THE MANTOUX AND MITSUDA REACTIONS IN HAMSTERS AND GUINEA-PIGS BEFORE AND AFTER VACCINATION WITH BCG

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While investigations were in progress elsewhere in Venezuela on the modifications of the reactions to tuberculin and lepromin after BCG vaccination in groups of humans in leprosy-endemic foci (2), parallel experiments were made with hamsters and guinea-pigs at the Cabo Blanco Leprosarium. The results are reported here.

The problem of lepromin sensitization in animals has attracted the attention of leprologists repeatedly, since Rodriguez in 1938 (5) reported the application of the test to a number of different animals and found that dogs, rabbits and a goat reacted positively.

Wade (6) conducted thorough experiments with dogs at Culion in 1941, and one of his conclusions was that time is a more important factor in the production of positivity than the quantity of antigen injected.

Chaussinand, in his book La Lèpre (1), affirms that guinea-pigs normally do not react positively to lepromin, an experience which differs from ours with the strain of albinos kept at Cabo Blanco.

Olmos Castro (4) studied the effect of BCG vaccination in causing positive reactions to lepromin in guinea-pigs. In animals which he considered to be normally nonreactors he observed that the degree of positivity increased with increase of the dose of BCG. He ascribes more importance to the late than to the early or Fernandez reaction, and he does not consider repeated inoculation with BCG to be necessary to bring about either.

Hadler (3) studied the immunologic response of tuberculin-negative guinea-pigs to heat-killed BCG and to lepromin and found that both mycobacteria evoke histologically similar skin reactions, but that the enzymatic elimination of the leprosy bacillus is much slower than is the case with the tubercle bacillus (BCG).

A. INVESTIGATION IN HAMSTERS

MATERIALS AND METHODS

The antigens used to carry out these tests were PPD, 5 units per 0.1 cc., prepared by the Statens Seruminstitut in Copenhagen, and regular lepromin prepared in the Cabo Leprosarium according to the Mitsuda-Hayashi method. The Calmette-Guerin vaccine (BCG) was prepared by the National Institute for Tuberculosis in Caracas. The rodents used were Syrian hamsters (*Cricetus auratus*).

A total of 120 animals were given intradermal injections of PPD and of lepromin prior to their vaccination with BCG. The animals were examined after 72 hours for the tuberculin reaction, and after one month for the Mitsuda reaction. For the

administration of BCG by various routes and for control, the hamsters were divided into the following 4 groups: (1) oral, 30; (2) intradermal, 30; (3) intracardiac, 10; (4) unvaccinated controls, 50.

Groups A, B and C were vaccinated with BCG 30 days after they had been given the PPD and lepromin injections. The dose given to the intradermal and the intracardiac groups was 0.5 mgm., while the oral dose was 100 mgm. Each of the three BCG groups was subsequently divided into 5 subgroups to be tested with PPD and lepromin in the 4th, 6th, 8th, 12th and 16th weeks following. The animals of the control group were given the same tests at the same time as these subgroups.

RESULTS

In all the 120 hamsters tested prior to the selection of groups for BCG vaccination and for the control, the tuberculin and Mitsuda reactions were negative. All tests with PPD and lepromin given to the subgroups after they had received BCG gave only negative results. The control group also remained negative in every test.

DISCUSSION, PART A

The absence of all reaction phenomenon, both before and after the administration of BCG, seems to demonstrate that the hamster is specifically negative to the tuberculin and lepromin reactions, and that BCG vaccination of this animal does not modify its natural lack of reactive capability.

B. INVESTIGATION IN GUINEA-PIGS

MATERIAL AND METHODS

The same antigens and the same BCG from the same sources as those used in the work with hamsters were used in its sequel with guinea-pigs. The animals used were 45 individuals of the albino variety of *Cavia cobaya*. Our strain of this variety may weigh as much as 1,200 gm. when fully grown, and thus it is considerably larger than the common piebald type.

The following groups were formed for BCG vaccination and for control: (1) for oral administration, 5; (2) for intradermal injection, 5; (3) by cardiac puncture, 5; (4) by all three routes, 5; (5) unvaccinated controls, 25.

