

IMMUNOBIOLOGIC RELATION BETWEEN TUBERCULOSIS
AND LEPROSY. X. COMPARATIVE STUDY OF THE RE-
SULTS OF THE LEPROMIN TEST IN SUBJECTS SUB-
MITTED TO SERIAL INJECTIONS OF MITSUDA'S
ANTIGEN AND TO ORAL BCG VACCINATION

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The purpose of the present paper is to compare the capabilities of two procedures to induce lepromin reactivity, one procedure being the repeated injection of the Mitsuda antigen at relatively short intervals (every three months), the other procedure being oral BCG vaccination about which we have written repeatedly (³⁻¹¹). The study permits answering objections raised by some authors that conversion of Mitsuda negativity after BCG vaccination is caused by the antigenic action of the lepromin test made previous to the vaccination.

MATERIAL AND METHOD

Sixty-three children of leprous parents were employed in this study. All of them were isolated from their parents at birth, and all live at the Creche Morato Coelho of the Associação Santa Terezinha in São Paulo. In this institution the children grow up in a closed environment, and are kept absolutely apart from any tuberculous or leprous contagion whatever. Nurses and auxiliary personnel are engaged only after negative radiologic and clinical examinations, especially as concerns tuberculosis.

At the outset of the experiment their ages varied from 3 days to 12 months 6 days. They were divided by lot into three groups as similar statistically as possible as regards age, sex and total numbers, as shown in Table 1.

Group A: 21 children, 14 males and 7 females, average age 6 months 9 days (subjected to repeated lepromin injections);

Group B: 21 children, 14 males and 7 females, average age 6 months 21 days (vaccinated orally with BCG);

Group C: 21 children, 9 males and 12 females, average age 6 months 24 days (kept as controls).

All of the children were kept under the same environmental conditions. All had the same diet, according to age. Tuberculin tests were performed on all three groups, and will be discussed in due course.

Experimental.—The experimental part of this study is divided into two parts.

Part I: The measures taken in the first part are as follow; those taken in the second part will be presented shortly, and are discussed later in this article.

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TABLE 1:—Original distribution of the 63 children of the study into three experimental groups, by sex and age.

Group	Sex	Age (months)					Totals
		0-3	3-6	6-9	9-12	>12	
A. Repeated (4) lepromin injections	Male	5	2	3	4	0	14
	Female	0	4	1	2	0	7
	Total	5	6	4	6	0	21
B. Oral BCG vaccination (3 doses)	Male	4	3	3	3	1	14
	Female	0	3	1	3	0	7
	Total	4	6	4	6	1	21
C. Control	Male	2	3	1	2	1	9
	Female	2	3	3	4	0	12
	Total	4	6	4	6	1	21
Total	Male	11	8	7	9	2	37
	Female	2	10	5	9	0	26
	Total	13	18	12	18	2	63

Group A was given 4 injections of integral lepromin,⁴ the second 93 days after the first one, the third 102 days after the second, and the fourth 84 days after the third (average about 13 weeks). Readings were made 30 and 90 days after each injection.

Group B was vaccinated with three doses of BCG at weekly intervals.⁵ Doses were 100 mgm. for children less than three months of age (total 300 mgm. of vaccine), and 200 mgm. for the older ones (total 600 mgm.). This group got its first and only lepromin injection, in this first part of our study, 9 months after the first dose of BCG.

Group C, kept as a control, was not vaccinated and got its first and only lepromin injection at the end of the observation period.

The schedule of treatments of these groups with lepromin and BCG vaccine, and of skin tests, in this first part of the experiment is summarized as follows:

Group A		Group B		Group C	
1st Mitsuda,	3/25/57	1st BCG,	3/26/57	-----	
2nd Mitsuda,	6/25/57	2nd BCG,	4/2/57	-----	
3rd Mitsuda,	10/3/57	3rd BCG,	4/9/57	-----	
Mantoux,	11/5/57	-----		Mantoux,	11/5/57
4th Mitsuda,	12/26/57	Mitsuda,	12/26/57	Mitsuda,	12/26/57
-----		Mantoux	2/1/58	-----	

Part II: The second part of the experiment consisted of determining the effect of BCG vaccination on the children of Group A who had not become lepromin positive as a result of the repeated injections of lepromin.

