POSITIVE "LEPROMIN" REACTIONS WITH SUSPENSIONS OF NORMAL TISSUE PARTICLES

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Kensuke Mitsuda reported first in 1919 (13), and again in 1923 at the Third International Leprosy Conference at Strassbourg (14), that the intracutaneous injection of a suspension of boiled leprous nodules (now called "lepromin") usually gives a negative reaction in cases of nodular leprosy, but a positive reaction in neuromacular cases. This so-called "Mitsuda reaction" has found world-wide application. It is a late reaction, starting the first week after the injection and reaching maximal intensity between the third and fourth weeks. Besides this late reaction an early one, the so-called "Fernandez reaction," may be observed between 24 and 72 hours after the injection of lepromin. At first this early reaction was supposed to be nonspecific and of no real significance, but Fernandez (3) found that its occurrence coincides with that of the classical Mitsuda reaction. Lowe and Dharmendra (11), among others, confirmed this.

The criteria of positivity of both of these reactions have changed during the years, which makes comparison of the results of different workers difficult. The following are the criteria adopted by the Sixth International Congress of Leprology held at Madrid in 1953 (2).

Readings of the early (Fernandez) reaction are made at 48 hours, those of the late (Mitsuda) reaction on the 28th day. The early reactions are recorded as measurements of the average diameter of the area of edema: — (negative), less than 5 mm.; ± (doubtful), 5-9 mm.; 1+, 10-12 mm.; 2+, 15-19 mm.; 3+, 20 mm. and over. The late reactions are recorded as follows:—(negative), less than 3 mm.; ± (doubtful), 3-4 mm.; 1+, 5-7 mm.; 2+, 8-9 mm.; 3+, 10 mm. and over, and all reactions with ulceration.

A positive lepromin reaction is regarded as an expression of resistance, directly proportionate in amount to the degree of positivity. A negative reaction is interpreted as: (*a*) in leprosy patients, and in contacts of open cases, a sign of deficient resistance; (*b*) in healthy individuals not contaminated with leprosy, without significance. The lepromin reaction is considered useful in respect of prognosis and classification in leprosy patients. It has no definite diagnostic value.

There are three kinds of lepromin. 1. Integral lepromin (L. I.), or ordinary lepromin, made by the Mitsuda-Hayashi procedure (technique improved by Wade). It contains all the elements of the leproma: bacilli, cells, tissue detritus, etc. It is

most widely used because of the simplicity of its preparation. Lepromatous tissue is autoclaved for 20 minutes and ground in a mortar with gradual addition of saline up to 20 cc. per gram, and the suspension is then filtered through nylon gauze. The residue left on the filter may be reground and suspended in fresh saline (10 cc. per gram). Phenol, 0.5%, is added to the filtrate.

2. Bacillary lepromin (L. B.), prepared from a pure suspension of the Hansen bacillus as obtained by the method of Fernandez and Olmos Castro (4) or that of Dharmendra (2). In the latter method, which gives an antigen which can be standardized with minimal loss of bacilli, the autoclaved lepromatous tissue is ground in chloroform and the chloroform pipetted off. The grinding is repeated in fresh lots of chloroform until a smear of the remaining tissue is almost free from bacilli; about 25 cc. of chloroform is necessary to extract almost all of the bacilli from 1 gm. of tissue. After the chloroform is evaporated off there remains a residue consisting of lipids and bacilli, which is then suspended in ether. This ether suspension is centrifuged to deposit the bacilli, the deposit is again suspended in ether to remove the lipids more completely, and the final bacillary deposit is dried *in vacuo*.

3. Purified lepromin protein (L. P. P.), which is a group of antigens made of active, water-soluble substances of leprosy bacilli, obtained either by filtration as by Fernandez (3), or by chemical extraction as by Paras (16) or Rabello and Villela (17) or Dharmendra (2).

