SENSITIZATION OF THE DOG WITH LEPROMIN AND BCG, AND EVIDENCE OF CROSS SENSITIZATION[1]

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In previous reports (2-4), one of us showed that guinea-pig nonreactive to lepromin and tuberculin would, after inoculation with BCG, show reactivity to intradermal injections of integral lepromin. It was thus demonstrated that the phenomenon of cosensitization between BCG and integral lepromin, reported by Fernandez (1) in man, could be verified experimentally. At the same time, however, it was found that guinea-pigs could not be sensitized to lepromin by repeated injections of that antigen, given in varied doses and by different routes.

The primary object of the study here reported was to ascertain whether integral lepromin can create, experimentally, a state of sensitization to BCG, as BCG can to lepromin. We believed that it would be of interest to carry out such an experiment, not only to further the earlier investigations made with the guinea-pig, but also because to our knowledge no such experiments have been made. The guinea-pig could not be used for the experimental animal for this work, because as said we had repeatedly failed to sensitize it with lepromin. Consequently, we decided to use the dog, which had been shown by Wade (6, 7) to be susceptible of sensitization by integral lepromin (Wade phenomenon).

To that end we performed three experiments. (1) The first was designed to study how the dog reacts to single intradermal injections (*a*) of lepromin, and (*b*) of BCG. (2) The second was to find out (*a*) how the dog sensitized to BCG reacts to a second injection of BCG, and (*b*) how a lepromin-sensitized dog reacts to a second injection of lepromin. (3) The third experiment concerned the question of existence of cross sensitization between lepromin and BCG.

MATERIALS

Ten dogs, each of which had been proved negative to an intradermal injection of 0.0002 mgm. of PPD (Parke, Davis), were used.[2] The number of dogs that could be obtained and maintained was limited. Because several of those used in the first experiment had to be reserved for later tests (5), only a single animal could be em-

[1] Communication presented at the third meeting of the Sociedad Argentina de Leprología, Rosario, December 1955. Translation of the Spanish original, approved by the authors.

[2] Three other dogs were also involved, but one died and two escaped. One of the latter ultimately returned and was used in later work.

ployed for each phase of the second and third experiments. However, since the results were clear-cut and consistent they are considered significant.

The antigens used were: (1) a BCG suspension each cubic centimeter of which contained 0.15 gm. of the bacillus,[3] and (2) integral lepromin prepared according to the Mitsuda-Hayashi technique.

Observations of the effects of the injections were made periodically, for the most part at 2-day intervals but not rigidly on that schedule. The days mentioned in the reports of observations are those on which definite changes were seen.

FIRST EXPERIMENT, SINGLE INJECTIONS

A. REACTIONS TO SINGLE INJECTIONS OF BCG

Four dogs were inoculated intradermally in the abdominal region, each with 0.1 cc. of the BCG suspension, the dose containing 15 mgm. of bacilli.

DOG. NO. 1: Inoculated on November 24, 1954. A local reaction began on the 7th day, consisting of a papule which had transformed into an erythematous nodule by the 14th day. This ulcerated 1 week later, and it cicatrized by the 42nd day.

DOG NO. 3: Inoculated on December 8, 1954. On the 14th day a small nodule was observed, which increased in size thereafter until it ulcerated on the 28th day. It healed by the 43rd day.

DOG NO. 6: Inoculated on July 19, 1955. The reaction began with a papule on the 9th day but progressed relatively rapidly. It became a nodule in another 2 days, ulcerated 5 days after that, and healed on the 28th day.

DOG. NO. 7: Also inoculated on July 19, 1955. The reaction started with a papule on the 9th day which developed into a nodule by the 14th day, ulcerated 2 weeks later, and healed by the 41st day.

B. REACTIONS TO SINGLE INJECTIONS OF LEPROMIN

Six dogs were injected intradermally in the abdominal region, each with 0.1 cc. of integral lepromin.

DOG NO. 2: Injected with lepromin on November 24, 1954. On the 14th day there was observed a papule which had transformed into a nodule 1 week later. It ulcerated on the 30th day after the injection, and healed by the 52nd day.

DOG NO. 5: Injected with lepromin on July 13, 1955. On the 15th day a small nodule was seen which increased in size until the 43rd day. Subsequently it regressed without ulceration; it was reabsorbed by the 67th day.

DOG NO. 10: Injected with lepromin on August 4, 1955. After 16 days a small nodule appeared which later increased in size until it became ulcerated on the 28th day. It healed by the 57th day.