The criteria of positivity of the Mitsuda reaction used were those of the Madrid Congress. The early, or Fernandez, reaction has not been taken into account in this study. We recorded "pigmentation" ("Pig.") among negative and doubtful results when, in the absence of nodulation, there was residual pigmentation at the site of the test. It was essentially a negative result, and has been so treated in our figures. For evaluation of the results as positive or negative, we chose to group the 2+ and 3+ results as positive, and all others—including 1+ and ±—as negative. This grouping was adopted in order to avoid misunderstandings with reference to 1+, whose prognostic significance has

⁴The lepromin was prepared by Dr. Murilo Pacea, of the Biochemistry Laboratory of the Department of Leprosy Prophylaxis of the São Paulo State Department of Health.

⁵The BCG vaccine was prepared by the Instituto Butantan of the Health Department, and was employed 48, 96 and 144 hours after preparation. The utmost care was observed in the conservation of the vaccine, and in the correct oral administration.

of late been held doubtful, although we ourselves think differently. For us, these cases—especially in very young children—simply show a lesser capacity of reactivity at the time, and not an absence of it. We believe that further stimuli in the course of time will make them react more strongly later on.

RESULTS, PART I

We now proceed to report the general results of the first part of our study, which concerns the Mitsuda reaction and which ended March 27, 1958. During this period 2 children were lost to the experiment, one by death and the other by removal to another institution.

In Group A, of 21 children, 12 (or 57%) were classed as negative (including, as explained, 5 doubtfuls), and 9 (or 43%), as positive (2 being 2+ and 7 being 3+). In Group B, of 20 children, 3 (or 15%) were classed as negative or weak positive, and 17 (or 85%) were strongly positive (3+). Of Group C, finally, none of the 20 children gave a positive reaction—at most doubtful in 2 cases—so they were 100 per cent negative. These data are given in Table 2.

TABLE 2.—*General results of the Mitsuda reaction in the three experimental groups.*

Group	No. of cases	Mitsuda reactions			
		Negative (-)	Subpositive ^a ± 1+	Positive 2+ 3+	
A. Lepromin, repeated tests	21	7 33%	5 0 24%	2 7 43%	
B. BCG, oral vaccination	20	2 10%	0 1 5%	0 17 85%	
C. Control	20	18 90%	2 0 10%	0 0 0%	

^aDoubtful and 1+ cases, neither counted as positive for reasons explained in the text.

Serial lepromin injections.—The figures given in Table 2 are the final ones, for comparison of the different groups. In Table 3 are given the step-by-step results of Group A, the readings after each of the four lepromin reactions, showing the progressive total changes in reactivity. It is to be borne in mind that in speaking of the results of the lepromin test we always refer to the *late* reaction, as read after about 30 days, and to the *remote* reaction, as read after about 90 days.

These data will be considered further in the general comments on this paper. Let it suffice now to note that at the end of the experiment, after 4 injections of lepromin antigen, the reading of the last reaction presented a final result of 57 per cent of negative and doubtful cases, and 43 per cent of unquestionably positive cases.

BCG vaccinated.—Group B, the children who were vaccinated with BCG, presented the following results of the Mitsuda test performed 9 months afterward: 15 per cent negative (including a 1+ reaction) and 85 per cent positive (all 3+). See Table 4 for details.

TABLE 3.—*Progression of reactivity after repeated lepromin tests in Group A; numbers showing the various readings at 30 days and 90 days after tests.*

Readings	Test and days of reading								Final results (90 days) No. and %
	First 30d.	90d.	Second 30d.	90d.	Third 30d.	90d.	Fourth 30d.	90d.	
Neg.	19	14	5	5	5	7	0	3	7 33
Pig. ^a	2	7	11	7	3	0	3	4	
±	0	0	1	5	5	6	4	5	5 24
1+	0	0	4	4	5	5	6	0	(57)
2+	0	0	0	0	3	3	4	2	9 43
3+	0	0	0	0	0	0	4	7	

^a Pigmented, without nodular infiltration; negative significance.