The results obtained with these different kinds of lepromin are not the same. With the partly-defatted bacillary lepromin (Dharmendra), the Mitsuda reaction is weaker than with ordinary lepromin. The purified lepromin protein produces only the early reaction. It seems that there is a great need for a standardized method of preparing lepromin for international use.

THE NATURE OF THE LEPROMIN REACTION

Nearly all leprologists believe, as Hayashi (7) did, that the Mitsuda reaction is dependent on the presence of bacilli. He had found that a filtrate did not give the late reaction, and that a suspension of lymph nodes from a resolved case, consisting almost entirely of leprous tissue but very few bacilli, gave only feeble reactions. Mitsuda (13) had used 0.5% phenol-saline as a control, with negative results. Kitano and Inoué (8) found that a leproma suspension in which the bacilli had been broken down by ultrasonic treatment until no acid-fastness remained still caused positive reactions, although less strong than the original suspension.

Dharmendra holds that of the constituents of the lepromatous nodule only the bacillary matter is antigenic, having found that the nodule tissue freed from bacilli is not active. Wade (18), in experiments with dogs, carried out control tests for human-tissue sensitivity with a mixture of lymph node, spleen and fat-free omentum and saw only slight and relatively brief reactions in animals highly sensitized to lepromin. Lopes de Faria (10) reported weak positive Mitsuda reactions with an extract of normal skin in patients with tuberculoid leprosy.

Lowe and Dharmendra (11) explained both the early and late reactions on the basis of one substance, the early reaction being caused by the free protein in the injected material, and the late reaction by protein

which is slowly liberated by the breakdown of the injected bacilli in the tissues. Dharmendra (1) carried out detailed studies on the active principle of lepromin. Of all fractions isolated from the bacilli, only the protein was found active.

Previously, however, Fernandez had postulated the existence of two different active substances, one for the early reaction and the other for the late one. In his comparative study of the two reactions he found that the ordinary lepromin produced late reactions in all the cases in which it had produced early responses, whereas the filtrate produced only the early reaction.

In his experiments on dogs Lopes de Faria obtained a positive Mitsuda reaction with the lipid fraction of Mitsuda-type lepromin, but not with the protein fraction.[1] He therefore deduced that only the early reaction is allergic in nature, and that the late one is independent of an antigen-antibody mechanism and is due mainly to the natural resistance of the organism. This resistance would be responsible for the destruction of the bacilli and the liberation of lipids, which then stimulated the granulomatous reaction.

According to Dharmendra, both the early and late lepromin reactions are an allergic phenomenon, although the allergy is not always specific but may in some persons be dependent on sensitization with other acid-fast bacilli, the most important being the tubercle bacillus.

Against the Mitsuda reaction being an allergic phenomenon we note: 1. The lateness and the nodular nature of the reaction. 2. Positive results seen in noncontacts. 3. Negative results in the lepromatous type of leprosy.

Because of doubt of the specificity of the lepromin reaction, we carried out experiments with normal tissue suspensions.

TABLE 1.—*Positive "lepromin" reactions with a normal skin preparation, compared with true lepromin, in four patients with tuberculoid leprosy.*[a]

Case No.	True lepromin[b]		Normal skin preparation[b]	
	Fernandez	Mitsuda	Fernandez	Mitsuda
12063	20	18	8 ±	5 ±
12070	14	13	5 ±	4 ±
12093	25	23	16 +++	No reading, discharged
12136	15	16	3 —	2 —

a. Six other patients tested gave negative results.

b. All readings in this and subsequent tables are in millimeters.

[1] Wade had reported (19), that, with lepromin, dogs regularly showed a positive Mitsuda phenomenon which was essentially the same as in man.

