THE PROTECTIVE ROLE OF BCG IN MURINE LEPROSY [1]

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Various reports, among them one presented by me at the Havana Congress in 1948 (1), have confirmed the finding of Fernandez in 1939 (3) that persons who react negatively to lepromin can be made to react positively by means of BCG vaccination. Because lepromin positivity is generally regarded as an indication of resistance to leprosy infection, I recommended at that time that children removed to preventoria should be protected by BCG vaccination.

The Havana Congress did not include BCG vaccination in their recommendations regarding preventive measures against leprosy. That was done, however, three years later by the Third Pan-American Conference, convened in Buenos Aires in December 1951. At present the National Leprosy Service of Brazil has two large-scale projects in progress, one in Goías under Nelson de Souza Campos, and another in North Iguaçú, State of Río, under Candido de Oliveira. Of necessity, however, an observation period of at least 5 years and perhaps 15 years is needed before we can properly evaluate from that work the role of BCG in the prevention of leprosy.

Although it is an established fact that natural reactivity to lepromin signifies resistance to infection, there are some leprologists who are uncertain that induced positivity resulting from BCG vaccination has the same significance. The final answer to this question, as said, can be reached only after long observation.

In the meantime, as a possible contribution to the subject, I undertook in 1952 a study of the effects of BCG vaccination in murine leprosy. A preliminary report (2) was presented at the Tenth Brazilian Congress of Hygiene, held in Belo Horizonte in October 1952, in which it was stated that the inoculation sites seemed to be more infiltrated in the controls than in the BCG-vaccinated rats, while in two of the four vaccinated animals the smears from those sites showed substantially fewer bacilli than those from the controls. No conclusions were drawn at the time, but a better-substantiated report was made in May 1953 before the Associação Brasileira de Leprologia. At present, with experience on a larger scale, it can be affirmed that BCG confers a certain degree of protection against murine leprosy.

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[1] Presented at the VI International Congress of Leprology, Madrid, October 1953. Condensed translation from the Portuguese, approved by the author.

TECHNIQUE

White rats were used, of approximately the same age and maintained under the same conditions of diet and environment. A total of 77 animals was used, in the six groups listed below. The inoculations with *Mycobacterium leprae murium* were made either subcutaneously in the hind leg or into the peritoneal cavity. In all instances the dose was 0.5 cc. of a rich suspension of a leproma four months old.

Group 1.—Nineteen rats were inoculated with 20 mgm. of BCG in the right hind leg, and 115 days later the test inoculations were made in the same leg.

Group 2.—Nineteen rats were inoculated with 20 mgm. of BCG in the right hind leg, and 115 days later the test inoculations were made in the left hind leg.

Group 3.—Nine rats were inoculated with 20 mgm. of BCG in the peritoneal cavity, and 115 days later the test inoculations were made in the same site.

Group 4.—Ten rats were inoculated with 20 mgm. of BCG in the peritoneal cavity, and 115 days later the test inoculations were made in the left hind leg.

Group 5 (controls).—Thirteen rats were inoculated with the leproma suspension in the right hind leg on the same day that the test inoculations were made.

Group 6 (controls).—Seven rats were inoculated with the leproma suspension in the peritoneal cavity on the same day the test inoculations were made.

RESULTS

The results of the experiment were evaluated on the basis of the clinical, bacteriological and anatomopathological findings.

Clinical evaluation.—Table 1 gives in condensed form the findings after four and six months in the animals inoculated subcutaneously with *M. leprae murium* (Groups 1, 2, 4 & 5). Regarding those inoculated intraperitoneally (Groups 3 and 6), the only clinical feature of interest

TABLE 1.—*Clinical changes seen after 4 and 6 months in BCG-vaccinated rats and in controls inoculated subcutaneously with* M. leprae murium.

Changes in the inoculation sites	Vaccinated rats				Control rats			
	4 mos. after inoculation		6 mos. after inoculation		4 mos. after inoculation		6 mos. after inoculation	
	No.	%	No.	%	No.	%	No.	%
Normal	24	57.1	1	2.7	2	16.6	----	----
Infiltrated	8	19.0	13	35.1	2	16.6	2	18.1
Nodule	5	11.9	8	21.6	----	----	----	----
Tumor, intact	4	9.5	8	21.6	4	33.3	4	36.3
Tumor, ulcerated	1	2.3	7	18.9	4	33.3	5	45.4
Total	42	99.8	37	99.9	12	99.8	11	99.8

is that at the end of six months there were infiltrations in the abdominal wall of all 7 of the controls, but not in any of the 9 which had been given BCG.