Control.—Group C, nonvaccinated cases, submitted to only one lepromin injection at the end of the observation period, presented only 2 doubtful reactions.

Significance of data.—These data are significant from the viewpoint of statistical analysis. The work and the general results have been evaluated by Prof. Walter Lesser, who drew the following conclusions:

"These data were submitted to Fischer's exact treatment for fourfold tables. We found the figure 0.006 for P, which means that the probability of occurrence, by chance, of results different from those expected (provided there is no difference between the groups), as much or more than observed, is equal to 0.6%. In view of this result we may reject the hypothesis . . . of there being no difference between the two groups, with less

than 1% risk of error. Value of $\chi^2 = \frac{1,284,489}{163,800} = 7.842$, with Yates's correction:

$$\chi^2 = \frac{1,004,182.25}{163,800} = 6.131."$$

Strength of reactions.—It is important to notice that, besides the marked percentage differences of positive responses to the Mitsuda test obtained by means of oral BCG vaccination as compared with lepromin retesting, the reactivity induced in the vaccination group was in general much stronger (17 necrotic responses) than that induced by repeated injections of the lepromin (only 1 case with necrosis). These differences may be appreciated from the photographs of reaction lesions of Groups A and B shown, Figs. 1 to 4 and 5 to 10.

TUBERCULIN REACTIONS

Having reported the results of Mitsuda reaction in the first part of our experiment, we now report our findings as concerns the Mantoux test. It had been planned that if, while experimenting, we observed positive Mitsuda results in Group A the Mantoux test was to be performed, in spite of the security of the children as regards tuberculosis infection.

TABLE 4.—*Results of the single lepromin test in the BCG-vaccinated group, made nine months after the vaccination.*

Readings	Mitsuda reaction		Final results (90 days) No. and %	
	30 days	90 days		
Neg.	0	2	}	2 70
Pig.	2	0		
±	0	0		
1+	1	1	}	5
2+	0	0		
3+	17	17	}	17 85

A few cases in Group A were read positive after the third lepromin injection, as shown in Table 3. Consequently, the Mantoux test, 1:1000 and 1:10, was applied to the entire group. Four of the children showed, after the 1:10 test, infiltrations 5, 7, 8 and 13 mm. in diameter, respectively.

At the same time, for assurance regarding freedom from tuberculous infection in our working environment, the Mantoux test was also applied to the children of Group C, the control group. The results were entirely negative.

For security's sake all children who showed any infiltration whatever in the Mantoux test were submitted to x-ray examination. Again the results were negative. As already stated, special precautions are taken with respect to tuberculosis infection in the working personnel of this institution.

Thus we found that tuberculin sensitization in Group A was negligible, and it was altogether absent from Group C, the control group. The subjects in Group B, given BCG vaccination and submitted to the Mantoux test at the end of the observation period, showed—as was to be expected—a significant amount of sensitivity.