EXPERIMENTS WITH EXTRACTS AND SUSPENSIONS OF NORMAL SKIN

Experiment 1.—From normal skin a preparation was made in the same way as lepromin is prepared from lepromatous tissue, following the Mitsuda-Hayashi procedure with the improved Wade technique, adding saline up to 20 cc. per gram of tissue. This preparation was tested

TABLE 2.—*"Lepromin" reactions with a concentrated normal skin preparation, and with fractions of preparation.*[a]

Case No.	Time of readings					
	48 hours			28 days		
	A	B	C	A	B	C
Patients with lepromatous leprosy						
8962	2	4	4	0	0	0
9759	5	2	5	2	0	2
12175	4	3	3	0	0	0
12176	3	4	2	0	0	0
12220	2	5	2	0	0	0
11458	2	5	3	0	2	2
12286	3	3	4	4	0	0
12290	2	4	2	3	3	0
12304	7	4	1	0	2	1
12312	3	5	10	1	1	1
Totals	33	39	46	10	8	6
Patients with tuberculoid leprosy						
12435	3	2	8	0	0	0
12448	5	2	5	3	0	5
12467	2	3	2	1	1	0
12498	2	4	3	3	1	4
12506	9	2	9	5	1	3
12424	2	—	5	—	—	—
12427	5	5	7	2	2	8
12471	2	4	5	2	1	6
12484	2	4	2	0	0	0
12492	10	5	12	7	0	10
Totals	42	31	58	23	6	36

a. A = 10X concentrated normal skin preparation (Mitsuda-Wade technique); B = lipid fraction; C = water fraction.

in ten patients with tuberculoid leprosy who previously had reacted strongly to lepromin. Positive reactions occurred in only four cases, and these were all weak (Table 1).

Experiment 2.—In this experiment a preparation was again made from normal skin by the same procedure, but it was concentrated ten times by dry freezing (A). Ether extraction of this concentrated product resulted in a lipid fraction (B), and a water fraction (C). The results of tests with these preparations are shown in Table 2. For simplicity, the totals of the readings of the different groups are compared, although the individual measurements are also given.

TABLE 3.—*"Lepromin" reactions with fractions of normal skin extracts.*[a]

Case No.	Time of readings							
	48 hours				28 days			
	A	B	C	D	A	B	C	D
Patients with lepromatous leprosy								
8962	2	4	5	2	0	0	0	0
9759	3	7	3	2	2	2	0	0
12175	3	5	5	3	0	0	0	0
12176	4	5	6	4	0	0	3	0
12220	3	4	6	2	0	0	3	1
11458	5	5	7	5	0	0	3	1
12286	5	6	6	4	0	0	3	0
12290	4	5	8	4	0	0	6	1
12304	8	10	8	5	4	3	2	1
12312	2	5	4	6	0	0	2	0
Totals	39	56	58	37	6	5	22	4
Patients with tuberculoid leprosy								
12484	3	8	6	4	0	0	3	0
12492	8	5	5	2	6	0	3	0
12526	2	5	7	3	0	0	3	1
12540	3	4	6	2	3	5	2	0
12548	—	5	4	3	0	0	0	0
12467	2	3	4	3	2	1	3	0
12498	1	4	4	2	0	0	2	0
12506	1	3	2	7	0	0	2	0
12528	3	7	8	5	0	0	3	0
12547	2	4	4	2	0	0	0	0
Totals	25	48	50	33	11	6	21	1

a. A = protein fraction soluble in 1% NaCl; B = lipid fractions plus emulsifier; C = protein fraction soluble in 1-5% NaCl; D = emulsifier.

The totals were slightly higher for the A and C preparation in the tuberculoid cases, especially for the late reaction, although on the whole the reactions were weak and the differences not significant.

Experiment 3.—We now made three preparations from a normal skin extract.

This was done by extracting 50 gm. of normal skin with 200 cc. of 5 per cent NaCl in the Waring blendor. After centrifuging for 20 minutes at 4,000 r. p. m., the clear supernatant was extracted with ether to obtain the lipid fraction (C). The water layer was worked up into two protein fractions, one containing the proteins soluble in 1 per cent NaCl (A), and the other the proteins soluble in 1-5 per cent NaCl (B). Because of the possibility that the poor results with the lipid fraction in Experiment 2 might have been due to the fact that the lipids were not well dissolved, in this experiment special care was taken in this respect. An emulsifier was added to the lipid fraction to enable injection of this fraction also in 0.5 per cent phenol-saline. For control, the emulsifier was tested separately (D).

