SENSITIZATION TO LEPROMIN IN PRESUMABLY NON-LEPROUS INDIVIDUALS*

By

DR. JOSÉ M. M. FERNANDEZ

Professor of Dermatology
on the Faculty of Medicine of Rosario, Argentina

I. INTRADERMAL REACTION TO LEPROMIN

The intradermal injection of "integral lepromin" or "bacillary lepromin"** provokes, in positive cases, a double response:

a) An early reaction within 24-48 hours; we have been the first to call attention to the frequency and significance of this reaction. A reaction with erythema and infiltration (edema) is positive.

---

* From the "Professor E. P. Fidenza" Leprosy Service: Chiefs, Professor Salomón Schujman and Professor José M. M. Fernandez. Communication presented at the Dermatological Society of Rosario, Argentina.

Translated from the Spanish by Dr. J. A. Doull and Dr. R. Mercau.

** With Olmos Castro (1) we have proposed the following nomenclature concerning lepromin:

INTEGRAL LEPROMIN (Lepromina Integral) (L. I.) is the antigen obtained by Mitsuda-Hayashi's procedure (2), or similar procedures, which contains all the elements of the "leproma": bacilli, cells, tissue detritus, etc.

BACILLARY LEPROMIN (Lepromina Bacilar) (L. B.) is the antigen prepared from a pure suspension of Hansen's bacilli as it is obtained by the method proposed by us (3) or by Dharmendra (4).

PURIFIED LEPROMIN PROTEIN (Lepromina Proteica Purificada) (L. P. P.) is a group of antigens formed from active water soluble substances of Hansen's bacilli, obtained either by filtration as we have made it or by chemical extraction as employed by Paras (5), by Rabello Y Vilella (6), and recently by Dharmendra (4).

b) A late reaction (Reaction of Mitsuda), which starts after the first week and reaches maximal intensity between the third and fourth week. This reaction is represented by a "papule" or a "nodule" which may ulcerate.

If instead of integral lepromin or bacillary lepromin we use a filtrate of this antigen (2), or a protein derivative, obtained by the procedure of Dharmendra, only the early reaction is produced. This has been confirmed by Lowe and Dharmendra (12). In agreement with Olmos Castro (1), we have maintained that the early reaction expresses a state of allergy of the entire organism, attributable to a previous sensitization produced by Hansen's bacilli and occasionally by Koch's bacilli. The late reaction, in the opinion of Wade (8) "is not a test of the existence of allergic hypersensitiveness, but rather one of capability of developing an allergic state after the introduction of the antigen."

According to this interpretation, it is possible to explain why in non-leprous persons the early reaction is negative in most of the cases (lacking previous contact or sensitization with the *Mycobacterium leprae),* whereas, by contrast, the late reaction is positive in more than 70 per cent of the cases. Although these individuals are not sensitized, they have the capacity to react in allergic fashion (potentially allergic) when the bacilli are introduced into their bodies. In the publication previously mentioned (1) we suggested also, as a result of our experience, the possibility of sensitizing by injections of lepromin non-leprous individuals whose early reactions to lepromin were negative.

In the present report, we shall attempt to show that this sensitization is possible and to describe the factors that condition it.

II. Materials and Methods

Our experiments have been carried out on a group of presumably non-leprous adults, most of whom were female patients in the "Hospital de Alienados." To investigate allergy to lepromin we employed a purified protein antigen derived from *Myco. leprae* (Dharmendra's method), and, as sensitizing agents: "lepromin integral" (Mitsuda- Hayashi); a suspension in mineral oil, of Hansen's bacilli killed by heat; a suspension, in mineral oil, of Koch's bacilli killed by heat; and as a control a suspension, in mineral oil, of Eberth's bacilli killed by heat. The technic of preparation will be explained later.

In all cases, intradermal injections of 0.1 cc. to 0.2 cc. were made. The reactions were read 48 hours and 4 weeks after injection of the antigen. In reading the results, the following standard criteria were adopted:

a) Early reaction was considered positive when presenting at 48 hours an infiltrated erythematous halo, not less than 10 mm. in diameter. b) Late reaction was considered positive when presenting at 3 or 4 weeks a nodule or papule not less than 5 mm. in diameter.

Method of preparation of the antigens employed.

