active tuberculosis (anti-tb serum). **3.** Polysaccharide I, isolated from *Nocardia brasiliensis* (PolyINb). **4.** Serum from patient with lepromatous leprosy (one extra band). **5.** As in No. 2. **6.** Serum from patient with lepromatous leprosy (two extra bands).

of which gave a reaction of identity with an extract of *Mycobacterium tuberculosis* obtained by mechanical rupture of the bacterial cells.

The purified fraction gave positive Molisch and phenol-sulfuric acid reactions. The total amount of polysaccharide obtained, as estimated by the phenol-sulfuric acid method, was 1.2 mgm., a mixture of D-arabinose and D-galactose in a molar ratio 3:1 being used as a standard. After concentration in the evaporator, the final concentration of polysaccharide (in 0.15 *M* saline) was 400 μg./ml. This purified fraction did not precipitate in capillary tubes with the sera of six healthy persons, but precipitated with the serum from a patient with active tuberculosis (anti-tb serum) and with two sera from patients with lepromatous leprosy; the latter sera also contained precipitins against the group antigen PolyINb.

A test of identity between PolyINb and the purified fraction was made in an agar plate as follows: 4 wells were arranged in a rhomboid; in two opposite wells, PolyINb from *Nocardia brasiliensis* and the purified fraction were placed, and in the other two wells the anti-tb serum and one lepromatous serum. The PolyINb and the purified fraction gave a band of identity with both sera (Fig. 1). On the same plate (in order to save antigen) one well containing anti-tb serum and one containing another lepromatous serum were arranged near the one with the purified fraction, and a band of identity was obtained. However, the purified fraction gave two extra bands with one lepromatous serum (No. 6) and one only with the other (No. 4), which were not identical with that seen with PolyINb (Fig. 1).

The anti-tb serum absorbed with PolyINb no longer precipitated with the purified fraction, and two sera from two rabbits immunized against *N. brasiliensis* gave only one precipitation band with the purified fraction. No precipitation was found with three other sera from lepromatous patients or with one from a tuberculoid patient. Eighteen sera from healthy persons did not precipitate at all.

DISCUSSION

From the experiments described above it is clear that after physical and enzymatic treatment of leproma tissue, to remove proteins and nucleic acids, antigenic material of polysaccharide nature remained. The scarcity of the active substance precluded determination of its constitutent sugars, but their inability to dialyze was a proof of their high molecular weights.

The existence of identity bands between PolyINb and the purified fraction, and the absorption of this precipitin with PolyINb obtained from *N. brasiliensis* cells, indicated the presence of an immunologically identical group antigen in *M. leprae*. On the other hand, the fact that some sera from patients with lepromatous leprosy gave extra bands with the purified fraction, and that this was not given by anti-tb serum or sera from the rabbits immunized with *N. brasiliensis*, indicated that leproma tissue might contain other species-specific antigens of polysaccharide nature as well.

The fact that the crude extract gave five precipitation bands in agar plates, indicated that other antigens were present in extracts of leproma tissue.

SUMMARY

A protein-free leproma extract containing polysaccharides gave a precipitation band in agar, immunologically identical with that produced by the group-specific antigen Polysaccharide I (PolyINb), isolated from *Nocardia brasiliensis*. Precipitation was given by sera from rabbits immunized against *N. brasiliensis*, with serum from a patient with active tuberculosis and with two sera from lepromatous patients. Each of the latter, however, gave an extra precipitin band, suggesting the presence of another species-specific polysaccharide antigen. Sera from eighteen healthy donors failed to precipitate the lepromatous extract. A crude extract obtained from leproma tissue gave five precipitation bands with the serum of a lepromatous patient.

RESUMEN

Un extracto de leproma libre de proteínas y conteniendo polisacáridos produjo en agar una banda de precipitación idéntica con la producida por el antígeno específico de grupo PolyINb, aislado de cultivos de *Nocardia brasiliensis*. La misma banda de precipitación se obtuvo con sueros de conejos inmunizados con *N. brasiliensis*, de un paciente con tuberculosis activa y de dos leprosos lepromatosos. Los sueros de los enfermos con lepra, sin embargo, dieron lugar a otra banda, apuntando la presencia de otro antígeno polisacárido posiblemente especie específico. El extracto crudo de leproma produjo cinco bandas de precipitación con un suero de paciente lepromatoso, y 18 muestras de personas sanas no dieron ninguna precipitación.

RÉSUMÉ

Un extrait de lépromes libre de protéines et contenant des polysaccharides donne une bande de précipitation sur agar, qui est immunologiquement identique à celle produite par l'antigène Polysaccharide I (Poly INB) doté d'une spécificité de groupe, qui est isolé de *Nocardia brasiliensis*. La précipitation a été obtenue avec des sérums de lapins immunisés contre *N. brasiliensis*, avec le sérum d'un malade atteint de tuberculose active, et avec deux sérums de malades lépromateux. Chacun de ces derniers sérums donnaient toutefois une bande complémentaire de précipitines, ce qui suggère la présence d'un autre antigène polysaccharidique doté d'une spécificité d'espèce. On n'a pas réussi à observer de précipitation de l'extrait lépromateux avec les sérums obtenus chez dix-huit donneurs en bonne santé. Un extrait brut préparé à partir de tissue lépromateux a donné cinq bandes de précipitation avec le sérum d'un malade lèpromateux.

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