

ATTEMPTS TO OBTAIN AN ANTIGEN (LPT) SUITABLE FOR
STUDY OF HYPERSENSITIVITY IN LEPROSY¹

NORBERTO OLMOS CASTRO, M.D.
AND PASCUAL B. ARCURI, M.D.
Leprosy Control Section (Provincial)
and Leprosy Control Dispensary (National)
San Miguel de Tucumán, Argentina

It is generally accepted in leprology that the intradermal injection of lepromin, either integral or bacillary, is capable of revealing the state of hypersensitivity to the proteins of *Mycobacterium leprae* and also of demonstrating the capacity of the individual to resist infection by the leprosy bacillus.

To Fernandez goes the credit of having called attention to the reaction of hypersensitivity, the early one, now known as the Fernandez reaction. We believe that this is the most valuable contribution to the knowledge of immunology of leprosy since Mitsuda discovered the late reaction, the one of resistance, which bears his name.

Although sixteen years have passed since Fernandez first published on the early reaction, strangely few of the investigations made in the field of immunology of leprosy—except those of Dharmendra with extracted bacilli—have been concerned with the condition of hypersensitivity. The literature shows that leprologists usually refer to the Mitsuda reaction in interpreting immunological phenomena, and when they do refer to the early reaction they do so superficially, giving little more than statistical percentages without undertaking to analyze its biological significance. Thus the great majority of the investigations on immunity in leprosy concern only one phase of the problem, that of the capacity of resistance to *M. leprae*.

Although it is true that there is not sufficient evidence to prove that hypersensitivity in leprosy is a form of immunity, and also that the interdependent relationships between resistance and hypersensitivity are not thoroughly established, yet we believe that the study of the latter phenomenon is as important as the study of the former, if not more important, for the interpretation of the numerous phenomena relative to immunity which as yet are not understood.

Furthermore, the existing confusion in terminology confounds the interpretation of results. We speak of allergic or anergic leprosy patients, of positive or negative immunology, based solely on the Mitsuda reaction, which expresses only a part of the immunological phenomena. Sometimes the reaction of resistance is given the character of the reaction of hypersensitivity. Thus, for example, there have been carried out comparative studies of the state of hypersensitivity and of the state of resistance, and conclusions have been drawn which refer to the two phenomena without distinction.

¹ Communication presented at the second annual meeting of the Sociedad Argentina de Leprología, November 18, 1956.

We believe that this confusion in terminology, and the inadequate specification of the phenomenon under study, is the primary reason why the study of hypersensitivity has not been given the place which its importance deserves.

Another factor which perhaps has had an influence in the lack of interest in the study of this valuable aspect of immunity is that there is not available a suitable routine antigen for detecting hypersensitivity. It is true that either integral or bacillary lepromin is capable of revealing that condition, but both have many defects in this respect. In the first place, lepromin is a sensitizing antigen, an allergen, and for that reason it cannot be used more than once in nonhypersensitive individuals. In hypersensitized subjects it provokes strong late reactions, usually of long duration, and its repetition in the same person may have disagreeable effects. It is also to be considered that we do not know what immunological changes may be provoked in such persons.

On our part we found it impossible to undertake studies of many interesting aspects of hypersensitivity in leprosy because of the lack of a suitable antigen. The known preparations of soluble leprosy-bacillus proteins are not suitable for routine work. The filtrates of lepromin, used first by Hayashi and later Fernandez and Olmos Castro, are only useful for research work, not at all for daily clinical use. The ideal antigen would be the purified protein of Dharmendra, but the present difficulties of providing enough raw material for its preparation make its routine use impossible. Furthermore, we have found that its antigenic activity decreases considerably with age, so that it becomes practically inactive within 60 days after its preparation.

For these reasons we have been induced to make attempts to obtain a type of antigen suitable for the investigation of hypersensitivity. This type of antigen must possess at least the following qualities: (a) a high degree of specificity and sensitivity; (b) lack of sensitizing effect; (c) capability of standardization; (d) prolonged antigenic activity after preparation; (e) ease of preparation; (f) a yield at least equal to that of the lepromins actually in use.

It would seem to have been proved that the antigenic activities of integral and bacillary lepromins for demonstrating hypersensitivity are similar, and that they depend exclusively upon a soluble antigenic substance derived from the leprosy bacilli themselves. This fact led us to attempt the preparation of a protein antigen directly from lepromas, a method which simplifies the technique and provides a greater yield.

