Delayed Lepromin Reaction and BCG Application in First Grade School Children in S. Paulo, Brazil[1, 2]

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Several studies have claimed that BCG seems to induce a positive Misuda lepromin reaction and to increase resistance to leprosy ([8, 9, 21]).

We observed, in cell cultures, that human macrophages from healthy or tuberculoid Mitsuda-positive people, cause the lysis of *M. leprae* and that the macrophages from healthy or lepromatous Mitsuda-negative people phagocytose but can't destroy the bacilli. We have classified those whose cells destroy the bacilli as LPT positive and the others as LPT negative, because our test was named the Leprosy Prognostic Test ([2, 3]).

According to the literature the percentage of Mantoux and Mitsuda positive people increases with age ([4, 5, 6, 10, 13, 14]) so we decided to study these reactions in children of the youngest age which can be found together through social circumstances, these being children in the first grade of three schools. The objective was to study the positive Mitsuda reaction conversion as induced by BCG.

MATERIALS AND METHODS

Eight hundred and sixty-three healthy children, 7 through 12 years old, from both sexes, statistically distributed in three groups designated A, B and C, having 282, 289 and 292 children each respectively.

All of them were subjected to the Mantoux and Mitsuda tests in the left and right arms respectively, with 2 T.U. PPD tuberculin and 0.1 ml of standardized lepromin ([11]) containing 40 x 10^6 Hansen's bacilli per milliliter[4].

The Mantoux reaction was read after 72 hours and the Mitsuda reaction at 28 days. Children positive to one or both reactions were excluded from the study.

The negative children received the following treatment:

Group A—intradermal BCG (99 children)
Group B—oral BCG (99 children)
Group C—no BCG (control group, 142 children)

The intradermal BCG was given in one injection while oral immunization was by weekly doses of 20 mg of bacilli for three weeks.

After 90 days all three groups were tested for tuberculin and lepromin delayed hypersensivity and after 180 days group A was tested for lepromin reactivity only.

Before giving BCG the LPT was performed with macrophages from ten negative children from each group.

Ninety days after BCG innoculation the LPT of 25 positive (++) children of Group A, and 15 of Group B was determined. In Group C the LPT was performed on one child who became lepromin positive. The test was also performed on five other lepromin positive children who were positive (+++) before BCG innoculation.

RESULTS

Table 1 summarizes the results of the Mantoux and Mitsuda reactions before BCG innoculation.

Only the Mantoux negative children (column a) were given the lepromin test; therefore the totals in columns a and i are identical.

The LPT on 30 children who were negative to both reactions was also negative in

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4 PPD from the Statens Seruminstitut of Denmark supplied by the Nat. Serv. Tuberculosis, Rio de Janeiro, Brazil. Lepromin from Dr. M. Tuma, Inst. of Leprology, Rio de Janeiro, Brazil. BCG from Dr. K. B. Christensen, Statens Seruminstitut Denmark.

TABLE 1. *Reactions of children to tuberculin and lepromin before BCG innoculation.*

	Mantoux				Mitsuda					
	a	b	c	d	e	f	g	h	i	j
Reaction / Group	0–4 mm (−)	5–9 mm (±)	10 & + mm (+)	Total	<3 mm (−)	3–5 mm (+)	>5 mm (++)	ulceration (+++)	Total	percentage of positives
A	231	18	33	282	99	78	51	3	231	54/231 23.4%
B	257	10	22	289	99	97	59	2	257	61/257 23.7%
C	262	16	14	292	142	86	29	5	262	34/262 13.0%
TOTAL	750	44	69	863	340	261	139	10	750	149/750 19.8%

all instances; that is, their macrophages were unable to destroy *M. leprae*.

Table 2 presents the results of Mantoux and lepromin testing subsequent to BCG innoculation in those children who were initially negative to both sensitivity testings.

All the Mitsuda positive children were LPT positive.

DISCUSSION

The number of children initially sensitive to tuberculin and lepromin, as shown in Table 1, indicates that in nature the occurrence of antigens sensitizing to lepromin is close to double that of tuberculin sensitizing antigens (compare columns c and g-h).

In some countries where leprosy is more prevalent, sensitivity to lepromin is greater than in other countries ([10]). This appears to be related with a genotypic factor that is responsible for LPT positivity when in contact with the appropriate antigens ([1, 2, 3, 7]).

Table 2 indicates that 15 of 99 children of Group A did not convert from Mantoux negativity to Mantoux positivity and in Group B 57 of 99 did not convert to Mantoux positivity. In other words, BCG induction of Mantoux positivity was much better by the intradermal than by the oral route.