1) Purified lepromin protein (L.P.P.). The method of Dharmendra was adopted (4) : "The pieces of lepromatous material, usually nodules cut from ears, are ground up with chloroform in a glass pestle and mortar. The chloroform is pipetted off. The grinding in chloroform is repeated till a smear from the remaining tissue is almost free from bacilli. (About 50 cc. of chloroform are necessary to extract almost all the bacilli from 2 gm. of lepromatous tissue). All the lots of chloroform used in grinding are pooled, and the remaining tissue is discarded. A smear from the pooled lot of chloroform shows bacilli in very large numbers and the absence of any tissue.

"The chloroform is then completely evaporated over a water-bath; the residual substance consists of lipoids and bacilli. This residue is then suspended in ether and the ethereal suspension is centrifugalized, at a low temperature at 3,000 r.p.m. The lipoids remain in the supernatant ether and the bacilli are deposited at the bottom. The ethereal extract is pipetted off. To remove the lipoids more completely, the bacillary deposit is again suspended in ether, the suspension centrifugalized, and the deposited bacilli separated and dried. The deposit forms a dry powder and smears made from it show only bacilli and no tissue." A suspension in carbol-saline of bacillary powder was " made by putting the substance in a mortar, adding a few drops of N/10 NaOH, grinding with a pestle, adding carbol-saline and completing the suspension by grinding." The suspension was made of a concentration of 1 mgm. of the substance to 1 cc. of saline.

"When the ground bacilli were suspended in saline, it was found that, while a portion of the material would easily go into solution, the remaining portion could not be dissolved even with the help of N/10 NaOH. The soluble and the insoluble portions were separated from one another by allowing the suspension to stand, and then pipetting off the supernatant fluid." The saline extract was antigenically active and was "shown to contain protein; the next step was to isolate the protein and test the antigenic activity of the isolated protein. This was done by precipitating the protein by trichlor-acetic acid. An equal volume of 20 per cent trichlor-acetic acid was added to the saline extract, and the mixture allowed to stand overnight. Next morning the precipitate was separated from the supernatant fluid. The precipitate was then washed in ether to remove traces of the trichlor-acetic acid, and was

dissolved in carbol-saline with the help of a few drops of N/10 NaOH. The strength of the solution was such that 0.1 cc. contained 0.0133 mgm. of the precipitated protein."

2) Lepromin integral (L.I.). Mitsuda-Hayashi's method (2) was adopted. "Fresh nodules are boiled in physiological salt solution for from 30 to 60 minutes, and ground in a mortar. To 1 gram of the ground nodular material are added 20 cc. of the salt solution used in the boiling, fresh salt solution being added if necessary, to make up the required volume. The whole is filtered through gauze and the filtrate heated at 60° C. for one hour. Carbolic acid is added to make a 0.5 per cent concentration."

3) Suspension, in mineral oil, of Hansen's bacilli killed by heat (antigen S.H.V.). From the lepromatous nodules pure bacilli were obtained by the method of chloroform extraction as employed by Dharmendra, which was described previously. The material (pure bacilli) was finely pulverized in a mortar.

By gradual addition of small quantities of mineral oil grinding was continued until a homogenous suspension was obtained. The concentration of this suspension is 20 milligrams of powdered bacilli in 100 cc. of mineral oil (1:5000). The material was fractionated into ampules and sterilized in autoclaves 30 minutes at 120° C.

4) Suspension, in mineral oil, of Koch's bacilli killed by heat (antigen S.K.V.). Koch's bacilli, human type, cultivated eleven days in potato medium with glycerin were heated for one hour at 95° C. With this material a suspension in mineral oil was prepared as described for S.H.V. above.

5) Suspension, in mineral oil, of Eberth's bacilli killed by heat (antigen S.E.V.). A 24-hour culture of Eberth's bacilli, in agar medium, was washed off with sterile normal saline solution, heated at 60°C., centrifuged, washed and dried. A suspension was then made in mineral oil, after pulverizing the bacilli in a mortar. The material was sterilized in an autoclave at 120°C., for 30 minutes. The concentration was 5:1000.

III. Results Obtained

1) The early reaction to lepromin in non-leprous individuals.

In making these experiments, we had the opportunity again of studying the early reaction among a group of persons presumably non-leprous. The subjects were 87 women and 13 men. The results of testing with L.P.P. were as follows:

Positive reaction (48 hours), 30 cases, 30 per cent.

Negative reaction (48 hours), 70 cases, 70 per cent.

The proportion of positives is unusually high but in spite of this fact we conclude that the early reaction is more often negative in non-leprous persons than is the late (Mitsuda's) reaction, made with integral lepromin or bacillary lepromin. It is possible that in most instances the early positive reaction is attributable to a co-sensitization to tuberculin as Schujman (9) thinks, although it may be that some of the subjects had had contact with leprosy.