TECHNIQUE

1. Heat the fresh lepromas in a boiling waterbath for 30 minutes.
2. Remove the epidermis if present, trim away all extraneous tissue, and desiccate the cleaned nodules in an oven at 56°C.
3. Weigh the desiccated nodules and grind them to a fine powder in a mortar.

4. Cover the powder with chloroform and continue the grinding, adding a fresh lot of chloroform from time to time whenever the last one has evaporated. Check microscopically for the presence of acid-fast bacteria, and continue the grinding in chloroform until no more bacilli are found.
5. Suspend the dry leproma powder, after the final chloroform aliquot has evaporated, in ether and centrifuge for 15 minutes at 4,000 r.p.m.
6. Wash the sediment several times with ether.
7. Return the sediment to the mortar and suspend it in distilled water in a proportion of 4 parts by weight of the original dried nodule per 1,000 cc.
8. Centrifuge at 4,000 r.p.m. for one hour, and distribute the supernatant fluid in 1 cc. lots into small bottles, such as penicillin (etc.) vials.
9. Desiccate in the oven at 56°C.
10. Sterilize in dry oven at 120°C. for 30 minutes, after closing the vials.

Comments.—The addition of chloroform during the grinding of the tissue powder greatly accelerates the destruction of the bacilli and thus provides the maximum amount of the antigenic bacillary substance.

How much chloroform will be used and how much grinding will be needed cannot be predicted in any given instance. The treatment is to be continued until the bacilli are destroyed, and that varies with different lots.

The addition of ether to the powder and centrifuging serves a double purpose: it eliminates the lipids, which have no antigenic effect in the hypersensitivity test, and it provides lipids for the preparation of the antigen for the Olmos Castro-Bonatti serological test.

The product is dried to preserve the antigenic potency of the protein.

Name.—We call this product *leprolina proteica total* (LPT) to distinguish it from lepromins of the usual types.²

Technique of use.—The antigen thus prepared appears as a thin, opalescent pellicle at the bottom of the vials. For administration this deposit is dissolved in 1 cc. of physiological saline. It is given in 0.1 cc. doses, hence one vial is good for 10 doses. The results are read according to the Madrid congress recommendations for the early reaction.

Concentration and yield of LPT.—The concentration of the LPT depends on the water-soluble fraction of the product of grinding, in the proportion of 4 gm. of the original dried leproma in 1,000 cc. of distilled water. Biological titration is being attempted, using dogs sensitized by integral lepromin. Up to the time of writing we have prepared 5 lots of LPT, and comparisons have shown them to be very similar in antigenic potency.

The yield is 50 cc. per 200 mgm. of desiccated leproma, which quantity is appropriately equivalent to 1 gm. of the wet, boiled leproma. This is more

² The term "leprolin" is applied in analogy with tuberculin, because of fundamental similarities of the two substances. It is unfortunate, perhaps, but not necessarily confusing, that in the past "leprolin" was applied to other products—first to suspensions of acid-fast bacilli other than *M. leprae*, and then briefly to the Mitsuda-Hayashi "vaccine" itself until the term "lepromin" introduced by Bargehr was adopted.

than double the yield of the classical Mitsuda-Hayashi integral lepromin. Whereas 1 gm. of fresh-boiled nodule gives 20 cc. of ordinary lepromin, or 200 doses, with LPT 1 gm. of boiled leproma, equivalent to about 200 mgm. of desiccated leproma, yields 50 cc. of the antigen which is good for 500 doses.

Tests for sensitization by LPT.—Four noncontact persons, supposedly free from leprosy, were given intradermal injections of 0.1 cc. of LPT. The results after 48 hours were negative. Repeated injections of the same dose after 28 days also gave negative results.

Considering the extraordinary capacity of the dog to develop hypersensitivity to integral lepromin, we tested their reactivity to repeated injections of LPT. Four dogs were injected intradermally each with 0.1 cc., with negative results. Retesting 26 days later again gave negative results. Subsequently 12 similar injections were given in the same way and at the same intervals, with consistently negative results.

These results in the dog, especially, have led us to conclude that LPT is without sensitizing capability. The results in human beings were similar, but that investigation would have to be extended to obtain results of statistical significance.