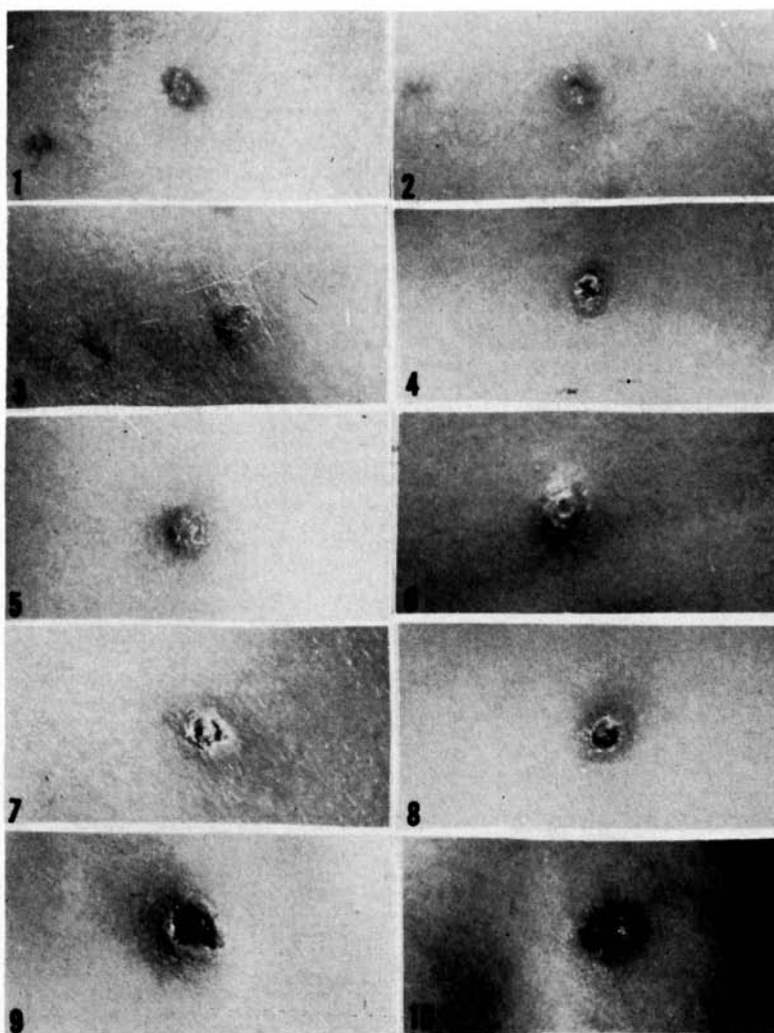
RESULTS, PART II

As has been seen, 12 of the 21 children of Group A remained Mitsuda negative after the fourth lepromin injection. They were then subjected to oral BCG vaccination (4 weekly doses, 0.2 gm. each). A fifth lepromin injection was made 36 days after the last ingestion of BCG. The results of this last lepromin test, read after 30 days, were as follows: 5 subjects 3+, 2 subjects 2+, and 5 subjects 1+. At the remote reading after 90 days, one of the 1+ cases had become negative, and a 2+ case had become 1+. From these results we infer that BCG vaccination constitutes a more efficient stimulus of lepromin reactivity than the lepromin retesting.

Of the 20 children of Group B (the BCG group), when given their one and only Mitsuda test 9 months after the BCG vaccination, two were lepromin negative and one gave a 1+ response. These 3 individ-

uals were given a second course of BCG vaccination, 4 weekly doses of 0.20 gm. each. A second lepromin test performed 36 days after the last vaccine ingestion showed that 2 cases were now 3+ Mitsuda positive, while the third cases was 1+.

To close this report on the results of the second part of our study, we may say that second Mantoux tests up to 1:10 were performed on the 4 children of Group A which had shown any degree of tuberculin sensitivity, and all were then negative. These tests were performed 12 months after the fourth lepromin injection. Thus they demonstrated



FIGS. 1-4. Examples of the stronger lepromin reactions in the children of Group A, subjected to four serial injections of lepromin. There were various degrees of positivity, but in only one instance (Fig. 4) was there necrosis and ulceration among the children of this group. FIGS. 5-10. Examples of the positivity of the Mitsuda reaction to the single lepromin test made in Group B, nine months after the BCG vaccination. All of these reactions presented more or less marked necrosis.

the fleeting character of the slight lepromin-induced tuberculin sensitivity presented by the cases studied.