The control group was subdivided into two age groups, one of ten animals over six months old and another of 15 aged from 2 to 3 months.

Groups 1 to 4 inclusive were vaccinated 36 days after the animals had received the initial tuberculin and lepromin tests. The oral administration of BCG in Groups 1 and 4 inclusive was effected in small doses, until each animal had received a total of 100 mgm. The intradermal group received 0.1 mgm. net of BCG in the mid-abdominal line, and the same dose was given to Group 3 by intracardiac injection. Group 4 received, besides the 100 mgm. orally, 0.1 mgm. intradermally and 0.1 mgm. by intracardiac injection.

The tuberculin and lepromin antigens were injected in symmetrically opposed abdominal sites in quantities of 0.1 cc. each. The readings of the Mantoux test were made 24, 48 and 72 hours after the injections, and at the same time the animals were examined for the early lepromin (Fernandez) reaction. The final readings of the Mitsuda test were made on the 36th day, and the BCG was administered immediately thereafter. The initial tests were read again on the 44th, 61st and 80th days after the first injections, i. e., on the 9th, 24th and 44th days after the animals had received BCG.

The vaccinated animals, as well as those of the control group, were given PPD and lepromin again on the 40th day following vaccination. These second tests were read after 48 hours for the Fernandez reaction and after 72 hours for the Mantoux reaction. The Mitsuda reaction was read 30 days later, i. e., on the 70th day after BCG vaccination.

RESULTS

The readings of the initial tuberculin tests were negative in all the groups, and this was also the case with the Fernandez reaction. The readings of the Mitsuda reaction after 36 days were negative in all the groups, except in 3 of the 5 animals in Group 2, selected for intradermal BCG vaccination; they were spontaneously positive. A biopsy specimen from 1 of the 3 proved to be histologically positive. In this same group, 4 of the animals were positive on the 24th day after BCG vaccination, and all 5 were positive 70 days after.

In Group 1, which received BCG orally and in which all reactions were negative before vaccination, the tuberculin reaction remained negative, but the Fernandez and Mitsuda reactions became positive in the three animals which remained after two had died. The Mitsuda reaction was still positive in those 3 on the 70th day after they had received BCG.

In the 5 animals of Group 3, which received BCG by intracardiac injection, also entirely nonreactive before vaccination, the tuberculin reaction remained negative but the Fernandez one became positive. On the 24th day after vaccination, 4 of the animals were Mitsuda positive and 1 negative, but all 5 were positive on the 70th day.

In the unvaccinated Group 5, it was observed that of the subgroup of 10 older animals, which had been Mantoux and Fernandez negative in the early examinations and were Mitsuda negative when examined 36 days after the injection of the antigens, 5 had become spontaneously positive on the 44th day, and a total of 7 were positive on the 61st day. One animal of this subgroup died. The remaining 2 remained negative. After the second inoculation with PPD and lepromin, the Mantoux test was still negative in all the 9 animals; but the Fernandez was positive in 7, and the Mitsuda remained positive in the 7 animals which were positive on the 44th day. The two negative animals died before the experiment was terminated.

In the subgroup of 15 younger animals, the Mantoux reaction remained negative in all examinations, and in the first one all were likewise negative for the Fernandez and the Mitsuda reactions. However, by the time they received the second inoculations they were nearly adult, and 8 were Fernandez- and 10 Mitsuda-positive.

DISCUSSION, PART B

It is necessary to consider the spontaneous reactions of the control group in any attempt to draw conclusions about the influence of BCG vaccination in the production of positivity to the Mitsuda reaction.

In the subgroup of adult animals it was seen that, although the

Fernandez reaction was negative, at least 7 out of 10 became spontaneously positive for the Mitsuda reaction after the initial test. It was impossible to determine the increase in positivity of the Fernandez reaction on account of the death of the two animals that were needed for comparison.

The different results observed in the two subgroups of control animals may be explained by the difference in age, but we expect in a subsequent investigation to throw some light on the question of whether or not repeated injections of the tuberculin and lepromin antigens have any influence on the positivity. There were no modifications of the Mantoux reaction in spite of the reinoculation, which of course was not to be expected.