On the other hand, 90 days after BCG, 47.5% and 27.3% of the children from groups A and B respectively became positive to lepromin while only 0.7% from Group C did the same. We had expected that BCG, by the intradermal route would induce lepromin positivity in 70% to 80% of the children as found previously in other countries and in Brazil ([2, 3, 10]) but we obtained only 47.5% positive conversion. Two possible explanations of this are offered. The children were from an abnormal (vitiated) population since they were from the disadvantaged segment of the population. Another factor might be that lepromin sensitivity induced by BCG may take more than 90 days to develop in some of the treated children. Since Group A reacted more strongly to the Mantoux and lepromin tests, an additional lepromin determination was made in the 51 children of this group at 180 days after the BCG (column 3-5 mm, Table 2).

The results, also presented in Table 2, showing that the lepromin reacting children increased from 47.5% to 60.1% indicates that some children react more slowly to BCG as an inducer of lepromin hypersensitivity.

TABLE 2. *Mantoux and lepromin test results following BCG innoculation.*

	Mantoux 90 days after BCG				Mitsuda 90 days after BCG					Mitsuda 180 days after BCG				
Reaction / Group	0–4 mm (−)	5–9 mm (±)	10 & + mm (+)	% of positives	<3 mm (−)	3–5 mm (+)	>5 mm (++)	ulceration +++	% of positives	3 mm	3–5 mm	5 mm	ulcera	% of positive
A	6	9	84	84/99 85.0%	1	51	47	0	47/99 47.5%	1	37	61	0	61/99 60.1%
B	35	22	42	42/99 42.5%	23	49	27	0	27/99 27.3%		not done			
C	140	1	1	1/142 0.7%	97	44	1	0	1/142 0.7%		not done			

The results of the LPT confirmed previous work ([2, 3]) since all those initially sensitive or having BCG-induced lepromin sensitivity were LPT positive and all who were Mitsuda negative were also LPT negative.

It had been initially decided not to give BCG to children with a tuberculin reaction greater than 5 mm and to give BCG only to children with less than 3 mm response to the first lepromin test. This was decided in order to obtain only very clear conversions to lepromin positivity.

The results of this study support Bechelli *et al* ([4]) in their statement that BCG can only induce lepromin reactivity in children who are LPT positive, but it can not induce resistance in the LPT negative segment of population which will remain lepromin negative.

In this manner BCG can be expected to diminish the number of tuberculoid cases of leprosy but the number of lepromatous instances will remain the same in the population even if widespread BCG innoculation is used.

SUMMARY

Eight hundred and sixty three healthy children from first grade schools were distributed statistically in three groups, and tested for tuberculin and lepromin reactivity. Of those who were negative to both intradermal reactions one group received intradermal BCG, another received BCG by the oral route and a third group was kept as control, without any treatment.

After 90 days, the children were tested for Mantoux and Mitsuda reactivity and after 180 days the first group only, was retested for lepromin sensitivity. At 90 days, 47.5% of the intradermal BCG group became Mitsuda positive, while oral BCG converted only 27.3% and in the controls only 0.7% became Mitsuda positive. At 180 days, 60.1% of the intradermal BCG group were Mitsuda positive, indicating that some children respond more slowly to BCG induction of lepromin hypersensitivity. It was concluded that the intradermal administration of BCG in young people induces lepromin reactivity faster than naturally induced reactivity.

The lysis of *M. leprae* in cell cultures of macrophages (LPT) from the children demonstrated that lepromin reactive persons can destroy the bacilli in less than 20 days either in the naturally occurring or BCG induced Mitsuda positivity.

It is therefore concluded that BCG can induce protection in that segment of the population whose macrophages can be induced to become lytic for *M. leprae*. That segment of population which remains LPT negative is without resistance and, if infected, will be lepromatous.

In public health surveys the result of the lepromin reaction, on first determination is valid only when it is positive. When it is negative the Mantoux reaction should be done and if it is negative, intradermal BCG should be given and the lepromin reaction then be repeated after 90 and 180 days and only then scored as negative if it remains so. When the Mantoux test is positive, a negative lepromin test is valid.

The positive Mitsuda reaction is a macroscopic demonstration of the macrophagic capacity to lyse *M. leprae*.

RESUMEN

Ochocientos sesenta y tres niños sanos, de escuelas primarias, se distribuyeron en forma estadística en tres grupos y se les hicieron pruebas con tuberculina y lepromina. De aquellos que resultaron negativos a ambas reacciones intradérmicas, un grupo recibió BCG en forma intradérmica, otro recibió BCG por vía oral y un tercer grupo se mantuvo sin ningún tratamiento, como control.