GENERAL CONSIDERATIONS

The findings here reported confirm our previously published experiments concerning the effects of orally-administered BCG. Mitsuda positivity can be induced in nonreactors by BCG vaccination, the lepromin test then causing strong Mitsuda reactions of the necrotic type in the great majority of cases. Thus, of the 20 children who got three doses of BCG administered at weekly intervals, 17 (85%) responded to the lepromin test 9 months afterwards with necrotic (3+) reactions. The 3 cases that remained negative (including one with only a 1+ reaction) were revaccinated with four doses of BCG, after which 2 of them were 3+ Mitsuda positive. This brought the final number of strongly positive reactors to 19, or 95 per cent of the group. The one case regarded as negative gave only a 1+ final reaction.

It should be emphasized that none of these children had had any lepromin test performed previous to oral BCG vaccination. This is important because it shows most definitely the capacity of oral BCG to cause the induction of Mitsuda reactivity. This fact proves incorrect the opinion sometimes expressed, namely, that the induction of lepromin positivity is not due to the action of the vaccine, but results from the effects of a lepromin injection made before vaccination.

This demonstration was also reported in one of our previous studies (⁶), in which oral BCG and intradermal lepromin were administered simultaneously to children who, also, were isolated from their parents at birth and kept in a closed environment. This resulted in a 100 per cent response to the Mitsuda test, whereas control children—not vaccinated and living under the same environmental conditions as the others—did not give a single positive reaction to lepromin injected on the same day.

The present experiment proves the same fact by other means. Of the 17 children who became Mitsuda positive, only 14 developed tuberculin allergy (1:10 Mantoux), 3 remaining tuberculin negative. This fact again confirms that which we have already exhaustively discussed in previous papers (^{4,5}), namely, that the absence of tuberculin sensitivity in BCG-vaccinated subjects does not mean that the vaccine has not been absorbed. The absorption has been proved in the three above-mentioned cases through the production of a positive response to the Mitsuda test.

The demonstration of the positivizing action of oral BCG is all the more eloquent if we consider that, in the present experiment, we also had a control group of the same number of children, kept under the same environmental conditions, and which was neither BCG-vaccinated nor lepromin tested. At the end of the experiment that test was per-

formed in this control group on practically the same day as in the other one. While of the vaccinated lot 85 per cent were Mitsuda positive, all of the control group remained negative.

We must add further that the security of this experiment lies in the important fact that it deals with children isolated in a closed environment from their first 24 hours of life. Thus they existed in complete absence of leprous or tuberculous infection, so that not a single child of the control group was positive to the 1:10 Mantoux test.

These truly privileged experimental conditions enabled us to verify, besides the work mentioned above—aside from any intervening causes which might alter the results—the capacity of lepromin itself to evoke in the organism conditions of Mitsuda positivity at subsequent tests.

In the present study we followed a group of 21 children, kept in the same environmental conditions as the other groups, not vaccinated but given four lepromin injections at intervals of about three months. On following the evolution of results at 30-day readings we observed (Table 3) the following results:

First test:	No positive reaction.
Second test:	4 cases 1+.
Third test:	5 cases 1+, 3 cases 2+.
Fourth test:	6 cases 1+, 4 cases 2+, and 4 cases 3+.

These readings were reviewed 90 days after the injections because of objections that have been raised as to the variation of results on very late readings. Regarding this matter, we draw attention only to the results of the fourth test, because they refer to the end of the study. Thus, of the 6 cases with 1+ reactions at 30 days, 5 had become negative by the 90th day and the other had increased to 3+. Of the four 2+ cases, 2 remained unchanged and 2 increased to 3+. The four 3+ cases remained unchanged.

Referring now only to the final percentages of positive lepromin results shown in this paper, we may summarize as follows:

Group A, Lepromin retested	43 per cent.
Group B, BCG vaccinated	85 per cent.
Group C, Control	0 per cent.

The analysis of these results, as has already been shown, is statistically significant, presenting $\chi^2 = 7.842$.

One must consider now that in the group that received repeated lepromin injections, contrary to what was observed in the BCG-vaccinated lot, who revealed mass conversion of the Mitsuda reaction of marked intensity (necrotic response), there was a slow progression of positive reactions, so that at the end of the experiment only 9 of the 21 individuals gave 2+ or 3+ reactions. There was only one necrotic reaction, and that was less marked than those usual in the BCG group, as can be seen in the pictures reproduced.