Clinical results.—The appearance of the 48-hour reaction induced by LPT in hypersensitive persons is similar to that observed after injections of lepromin filtrates or Dharmendra's purified protein. This is an infiltrated erythema, the infiltration varying in intensity with the degree of hypersensitivity. In highly hypersensitive persons the infiltration is very intense, with a pale center, and there may be slight vesiculation.

Among leprosy patients tested, the 19 lepromatous cases all gave negative reactions; whereas 14 of 17 tuberculoid cases (82%) were positive, most of them strongly so.

Of 81 supposedly healthy noncontacts, only 4 (5%) were positive. On the other hand, of 91 noncontacts who previously had been given two or more injections of integral lepromin, 71 (78%) were positive. The late readings, at 21 days, were negative in all cases.

Among a group of 56 persons supposedly free from leprosy, previously hypersensitized with integral lepromin, 26 persons showed on injection of the LPT reactivation of the lepromin nodules and the formation of an erythematous halo around them.

The results of the limited number of tests made so far are given in Table 1.

CONCLUSIONS

From what has been observed so far with the 5 different lots of "*leprolina proteica total*" (LPT) that have been prepared from lepromas obtained from different patients, the following conclusions may be drawn.

1. It seems to be feasible to prepare a new antigen of the soluble protein type suitable for the investigation of hypersensitivity in leprosy.

2. This LPT antigen at present under study possesses the following qualities:

(a) It seems not to be allergenic. It does not create hypersensitivity in the dog, and apparently it does not do so in supposedly healthy persons, although more people must be tested in order to obtain data of statistical significance.

(b) It seems to be specific. It causes no reactions in cases of lepromatous leprosy. It usually gives positive results in cases of tuberculoid leprosy,

TABLE 1.—*Results of the Fernandez test with the LPT antigen in leprosy patients and supposedly healthy noncontacts.*

Group	No. of cases	Positive	Degree of positivity		
			1+	2+	3+
Lepromatous	19	0 (0%)	0	0	0
Tuberculoid	17	14 (82%)	1	3	10
Healthy noncontacts	81	4 (5%)	2	1	1
Healthy noncontacts sensitized with lepromin ^a	91	71 (78%)	16	26	29

^a Mitsuda-Hayashi integral lepromin.

and in supposedly healthy persons who have previously been sensitized with integral lepromin. It is usually negative in noncontacts, but the number of persons tested must be increased to permit definite conclusions in this matter.

(c) Production on the basis of the weight of desiccated lepromas has given several lots of very similar antigenic activity. Biological titration is being attempted.

(d) It gives maximum yield, approximately 500 doses per gram of boiled lepromas.

(e) It is easy to prepare, not requiring special facilities or equipment.

3. The degrees of specificity and sensitivity have not yet been determined, the testing of large numbers of patients and healthy persons being needed. Nor has the duration of its activity been determined.

CONCLUSIONES

De lo llevamos observado a la fecha, con 5 tandas diferentes de L.P.T. preparadas con lepromas provenientes de diversos enfermos constatamos:

1. Que parece ser factible, la preparación de una nueva lepromina de tipo proteica soluble, apropiada para la investigación de la hipersensibilidad en lepra.

2. La L.P.T. actualmente en estudio, reúne las siguientes cualidades:

a). No crea hipersensibilidad en el perro, y parece que tampoco en personas supuestas sanas de lepra, aunque es necesario proseguir investigando en un número mayor de personas a fin de obtener datos de significación estadística.

b). Parece ser específica. Es negativa en lepra lepromatosa. Es frecuentemente positiva en lepra tuberculoide y personas supuestas sanas sensibilizadas previamente con lepromina integral. Es poco frecuente su positividad en personas supuestas sanas de lepra no convivientes. Será necesario ampliar el número de personas testificadas para deducir conclusiones definitivas.

c). La titulación a partir del peso de lepromas desecados provee diversas tandas de actividad antigénica muy semejante. Se están realizando ensayos para su titulación biológica.

d). Su rendimiento es óptimo. Aproximadamente con 1 gr de lepromas hervidos pueden prepararse 500 dosis.

e). Es de fácil preparación, no necesitándose laboratorio ni instrumental especializado.

3. El grado de especificidad y sensibilidad, aun no está determinado, necesitándose la testificación de grán número de personas sanas y enfermas. Tampoco aun ha sido determinado el tiempo que dura su actividad.