Noventa días después se les hicieron pruebas a todos los niños para determinar su reactividad ante el Mantoux y el Mitsuda y después de 180 días se volvió a probar la sensibilidad hacia el Mitsuda del primer grupo solamente. Después de 90 días 47,5% del grupo que había recibido BCG intradérmico se volvió Mitsuda positivo, mientras que el BCG oral convirtió solamente a 27,3% y de los controles solamente 0,7% se volvieron Mitsuda positivos. A los 180 días, 60,1% del grupor con BCG intradérmico dió una reacción de Mitsuda positiva, indicando que algunos niños responden más lentamente a la inducción de sensibilidad hacia la lepromina por BCG. Se concluyó que la administración intradérmica de BCG en personas jóvenes induce reactividad hacia la lepromina con mayor rapidez que la reactividad de inducción natural.

La lisis del *M. leprae* en cultivos celulares de macrófagos (LTP) de los niños demostró que

las personas reactivas hacia la lepromina pueden destruir el bacilo en menos de 20 días, ye sea en la positividad hacia el Mitsuda que se produce naturalmente o en la inducida por el BCG.

Se concluye, por lo tanto, que el BCG puede inducir protección en aquel segmento de la población cuyos macrófagos pueden ser inducidos a transformarse en líticos con respecto al *M. leprae*. Aquel segmento de la población que permanece LPT negativo no tiene resistencia y si se infecta será lepromatoso.

En encuestas sanitarias el resultado de la reacción hacia la lepromina en la primera determinación es válido solamente cuando es positivo. Cuando es negativo, se debe hacer la reacción de Mantoux y si ésta es negativa, se debe administrar BCG intradérmico y luego repetir la reacción con lepromina después de 90 y 180 días y sólo entonces marcarla como negativa si aún permanece así. Cuando el Mantoux es positivo, una prueba negativa de lepromina es válida.

La reacción de Mitsuda positiva es una demostración macroscópica de la capacidad macrofágica para lisar al *M. leprae*.

RÉSUMÉ

Huit cent soixante trois enfants en bonne santé, fréquentant des écoles primaires ont été testés quant à leur réactivité à la tuberculine et à la lépromine. Parmi ceux qui étaient négatifs à l'une et l'autre de ces réactions intradermiques, certains ont reçu du BCG par voie intra-dermique, d'autres ont reçu du BCG par la voie orale et un troisième groupe a été utilisé comme témoin, ne recevant aucun traitement.

Après 90 jours, les enfants ont été testés pour la réactivité de type Mantoux et de type Mitsuda; après 180 jours, le premier groupe, et celui-là seulement, a été testé à nouveau pour la sensibilité à la lépromine. Au 90 ème jour, 47,5 pour cent du groupe ayant reçu du BCG intradermique était devenu positif pour la réaction de Mitsuda; par contre, le BCG par voie orale n'a entraîné un virage de la réaction à la lépromine que chez 27,3 pour cent des individus, alors que chez les témoins, 0,7 pour cent seulement étaient devenus Mitsuda-positifs. Au cent quatre-vingtième jour, 60,1 pour cent du groupe ayant reçu du BCG intradermique était positif pour la réaction de Mitsude, ce qui indique que certains des enfants répondaient plus lentement à l'induction d'une hypersensitivité à la lépromine par le BCG. On en conclut que l'administration intradermique de BCG chez des individus jeunes provoque une réactivité à la lépromine plus rapidement que ne le font les facteurs naturels qui peuvent intervenir dans le développement d'une telle réactivité.

L'étude de la lyse de *M. leprae*, dans les cultures cellulaires de macrophages (LPT) provenant de ces enfants, a montré que les personnes réagissant à la lépromone peuvant détruire les bacilles en moins de 20 jours, tant les individus présentant une positivité naturelle de type Mitsuda que ceux chez lesquels cette réactivité avait été induite par le BCG.

On en conclut dès lors que le BCG peut protéger cette fraction de la population chez laquelle les macrophages peuvent être rendus lytiques pour *M. leprae*. Cette portion de la population restant LPT négative est dépourvue par contre de résistance; si elle est infectée, elle développera une lèpre lépromateuse.

Dans les enquêtes de santé publique, le résultat de la réaction de la lépromine est valide, à première vue, seulement lorsqu'il est positif. Lorsque cette réaction est négative, la réaction de Mantoux devrait être pratiquée; si celle-ci est négative, il faudrait administrer du BCG par voie intra-dermique et al réaction à la lépromine devrait alors être répétée après 90 et 180 jours; les malades ne devraient être considérés comme négatifs que si cette réaction à la lépromine reste négative. Lorsque le test de Mantoux est positif, une épreuve négative à la lépromine est valide.

Une réaction de Mitsuda positive constitue une démonstration macroscopique de la capacité macrophagique à lyser *M. leprae*.

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