The results of the tests with these preparations in ten tuberculoid and ten lepromatous cases are shown in Table 3.

In general, the reactions were weaker than those in Experiment 2. Especially the totals of the Mitsuda reactions in the tuberculoid cases were lower, while those in the lepromatous cases were somewhat higher. With regard to the lipid fraction, in spite of the special care taken in its preparation the results were still bad, especially with respect to the Mitsuda reaction. It may therefore be concluded that the *soluble* lipids are not responsible for the reaction. From the results so far it seems that, after concentration and centrifugation, the activity of the preparations decrease. This brought us to the hypothesis that probably the presence of particles and their size might be of importance, and that in the late lepromin reaction we are dealing with a kind of foreign-body reaction.

EXPERIMENTS WITH PREPARATIONS FROM NORMAL LIVER AND FROM LEPROMATOUS LIVER AND SPLEEN

Experiment 4.—The following preparations were tested in this experiment:

Preparation A: "Normal lepromin" prepared according to the Dharmendra method from lepromatous earlobes (control).

Preparation B: Lepromin prepared from a lepromatous spleen (bacillus index 4+) and a lepromatous liver (bacillus index 3+), also by the Dharmendra method.

Preparation C: The same lepromin as B, but refluxed for six hours with 98 per cent ethanol. This was done to split the lipoproteins from the outer layer of the bacilli, as it is claimed (Lopes de Faria) that the late reaction is due to lipids. After this alcohol treatment nearly all the bacilli had lost their acid-fastness.

Preparation D: A suspension of particles from normal liver, prepared according to the Dharmendra method.

These preparations were tested in ten tuberculoid and ten lepromatous cases. In the former experiments we used only patients with tuberculoid leprosy who gave positive lepromin reactions. In this experiment the tuberculoid patients were not selected for lepromin positivity, and about one-half of them were negative to lepromin. The readings were made after 48 hours, and after 1, 2, 3 and 4 weeks, as shown in Table 4.

It will be seen that, although the strength of the reactions varied, all the preparations reacted in the same way. From the results of the tuberculin test (PPD, 5 units) it is obvious that hypersensitivity to tuberculin can exist with negative reactivity to lepromin.

DISCUSSION

In these experiments it was demonstrated that with suspensions of tissue particles, prepared from normal skin or normal liver in the same

TABLE 4.—*"Lepromin" reactions with Dharmendra preparations from lepromatous earlobes (A), lepromatous liver and spleen before (B) and after (C) boiling with ethanol, and normal liver (D).*

Case No.	Time of readings																				Tuberculin reaction
	48 hours				1 week				2 weeks				3 weeks				4 weeks				
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	
Patients with lepromatous leprosy																					
12632	0	0	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10
12629	0	2	0	0	0	0	2	0	0	0	0	0	0	0	0	2	0	0	0	0	2
12617	0	?	0	0	1	2?	2	0	1	3?	2	0	1	?	1	0	0	0	0	0	3
12608	0	3	0	0	0	2	0	0	0	2	0	0	0	2	0	0	0	1	0	0	22
12606	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	11
12605	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
12603	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10
12576	0	5	0	0	0	2	0	0	0	2	0	0	0	2	0	0	0	1	0	0	14
12575	0	0	2	1	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12
12571	0	2	1	0	0	0	0	0	0	0	0	0	1	4	0	0	0	2	0	0	21
Totals	0	14	5	2	1	14	4	0	1	9	2	0	2	8	1	2	0	4	0	0	
Patients with tuberculoid leprosy																					
12625	0	0	1	0	0	8	10	7	0	7	6	5	4	7	6	5	0	6	4	2	6
12622	12	2	18	8	4	7	2	4	5	6	4	3	4	4	3	2	3	2	2	2	5
12616	12	0	12	0	5?	7	0	0	0	5	0	0	0	4	0	0	0	3	0	0	10
12601	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12
12581	0	3	0	0	0	0	0	0	0	0	3	1	2	0	4	0	1	0	2	0	8
12559	9	15?	8	?	8	9	13	6	5	7	7	4	4	5	6	2	4	4	6	1	12
12527	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	8
12521	0	0	8?	0	3	0	5	3	0	0	4	3	2	0	5	1	0	0	4	0	8
12553	7	2	10	2	0	0	0	0	0	0	2	0	0	0	1	0	0	0	1	0	15
12492	33	18	30	18	25	14	20	12	22	18	22	10	25	14	20	12	25	14	18	10	15
Totals	69	40	87	28	45	44	50	32	32	46	48	26	41	34	45	22	33	29	37	15	