2) Sensitizing action of integral lepromin:

Using as subjects 25 psychopathic females from 20 to 69 years of age, and supposedly free from leprosy, intradermal injections of 0.1 cc. each of integral lepromin and of L.P.P. were made in the right scapular region.

At the end of 48 hours, 9 of these individuals showed a positive reaction to both antigens; 3 were positive to L.P.P. and negative to integral lepromin. The remainder were negative to both.

At the end of the fourth week all the 25 subjects presented a positive late reaction to the integral lepromin, the intensity varying from a papule at least 5 mm. in diameter to a nodule, at times ulcerated. At this time the intradermal injection of 0.1 cc. of L.P.P. was repeated on the 13 subjects who formerly presented a negative early reaction to this antigen. It was found that the second reaction was frankly positive at 48 hours in 12 subjects and negative in one. Twelve days later the intradermal injection with L.P.P. was repeated again on the negative subject and the reaction was positive.

In other words, among 13 persons supposedly non-leprous, anergic to L.P.P., given an intradermal injection of integral lepromin, 12 presented after 4 weeks, and one after 6 weeks, a frank positive reaction to L.P.P.

3) Sensitizing action of a suspension, in mineral oil, of Hansen's bacilli killed by heat (antigen S.H.V.).

Using as subjects a second group of 25 psychopathic females, from 23 to 68 years of age, supposedly free from leprosy, intradermal injections of 0.1 cc. each of S.H.V. and of L.P.P. were made in the right scapular region.

At the end of 48 hours all subjects gave a positive reaction to the S.H.V. antigen; this reaction consisted of a red plaque or nodule more than 20 mm. in diameter, surrounded by an erythematous halo. In 4 subjects the L.P.P. antigen gave a positive result; in 21 the result was negative.

At the end of the fourth week, 22 subjects presented an infiltrated plaque or nodule at the site of injection of the S.H.V. antigen; 3 were not observed.

At this time, intradermal injection of 0.1 cc. of L.P.P. was repeated on each of those subjects whose early reaction to this antigen had been negative. Thirteen now gave a frankly positive reaction and 5 were still negative. Twelve days later a third injection of L.P.P. was given to 3 of these 5, with one positive result.

Thus among 18 supposedly non-leprous persons, anergic to the L.P.P. antigen and given an intradermal injection of S.H.V. antigen, 15 presented a positive early reaction to L.P.P. five weeks later and one six weeks later; two were persistently negative.

4) Sensitizing action of suspension, in mineral oil, of Koch's bacilli killed by heat (antigen S.K.V.).

Using as subjects a third group of 12 psychopathic females, of ages from 20 to 61 years and all supposedly free from leprosy, intradermal injections of 0.1 cc. each of the S.K.V. antigen and of L.P.P. were made in the right scapular region.

At the end of 48 hours, all subjects showed a positive reaction to S.K.V., in some instances with vesiculation and central necrosis. In 5 subjects the reaction to the L.P.P. antigen was positive, and in 7 it was negative.

At the end of the fourth week, 11 subjects showed a frankly positive late reaction to the S.K.V. antigen, consisting of a papule, more than 5 mm. in diameter, and in some instances an ulcerating nodule; one subject was not observed. At this time, the intradermal injection with 0.1 cc. of L.P.P. was repeated in 6 subjects formerly negative for early reaction; we obtained 4 positive and 2 negatives. A third test was made on these negatives ten days later but both remained negative.

Thus, in 6 persons presumably non-leprous, anergic to the L.P.P. antigen and given an intradermal injection of the S.K.V. antigen, 4 showed a positive early reaction to L.P.P. four weeks later.

5) Sensitizing action of a suspension, in mineral oil, of Eberth's bacilli killed by heat (antigen S.E.V.).

Using as subjects a fourth group of 25 psychopathic females, aged 20 to 72 years, all supposedly free from leprosy, intradermal injections of 0.1 cc. each of the S.E.V. antigen and of the L.P.P. antigen were made in the right scapular region.

At the end of 48 hours, all 25 subjects showed a positive reaction to S.E.V. It consisted of a plaque or nodule, with redness and swelling, and a diameter greater than 15 mm. In 6 subjects the reaction to the L.P.P. antigen was positive and in 19 it was negative.