With regard to the vaccinated groups, it is to be noted that the Mantoux, Fernandez and Mitsuda reactions became positive in all the animals of the groups which received BCG intradermally, including those that received it by all routes combined, so that we have here an agreement of results as regards these reactions.

In the groups that were vaccinated orally or by intracardiac injection, on the other hand, the Mantoux reaction remained negative in all the animals, while their Fernandez and Mitsuda reactions became positive, thus indicating a disagreement of effect in the tests when the BCG is given to these animals by those two routes.

SUMMARY

The reactions to tuberculin and lepromin have been studied in 120 hamsters (*Cricetus auratus*) and 45 albino guinea-pigs (*Cavia cobaya albina*), before and after the administration of BCG by different routes.

The hamsters were divided into subgroups to be vaccinated with BCG orally, intradermally and by intracardiac injections, and an unvaccinated control group was left. Before vaccination it was made certain that the animals were all negative, and after vaccination tests were made on the 4th, 6th, 8th, 12th and 16th days. None of the animals showed change to positivity. It is concluded that *Cricetus auratus* as a species is not immunoallergically responsive to either PPD tuberculin or lepromin, and that this specific lack of responsiveness is not modified by BCG vaccination.

In the experiment with guinea-pigs, 5 groups were formed and BCG was given by mouth and by the intradermal and intracardiac routes, as also by these routes combined, an unvaccinated group being again set aside as controls.

It is regarded as important that 7 of the 9 control animals became Mitsuda positive, and that the same animals were also Fernandez-positive, although weakly so. The animals that become spontaneously positive to the Mitsuda test were approaching full development, while those in the younger subgroup were negative; but when they were tested again—they then approaching adulthood—the proportion of positive individuals among them became about the same as in the older groups.

Of the vaccinated animals, all became Fernandez and Mitsuda positive. That was not so of the Mantoux reaction, however, for the groups which received BCG by mouth and by intracardiac injection remained quite negative, whereas those that were vaccinated intradermally became Mantoux positive.

RESÚMEN

Los autores estudian las reacciones de un grupo de 120 hamsters (*Cricetus auratus*) y de un grupo de 45 cobayos (*Cavia cobaya albina*) al PPD y Mitsuda antes y después de la administración de la vacuna BCG por diferentes vías.

Los hamsters fueron divididos en subgrupos a ser vacunados con BCG oral, intradérmico e intracardíaco. Un grupo de control fué agregado, que no recibió vacunación BCG. La dosis oral fué de 100 mgm., mientras que la intradérmica y la intracardíaca fueron de 0.5 mgm. Después de estar seguros que todos los animales eran negativos antes de la vacunación, fueron practicados tests en las cuarta, sexta, octava, duodécima y dieciseisava semanas después de la vacunación sin que se observara alteración en los tests iniciales. Los autores concluyen, que el *Cricetus auratus*, como especie, inmunológicamente no responde a los tests de PPD y lepromina y que esta falta de respuesta específica no se modifica por la vacunación BCG.

En los experimentos con cobayos fueron hechos 5 grupos con el propósito de administrar BCG por vía oral, intradérmica e intracardíaca, así como por todas estas vías combinadas y un grupo de control de animales no vacunados fué hecho para comparación.

1) Los autores señalan la importancia de la observación de que 7 de los 9 animales de un subgrupo de control se hicieron Mitsuda positivos y que estos mismos animales fueron también Fernández positivos. Llaman la atención sobre el hecho de que los animales, que se hicieron espontaneamente positivos, estaban próximos a su completo desarrollo, mientras que la negatividad al Mitsuda fué observada en aquellos del grupo mas joven. Tan pronto como estos últimos se aproximaban a la edad adulta, la proporción de los indivíduos espontaneamente positivos llegó a ser igual al del grupo de mayor edad.

2) En los animales vacunados se observó, que todos se hicieron Fernández y Mitsuda positivos después de la vacunación, pero que en los grupos, que recibieron BCG, ya por vía oral, ya por vía intracardíaca, la reacción de Mantoux persistió negativo en contraste con los animales, que recibieron BCG por vía intradérmica o por todas las vías combinadas, en los cuales el Mantoux se hizo positivo.

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