way as lepromin is prepared from lepromatous tissue, positive reactions can be obtained in cases of tuberculoid leprosy, but not in lepromatous cases. The same patients who react positively to lepromin from lepromatous tissue reacted positively to the normal-tissue suspensions, although as a rule these reactions seem to be weaker. We may therefore conclude that it is not only the leprosy bacilli which are responsible for the evoking of

the lepromin reaction, but that the tissue component also takes part in the reaction.

Confirmation of this concept can be found in the results of experiments of Ota and Nitto (15). They reported having performed serial transmission of human leprosy in fowls for seven generations by injecting a suspension of a human leproma into the breast muscle of chickens. By Hayashi's method they made, among others, preparations from the bacillus-containing liver of a fowl of the fifth generation and obtained with them positive Mitsuda reactions that paralleled results with ordinary lepromin used as a control on the same individual at the same time. The authors, erroneously, explained these results as a proof of the transmission of leprosy in fowl. In the light of our findings their results are no proof of successful transmission of leprosy. It is more likely that their positive Mitsuda reactions are evoked by suspensions of normal liver particles.

In our search for the active principle of the Mitsuda reaction it was found (Experiment 3 and 4) that after concentration and centrifugation the strength and the specificity of the reaction had decreased. This brought us to the concept that probably the size of the particles might be of importance, and that in the Mitsuda phenomenon we are dealing with a kind of foreign-body reaction. The histology of a papule evoked by normal skin extract (biopsy of Case 12492, Experiment 2, made 42 days after injection) was in agreement with that concept.

The leprosy bacilli, too, probably act as a foreign body, due to the fact that they are not broken down readily. In many cases bacilli can easily be found three months and more after injection. As bacterial filtrates of active lepromin do not evoke the Mitsuda phenomenon, it seems that the size of the particles is critical. Probably these particles have to remain unaltered for a relatively long period of time if a cutaneous reaction is to occur. Therefore, these substances must be rather inert to the enzymes of the living tissue.

In agreement with this view is the opinion of Kitano and Inoué referred to, who found that to produce a Mitsuda reaction it is necessary that the lepromin contain the solid components of the leprosy bacilli, although they may be broken down to the point of losing their acid-fast character. In our Experiment 4 we found, with Preparation C, that loss of acid-fastness does not make lepromin inactive.

Other substances which are not quickly absorbed may act as foreign bodies, e. g., collagen, elastic fibers, keratin, melanin, etc. These substances may be responsible for the positive reactions obtained with our "lepromin" preparations from normal tissue. Although this explanation holds especially for the late reaction, early reactions are also obtained with preparations from normal tissue. Therefore, the leprosy bacillus is not absolutely necessary for the early reaction. Much more work has to be done to elucidate the nature of the early reaction.

