IMMUNOLOGIC MECHANISM OF THE ACCELERATED FORMATION OF THE TUBERCLE. A CLINICAL STUDY[1]

NORBERTO OLMOS CASTRO, M.D.[2]
AND PASCUAL B. ARCURI, M.D.

National Council of Scientific and Technical Investigations
Tucumán, Argentina

In previous investigations, experimental and clinical ([1-8]), we have demonstrated the following facts.

1. Lepromin, when injected intradermally into a healthy man or dog not hypersensitized to it, provokes at about the end of the second week the formation of a small, slightly inflammatory nodule, which increases in size in subsequent days and becomes frankly inflammatory by the third week.

2. The intradermal injection of lepromin into a man or dog previously hypersensitized to it, causes the formation of a definite tubercle, frankly inflammatory, by the end of the first week. If the existing hypersensitivity is very high, the tubercle can be seen on the fourth day after the injection of the antigen, or even, in some cases, in 48 hours. In such cases the tubercle becomes manifest at the center of an intense and large inflammatory reactional area, erythematous and infiltrated.

This effect of the injection of organisms hypersensitive to lepromin, the formation *in situ* of a tubercle in a much shorter time than in organisms which are not hypersensitive, we call the "accelerated formation of the tubercle." Fernandez has called it the "Olmos Castro phenomenon."

In the present article are reported experiments of a study of the immunologic mechanism of the accelerated formation of the tubercle, performed with leprosy antigens of different constitutions.

METHOD

Antigens used.—1. Integral lepromin (I.L.M.H.), prepared according to the classical Mitsuda-Hayashi technique.

2. Bacillary suspension (B.S.): Bacillus-rich lepromas are boiled for 30 minutes, cleansed, and ground in a glass mortar to make a homogenous paste. This paste is suspended in saline solution, adding more sodium chloride until a specific gravity of 1,050 is reached. This suspension is centrifuged, the supernatant fluid removed, and to it absolute alcohol is added until the specific gravity is reduced to 0.900. This suspension is then centrifuged and the supernatant fluid discarded. The moist sediment is suspended in saline (10 cc. per gm. of leproma), shaken thoroughly, put up in ampules, and autoclaved.

3. Whole-leproma (integral) antigen (IA): Lepromas prepared as before are cut up into small pieces and dried. After weighing, this material is ground up in chloroform until bacilli can no longer be found. The powder obtained is suspended in distilled water

[1]Translated from the Spanish.
[2]Dr. Olmos Castro died on December 31, 1962.—EDITOR.

in the proportion of 4 x 1,000 with respect to the dried nodule and centrifuged at 4,000 r.p.m. for 1 hour, after which the supernatant fluid is put in ampules and autoclaved.[3]

Subjects utilized: The tests were made in 28 adults with tuberculoid leprosy.

Procedure.—Of each of these antigens, 0.1 cc. was injected intradermally in the scapular areas of the back. The readings were made after 2, 7 and 21 days.

The results were recorded according to the clinical characteristics of the reactions observed. Measurements were recorded in millimeters, referring to the average of the greater diameters. For the 48-hour or Fernandez reaction, and for the late nodular Mitsuda reaction, the criteria of positivity used were those recommended by the Tokyo Congress, i.e., a minimum of 10 mm. for the Fernandez reaction and of 3 mm. for the Mitsuda reaction.

The accelerated formation of the tubercle is characterized by the presence of an erythematous tubercle, minimum size 3 mm., which may or may not be surrounded by an inflammatory halo. The presence of an inflammatory reaction in the form of an erythematous infiltration, firm and elevated, is regarded as a large tubercle.

RESULTS

The lepromin test, performed with either the Mitsuda-Hayashi integral lepromin or with the Fernandez-Olmos Castro bacillary suspension, caused the "accelerated formation of the tubercle" in all of the cases tested in this series. This accelerated reaction was preceded by the Fernandez reaction in most of the cases—25 out of the 28. In

TABLE 1.—*Results of the lepromin tests in 28 cases of tuberculoid leprosy, performed with the Mitsuda-Hayashi integral lepromin, the Fernandez-Olmos Castro bacillary suspension, and the whole-leproma extract.*