In our previous experiments the intervals between the individual

lepromin tests were approximately one year as a rule, and when they were shorter the subjects had not been submitted to as many repeated lepromin injections. At that time (⁷) we had the opportunity of studying subjects who were persistently negative to lepromin given 3, 4, 5 and even 6 times at one-year intervals, but whose response turned positive after BCG vaccination. In our previous studies we did not have the opportunity to make repeated injections at relatively short intervals thus to introduce—as in the present experiment—a larger quantity of lepromin in a limited period of time. This latter procedure offers to the subjects of the experiment a greater chance to respond positively to a lepromin injection because of simple accumulation of more antigen.

We have shown elsewhere that lepromin may remain in the skin for a long time without being absorbed. In previous reports we stated that subjects in whom lepromin tests made 1, 2, or even 3 years before had been negative, and who were then subjected to oral BCG vaccination, might present positive reactions in the skin sites in which lepromin had been injected years before. This reaction we called “remote Mitsuda positivization.”

The possibility of lepromin being able to induce Mitsuda reactivity in organisms in which one can accumulate more antigen by repeated tests at short intervals, can be explained by comparison with the attainment of the Koch phenomenon by means of intradermal injections of dead human tubercle bacilli, or dead BCG. As it is, lepromin contains varying quantities of dead leprosy bacilli, according to the techniques of preparation employed. In fact, if the researcher is able to obtain large quantities of *Mycobacterium leprae* free from the tissues and other elements that are contained in lepromin, in order to inject them in higher doses, the late (or Mitsuda) reaction may be stronger. In leprosy infection, as well as in tuberculosis, one must consider the biologic and antigenic properties of the physicochemical constituents of the bacterial cell, independent of the factor of vitality.

It remains to be proved whether the positive Mitsuda response obtained by lepromin retesting has the same immunologic significance as has the positivity resulting from virulent infection or BCG vaccination. Regarding the immunology of tuberculosis, it is known that immunization is truly effective only when induced by live germs. However, only further research in leprology can in the future say whether or not the antileprosy immunity has the same biologic characteristics as that of tuberculosis.

Obviously, regardless of the fact that the immunizing potentiality of the dead leprosy germ is yet to be proved, the practical applicability of repeated lepromin injections for immunizing purposes is out of the question. This is so because of the prolonged period of time required to obtain positive effects, and also because of the increasing difficulty in getting the antigen for any purpose whatever. It is to be borne in

mind that of the 21 children submitted to four antigen injections, 12 still were Mitsuda negative after one year. With the BCG vaccination then performed, it was possible quickly to positivize 50 per cent of these negatives.

Before ending we should like to refer to a most interesting point, concerning the capacity of the Hansen bacillus to induce allergy to tuberculin. It is already known that it is possible to obtain allergic conditions (of varying duration), whether in "anima nobile" or in "anima vile," by means of dead virulent tuberculosis bacilli or dead avirulent bacilli (BCG). Many authors have reported tuberculin sensitivity, a truly cross allergy, as an effect of the injection of lepromin. The most rigorous experimental studies, as for instance that of Melsom (²), employing macerated lepromas suspended in water, or of Hadler and Zitti (¹), employing leprosy bacilli obtained from lepromas by means of chloroform, show that the tuberculin sensitivity thus obtained is of the hypoergic type and only transient, disappearing after some time.

In Group A of our present experiment, subjected to lepromin re-testing, 4 cases reacted to 1:10 Mantoux performed one month after the third lepromin inoculation. All of them, however, were tuberculin negative when tested again one year after the fourth and last lepromin injection. This verification of the evolution in children of transitory tuberculin allergy caused by repeated lepromin inoculations at relatively short intervals, we credit with a value practically comparable to the experimental verifications mentioned above, because of the special conditions of our observations, performed in a closed environment on subjects isolated on their first day of life. It is to be remembered that the BCG-vaccinated group showed tuberculin allergy on a much bigger scale, as was to be expected, and that the nonvaccinated control group who underwent only one Mitsuda test remained wholly anallergic.