It is evident from these data that the development of the lesions progressed more slowly in the BCG-vaccinated animals than in the controls.

Bacteriological evaluation.—Morphologically and tinctorially we saw no differences between the bacilli from either lot of rats, vaccinated or controls. On the other hand, from the point of view of the distribution of dissemination the differences were more marked, as is shown in Table 2. It is seen there that in the unvaccinated animals there was a greater tendency to dissemination of the infection than in the controls.

TABLE 2.—*Organs found bacteriologically positive in BCG-vaccinated rats and controls.*

Examined areas	Vaccinated rats		Control rats	
	Number of examinations	Positive	Number of examinations	Positive
Inoculation site	12	12 (100%)	7	7 (100%)
Lymph nodes	12	1 (8.3%)	7	4 (57.1%)
Spleen	12	3 (25%)	7	3 (42.8%)
Liver	12	2 (16.6%)	7	3 (42.8%)
Lungs	12	2 (16.6%)	7	3 (42.8%)

Anatomopathological evaluation.—Macroscopically, it was observed at autopsy there was a greater diffusion of the process in the animals not given BCG than in those that were vaccinated, although microscopically there were no differences in the reactivity of the tissues in the two groups of animals.

SUMMARY AND CONCLUSIONS

For the purpose of studying the possible protection by BCG in murine leprosy infection, 57 rats were inoculated with 20 mgm. of BCG subcutaneously or intraperitoneally, and 115 days later they were inoculated with *M. leprae murium*. Twenty rats were used as controls. The experiment extended over a period of 10 months.

Clinically, the unvaccinated animals showed earlier and larger lesions than the vaccinated ones. Bacteriologically, there was no morphological or tinctorial alteration of the bacilli in the vaccinated animals, but the percentages of positivity in the visceral organs (spleen, kidney, lungs) were lower than in the unvaccinated animals, which shows a lesser tendency of diffusion of the infection in those previously inoculated with BCG. From the anatomopathological viewpoint, there was a much more extensive involvement by the leprous process in the animals not

given BCG, although the histopathological reactions were the same in both groups.

We therefore conclude that BCG has a protective effect against leprosy infection in rats. Considering the clinical, bacteriological and anatomopathological analogies of the leprotic infections in man and the rat, it is assumed that the results of this experiment will serve to strengthen the view that BCG may be useful in the prevention of leprosy.

RESÚMEN

Con el propósito de estudiar el efecto protectivo de la vacuna BCG en la lepra murina, se inocularon 57 ratas con 20 mgm. de BCG por via subcutánea o intraperitoneal, y 115 días mas tarde se inocularon con *M. lepra murium.* Viente ratas sirvieron de control y el experimento tuvo 10 meses de duración.

Los animales no vacunados desarollaron lesiones mas grandes y mas rápidamente que los vacunados. Bacteriologicamente no hubo diferencias morfologicas entre los bacilos de ambos grupos, pero el porcentage de visceras positivas (vazo, riñon, pulmones) fué menor en el grupo no vacunado, lo que demuestra que mayor tendencia a la difusion de la infección en los animales previamente inoculados con BCG. Anatomopatológicamente se notó que las lesiones del grupo no vacunado fueron mucho más extensos aunque el carácter histológico de las lesiones fué parecido, en ambos grupos. Se concluye que la vacuna BCG tiene efecto protectivo en lepra murina y que éste hallazgo puede utilizarse par reforzor la opinión que BCG es útil en la prevención de la lepra humana.

REFERENCES

1. AZULAY, R. D. A ação do B.C.G. sobre a reação lepromínica. Mem. V Congr. Internac. Lepra, Havana, 1948; O Hospital **34** (1948) 853-856.
2. AZULAY, R. D. B.C.G. e lepra. O Hospital **43** (1953) 215.
3. FERNANDEZ, J. M. M. Estudio comparativo de la reacción de Mitsuda con las reacciones tuberculinicas. Rev. argentina Dermatosif. **23** (1939) 425-452.