If our hypothesis is correct that in the Mitsuda phenomenon we are dealing with a foreign-body reaction, then attempts to find a correlation between the results of the lepromin and tuberculin tests, to prove an immunological relationship between leprosy and tuberculosis, are incorrect. The same applies, but perhaps in a lesser degree, for comparisons with the results of tests with killed tubercle bacilli or BCG bacilli. However, one can imagine that tubercle bacilli may in special circumstances evoke, besides the Koch phenomenon, also a kind of foreign-body reaction. These questions need further study.

Assuming the foreign-body nature of the Mitsuda phenomenon, then the concept that this reaction is an expression of resistance also becomes doubtful. However, there may be a yet-unknown correlation. It is also possible that a Mitsuda phenomenon is merely a general tissue response to a chemical foreign body which occurs in a certain percentage of human beings.

In our tuberculoid cases we found about 50 per cent to give positive Mitsuda reactions. With lepromin prepared from lepromatous earlobes by the Dharmendra method, Kooij and Rutgers (9) have found about the same percentage (between 40 and 50 per cent) of positive Mitsuda reactions in healthy people and in patients suffering from tuberculosis. On the contrary, the lepromin reaction in lepromatous leprosy is nearly always negative. It therefore seems probable that an initially positive Mitsuda reaction becomes negative in lepromatous cases. The typical feature of the Mitsuda reaction is not its positivity in the tuberculoid type, but its negativity in the lepromatous type, although positive tuberculin reactions occur in the latter type (Table 4). Hale and Molesworth (5) report that they have observed many patients with lepromatous leprosy (probably borderline cases) with positive lepromin reactions which became negative as the disease progressed. These findings need confirmation. Another interesting point for study is the conversion of negative lepromin reactivity to positive by means of BCG vaccination.

Because of the scarcity of lepromin prepared from trimmed earlobes, we tried preparing lepromin from lepromatous liver and spleen. As shown in Experiment 4, the lepromin so prepared was as active as regular lepromin. This result, and the finding that positive reactions can be obtained with suspensions of particles of normal tissue, bring to an end the scarcity of lepromin. Now it will be possible to make investigations on a large scale with the same batch of lepromin, which is urgently needed. We hope in the future to prepare a more active suspension of normal tissue particles, and also to standardize it.

A plea is made for the Madrid congress criteria to be used in reports of lepromin readings, and in any case that the readings be given in millimeters so that comparisons will be possible.

SUMMARY

1. Positive early and late "lepromin" reactions have been obtained with suspensions of normal skin and of normal liver particles in patients with tuberculoid leprosy. These suspensions gave negative results in patients with lepromatous leprosy.

2. The results of the experiments support the hypothesis that in the Mitsuda reaction we are dealing with a foreign-body reaction.

3. Besides the particles of normal tissue, the leprosy bacilli may also act as foreign bodies, due to the fact that they are not broken down readily.

4. If the assumption is correct that the Mitsuda reaction is a foreign-body reaction, then it is incorrect to attempt to prove any immunological relationship between leprosy and tuberculosis by means of this reaction.

5. Active lepromin was also prepared from lepromatous liver and spleen.

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RESUMEN

1. Se han obtenido reacciones "lepromínicas" positivas tempranas y tardías con suspensiones de partículas de piel normal y de hígado normal en sujetos que padecían de lepra tuberculoidea. Esas suspensiones dieron resultados negativos en enfermos de lepra lepromatosa.

2. Los resultados de los experimentos apoyan la hipótesis de que, en la reacción de Mitsuda, nos confronta una reacción de cuerpo extraño.

3. Además de las partículas de tejido normal, los bacilos leprosos pueden obrar como cuerpos extraños, debido a no desintegrarse fácilmente.

4. Si está bien fundada la suposición de que la reacción de Mitsuda es una reacción de cuerpo extraño, resulta incorrecto tratar de demostrar por medio de esta reacción que exista alguna reacción inmunológica entre la lepra y la tuberculosis.

5. También se preparó lepromina activa del hígado y del bazo lepromatosos.

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