These appearances of tuberculin allergy, if only transient, in a number of cases repeatedly injected with lepromin might bear the same explanation given above of the summing up of antigenic quantities in these organisms as a result of repeated lepromin inoculations at short intervals. The similarity of chemical composition of some of the antigenic fractions which constitute the mycobacteria explains the fact, and opens new channels of speculation on the intricate problem of the immunobiologic relations between leprosy and tuberculosis.

SUMMARY AND CONCLUSIONS

The purpose of the present paper is to answer objections raised by some authors that conversion of Mitsuda negativity after BCG vaccination is caused by the antigenic action of the lepromin test previous to the vaccination. To this end there have been studied the effects upon the Mitsuda reaction of repeated lepromin injections, in comparison

with the effects of orally administered BCG without previous lepromin test.

Results obtained with 61 children of parents with leprosy are reported (2 children were lost from the experiment, one by death and one by removal to another institution). These children, taken from their parents in their first day of life, ranged in age at the beginning of the experiment from 3 days to 12 months 6 days. They were divided by lot into three groups so as to make the age relationship in each group approximately the same.

Group A: 21 nonvaccinated children who underwent 4 lepromin tests at intervals of about 3 months. After the fourth injection the Mitsuda reaction was positive in 9 cases (43%), only one of them strong enough to be necrotic. The 12 negatives were afterwards given BCG, and then 7 more (58% of the negatives) gave positive Mitsuda reactions.

Group B: 20 children BCG-vaccinated orally with three weekly doses of 0.10 or 0.20 gm., according to age. A lepromin test performed nine months after the vaccination gave 17 (85%) strongly positive (necrotic) Mitsuda reactions. The other 3 cases (one 1+ and two negative) were given another course of BCG vaccination, after which they were retested with lepromin; 2 of them then gave strongly positive (necrotic) Mitsuda reactions.

Group C: 20 control children did not get BCG and were tested with lepromin for the first time 9 months after the study was begun. All were completely negative.

Analysis of the results from the groups studied proved them to be statistically significant, with $\chi^2 = 7.842$, confirming the superiority of BCG concerning the conversion of the Mitsuda reaction.

On the other hand, it has been proved again that the Mitsuda reactivity after oral vaccination with BCG is stronger—all of the positive reactions being of the necrotic type—than that determined by repeated injections of the lepromin antigen.

The true immunologic significance of Mitsuda reactivity induced in such ways is discussed, bearing in mind what is known of mycobacterial immunity as in tuberculosis, in which it can be induced effectively only with living germs.

Repeated inoculations of lepromin at short intervals introduces into the organism an appreciable amount of dead leprosy bacilli, whose chemical fractions have antigenic action, to be compared with the possibility of producing the Koch phenomenon with dead tuberculosis bacilli, virulent or avirulent (BCG).

The orally-administered BCG is able to cause prompt mass conversion of the Mitsuda reactivity, whereas the positivizing effect of repeated lepromin injections is much less frequent, and is slower and weaker.

Investigation of tuberculin allergy showed the control group to be

totally negative to 1:10 Mantoux. In the BCG group of 17 children who gave positive Mitsuda responses, 14 were found tuberculin positive. The 3 tuberculin negatives confirm previous conclusions that the absence of tuberculin sensitivity after BCG vaccination does not prove that orally administered BCG has not been absorbed.

In the lepromin retest group, 4 children became tuberculin positive (1:10 Mantoux), but tests made 12 months after the last lepromin injection were all negative.

The results of this study are of great interest as concerns the better knowledge of similar chemical fractions of the Koch and Hansen bacilli, as well as for the development of studies on the immunobiologic relations between tuberculosis and leprosy.