Time of reading (days)	Integral lepromin			Bacillus suspension			Whole-leproma antigen		
	Reaction	Pos.	Neg.	Reaction	Pos.	Neg.	Reaction	Pos.	Neg.
2	I.E.[a]	19	3	I.E.	24	3	I.E.	25	3
	E.T.I.[b]	6		E.T.I.	1				
7	E.T.[c]	20	0	E.T.	16	0	E.T.	10	3
	E.T.I.	8		E.T.I.	12		E.T.I.	15	
21	E.N.[d]	28	0	E.N.	28	0	E.N.	25	3

[a]I.E. = Infiltrated erythema, i.e., the early or Fernandez reaction.
[b]E.T.I. = Erythematous tubercle central in an area of infiltration.
[c]E.T. = Erythematous tubercle without surrounding infiltration. Both ETI and ET signify the "accelerated tubercle formation."
[d]E.N. = Erythematous nodule, i.e., the late or Mitsuda reaction.

[3]The only difference in the procedure here described and that given for the production of the authors' leprolin [THE JOURNAL **26** (1958) 51-56] is that in the present case the powder obtained by grinding in chloroform is not treated repeatedly with ether, for the purpose of removing the lipids, "which have no antigenic value in the hypersensitivity test." The LPT antigen, or leprolin, was said not to be allergenic, whereas the whole-leproma, or "integral" preparation here described is very much so.—EDITOR.

all of the cases the accelerated tubercle formation preceded the late Mitsuda reaction. These results are to be seen in Table 1.

It is also to be seen in Table 1 that in the tests made with the whole-leproma extract, devoid of bacillary bodies and tissue debris, the early reaction of Fernandez was positive in 25 of the 28 cases tested, and that in 24 of the 25 cases the early reaction was followed by the accelerated formation of the tubercle. The late Mitsuda reaction was positive in 25 out of the 28 cases, and in 24 of these positive cases that reaction was preceded by the accelerated tubercle formation. In 6 of the cases the Mitsuda-Hayashi lepromin caused the production of an accelerated tubercle as early as the 2nd day, and the bacillus suspension did so in 1 case.[4]

SUMMARY AND CONCLUSIONS

The intradermal injection of either integral (i.e., Mitsuda-Hayashi) lepromin, or of the bacillus suspension of Fernandez and Olmos Castro, provoked the accelerated formation of the tubercle in all of the tuberculoid leprosy cases in the experiment reported. This confirms the results of previous studies, in which we have insisted that this tubercle is induced by the action of the bodies of the leprosy bacillus.

Nevertheless, using an antigen which contains the integral antigenic components of *M. leprae,* devoid of whole bacillus bodies and cellular detritus, we found that the formation of the same tubercle occurs in a large proportion (25 out of 28) of tuberculoid cases.

RESUMEN Y CONCLUSIONS

La intradermoinyección de lepromina integral o de suspensión bacilar, provocó la formación acelerada del tubérculo, en nuestra experiencia, en la totalidad de los casos, lo cual viene a ratificar trabajos nuestros anteriores, en los cuales insistíamos, que este tubérculo se formaba por la acción del cuerpo de *M. leprae.*

Sin embargo, utilizando antígenos que poseen los componentes antigénicos integrales de *M. leprae,* privado de detritus celulares y de cuerpo bacilar íntegro, hemos podido comprobar que la formación del tubérculo también se efectúa en un alto número de casos tuberculoides (25 sobre 28 cases).

RESUMÉ

L'injection intra-dermique, soit, de lépromine intégrale (Mitsuda-Hayashi), soit de la suspension bacillaire de Fernandez et Olmos Castro, provoqué la formation accélérée du tubercule dans tous les cas de lèpre tuberculoïde inclus dans cette étude. Ceci confirme les résultats de nos études antérieures, dans lesquelles nous avons insisté sur le fait que ce tubercule est provoqué par les corps bacillaires de *M. leprae.*

Néanmoins, utilisant un antigène qui contient les constituants antigéniques intégraux de *M. leprae,* et dépourvu de bacilles entiers ou de débris cellulaires, nous avons observé

[4]The authors consistently use "tubercle" in connection with the 7-day "accelerated" reaction, but "nodule" is frequently used in connection with the late Mitsuda reaction. No statement regarding the difference between the accelerated tubercle and the nodule has been seen.—EDITOR.

chez une grande proportion des cas tuberculoïdes (25 sur 28) la formation du même tubercule.

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