At the end of the fourth week, each of the 23 subjects presented a plaque, indurated, non-inflammatory, and pink in color. At this time the intradermal injections of the L.P.P. antigen were repeated in 17

of the subjects previously negative; these tests, however, were likewise negative.

Thus of 17 persons supposedly non-leprous, anergic to the L.P.P. antigen, and given an intradermal injection of the S.E.V. antigen, all were negative four weeks later to a second injection of L.P.P.

We wish to call attention to two phenomena that we have observed in this experiment:

1) Generally, when the first intradermal injection was positive to L.P.P., the second reaction, made after the injection of sensitizing antigen, was more strongly positive than the first. It means perhaps, that the pre-existent allergy was heightened by the injection of the bacillary antigen.

2) We observed also that at the end of the fourth week and after the second injection of L.P.P., a positive reaction occurred at the site of the first injection, even when it was negative the first time.

IV. Summary

The experiments which have been reported demonstrate that:

1) It is possible to sensitize presumably non-leprous persons to lepromin by intradermal injection of either an oily or an aqueous suspension of *Mycobacterium leprae* killed by heat. Furthermore, this sensitization ensues in a high percentage of subjects.

2) Sensitization to lepromin can be produced also by intradermal injection of suspensions of *Mycobacterium tuberculosis* killed by heat. This is in agreement with the opinion which we have expressed (10,11) in discussing positive lepromin reactions in patients with cutaneous tuberculosis and in persons who had been vaccinated with B.C.G.

3) The early lepromin reaction is attributable to previous sensitization which may be induced either by *Mycobacterium leprae* or by *Mycobacterium tuberculosis.*

4) Sensitization to lepromin was not induced by previous intradermal injection of purified lepromin protein (L.P.P.), nor by injection of a suspension of *E. typhosus* killed by heat.

5) As regards the duration of sensitivity to lepromin, a final answer cannot be given. We have observed positive early reactions to the antigen L.P.P. in presumably non-leprous individuals who have received endermal injections of integral lepromin five years previously.

6) As to the practical value of allergic response as an element of protection against later infection, we believe that it is more desirable

for an individual exposed to leprosy to have a positive lepromin reaction. Unfortunately it is not possible to provoke this supposedly protective allergy, since apparently it is dependent on an unknown factor which may be constitutional. When that factor is lacking, nothing can be gained by the intradermal injection of the antigen. However, if the factor is present in an individual as evidenced by his capacity to react in an allergic manner to the antigen, we believe that he will always have a high degree of immunity to leprosy, regardless of his previous state of sensitization.

V. References

1) Fernandez, J. M. M. and Olmos Castro, N. La reacción precoz provocada por la lepromina. Investigaciones efectuadas con diversos antígenos derivados del *M. leprae*. Rev. Arg. de Dermatosif. **26** (1942) 556.

2) Hayashi, F. Mitsuda's skin reaction in leprosy. Internat. J. of Leprosy. **1** (1933) 31.

3) Fernandez, J. M. M. and Olmos Castro, N. Estandardización de le lepromina. Rev. Arg. de Dermatosif. **25** (1941) 435.

4) Dharmendra. Studies of the lepromin test. The active principle of lepromin is a protein antigen of the bacillus. Leprosy in India.. **13** (1941) 89.

5) Paras, E. M. Chemical fractionation of leprotic nodules. Isolation of the lipoid fraction. Philippine J. Sc. **66** (1938) 155.

6) Rabello, Jr. and Villela, G. Use of an antigenic substance extracted from the leproma in the diagnosis of lepra. Rev. Bras. de Leprol. **6** (1938) Spec. No., 231-232.

7) Fernandez, J. M. M. The early reaction induced by lepromin. Internat. J. of Leprosy. **8** (1940) 1.

8) Wade, H. W. The lepromin reaction in normal dogs. Preliminary report. Internat. J. of Leprosy. **9** (1941) 39.

9) Schujman, D. Personal communication.

10) Fernandez, J. M. M. Estudio comparative de la reacción de Mitsuda con las reacciónes tuberculinicas. Rev. Arg. de Dermatosif. **23** (1929) 425.

11) Fernandez, J. M. M. Influencia del factor tuberculosis sobre la reacción a la lepromina. To be published in La Rev. Arg. Nort. de Cienc. Med.

12) Lowe, J. and Dharmendra. Studies of the lepromin test. The early reaction to lepromin, its nature and its relations to the classical Mitsuda reaction. Leprosy in India. **13** (1943) 133.