RESUMEN Y CONCLUSIONES

Tiene por objeto el trabajo actual refutar los reparos ofrecidos por algunos autores, al efecto de que el viraje de la reactividad de Mitsuda después de la vacunación con BCG se debe a la acción antigénica de la reacción a la lepromina, ejecutada antes de la vacunación. Con tal objeto, se han estudiados los efectos de repetidas inyecciones de lepromina sobre la reacción de Mitsuda en comparación con los efectos del BCG administrado oralmente sin previa reacción de la lepromina.

Se presentan los resultados obtenidos en 63 niños de padres leproso. Estos niños, separados de los padres el primer día de sus vidas, variaban en edad al comenzar el experimento de 3 días a 12 meses y 6 días. Se les dividió por lote en tres grupos de modo que la relación por edades en cada grupo fuera aproximadamente idéntica.

Grupo A: 21 niños no vacunados que experimentaron reacciones a la lepromina a plazos de unos 3 meses. Después de la cuarta inyección, la reacción de Mitsuda era positiva en 9 casos (43%), siendo en uno solo suficientemente intensa para resultar necrótica. Los 12 negativos recibieron luego BCG, y entonces 7 más (58% de los negativos) acusaron reacciones de Mitsuda positivas.

Grupo B: 20 niños vacunados con BCG oralmente con tres dosis semanales de 0.10 ó 0.20 gm., conforme a la edad. Una prueba de la lepromina ejecutada a los nueve meses de la vacunación mostró 17 (85%) Mitsudas intensamente positivas. Los otros 3 sujetos (uno + y dos negativos) recibieron otra serie de vacunación BCG, después de la cual fueron recomprobados con lepromina; 2 de ellos acusaron entonces Mitsudas intensamente positivas (necróticas).

Grupo C: 20 niños testigos no recibieron BCG y fueron comprobados con lepromina por primera vez a los 9 meses de comenzar el estudio. Todos resultaron absolutamente negativos.

El análisis de los resultados observados en los grupos estudiados demostró que eran estadísticamente significativos, con $\chi^2 = 7.842$, confirmando la superioridad del BCG en lo tocante al viraje de la reacción de Mitsuda.

Por otro lado, se ha demostrado de nuevo que la reactividad de Mitsuda es más intensa después de la vacunación oral con BCG—siendo todas las reacciones positivas de la forma necrótica—que la determinada por repetidas inyecciones del antígeno de lepromina.

Se discute la verdadera importancia inmunológica de la reactividad de Mitsuda inducida de tales modos, teniendo presente que no puede inducirse eficazmente más que con gérmenes vivos.

Las repetidas inyecciones de lepromina a breves plazos introducen en el organismo una cantidad apreciable de bacilos leproso muertos, cuyas fracciones químicas ejercen efecto antigénico, que hay que comparar con la posibilidad de producir el fenómeno de Koch con bacilos tuberculosos muertos, virulentos o avirulentos (BCG).

El BCG administrado por vía oral es capaz de provocar rápido viraje en masa de la reactividad de Mitsuda, mientras que el efecto positivante de las inyecciones repetidas de lepromina es mucho menos frecuente y más lento y débil.

La investigación de la alergia tuberculínica demostró que el grupo testigo era totalmente negativo a la Mantoux al 1:10. En el grupo becegetizado de 17 niños que acusaron Mitsudas positivas, 14 resultaron positivos a la tuberculina. Los 3 tuberculino-negativos restantes confirman conclusiones anteriores de que la falta de sensibilidad a la tuberculina después de la vacunación BCG no demuestra que no se haya absorbido el BCG administrado oralmente.

En el grupo de recomprobación con lepromina, 4 niños se volvieron tuberculino-positivos (Mantoux al 1:10), pero todas las pruebas ejecutadas a los 12 meses de la última inyección de lepromina resultaron negativas.

Los resultados de este estudio revisten mucho interés en lo relativo a un conocimiento mejor de las fracciones químicas semejantes de los bacilos de Koch y de Hansen, así como para la formulación de estudios acerca de las relaciones inmunobiológicas entre la tuberculosis y la lepra.

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