DOG NO. 11: Injected with lepromin on August 4, 1955. After 16 days there was seen a small nodule which increased in size until it ulcerated on the 28th day. It had healed by the 57th day.

DOG NO. 12: Injected with lepromin on September 6, 1955. On the 14th

[3] Prepared by the Instituto Malbrán of the Ministry of Social Welfare and Public Health.

day there was found a small nodule, which ulcerated on the 25th day and healed by the 61st day.

Dog No. 13: Injected with lepromin on September 6, 1955. On the 21st day there was observed a papule which increased in size until it became a nodule on the 31st day. This reaction lesion retained its nodular character, without becoming ulcerated, up to the 61st day, the time of the last observation.

Comments on the first experiment.—The intradermal injection of BCG in the dog produces, after a latent period, a local reaction which begins somewhere between the first and second weeks. This initial reaction has the dermatological characteristics of a papule which in the following days increases in size until it assumes the character of a nodule, about the end of the second week. Ulceration usually occurs between the third and fourth weeks, and healing takes place between the fifth and sixth weeks.

The intradermal injection of lepromin causes a reaction similar to that produced by BCG except that the latent period is somewhat longer, the beginning nodule appearing sometime between the second and third weeks. In 5 of the 6 animals the average was 16.4 days, but because in one of them the nodule did not appear until the 31st day the general average was 18.8 days. This nodular reaction increases in size until, at around the end of the fourth week, ulceration usually occurs; but in 2 of these dogs ulceration did not eventuate. Cicatrization is much delayed, to between the seventh and ninth weeks.

The course of evolution of the lesions in the animals of Experiment 1 is summarized in two parts of Table 1. In short, intradermal injection of either BCG or lepromin produces the same kind of local cutaneous reaction, the only difference being that the former appears earlier and progresses more rapidly than the latter.

SECOND EXPERIMENT, REPEATED INJECTIONS

B. REACTION TO A SECOND INJECTION OF BCG

A BCG-inoculated dog (No. 1) of the first experiment, originally inoculated on November 24, 1955, was given a second injection of the same dose of the vaccine on the 28th day after the first one (December 22). At that time the first reaction was still in the stage of ulceration.

After 48 hours there was seen at the injection site an erythematous, edematous infiltration 10 mm. in diameter, of the appearance of a tuberculin raction. On the 7th day this had evolved into an erythematous nodule, markedly elevated above the surrounding normal skin. On the 14th day this nodule had ulcerated, and later it became covered with a scab; it was healed by the 29th day.

B. REACTION TO A SECOND INJECTION OF LEPROMIN

One of the dogs (No. 2) which had been injected with lepromin in the first experiment on November 24, 1954, was given a second intradermal

injection of lepromin. This was on the 28th day after the first one (December 22), when the first reaction was in the nodular stage, shortly before it became ulcerated.

In 48 hours after this second injection there was observed an edematous, erythematous infiltration measuring 10 x 15 mm., which in 5 more

TABLE 1.—*The development of the reactions in dogs to intradermal injections of BCG and lepromin, with evidence of sensitization induced thereby (Experiments 1 and 2).*

Dog[a] No.	Time of observation of each stage (days).			
	Onset[b]	Nodule	Ulceration	Healing
BCG, first injections				
3	—	14	28	43
6	9	11	16	28
7	9	14	28	41
1	7	14	21	42
BCG, reinjection after 28 days				
1	2	7	14	29
Lepromin, first injections				
5	—	15	(none)	67
10	—	16	28	57
11	—	16	28	57
12	—	14	25	61
13	21	31	(none)	(+61)
2	14	21	30	52
Lepromin, reinjection after 28 days				
2	2	7	14	45

a Dogs 1 and 2 are listed out of numerical (and chronological) order to facilitate comparison of the results of the first and second injections.

b First observation of the beginning papule or infiltration.

days developed into a nodule. This nodule became ulcerated on the 14th day, and cicatricized by the 45th day.

Comments on the second experiment.—A second intradermal injection of BCG in a dog inoculated 28 days previously with that antigen produced a local reaction of dermatological characteristics essentially similar—except for the early response—to that produced by the first one, differing mainly as regards the time of its appearance; the reaction to the first injection began on the 7th day, while that to the second one appeared within 48

hours. It is therefore evident that the first injection had altered the normal state of reactivity. In other words, there had been established a state of allergy, or hypersensitivity, to the antigen used.

A second intradermal injection of lepromin in a dog previously injected with that antigen also produced a local reaction essentially similar to that induced by the first injection, again differing mainly as regards the time of its appearance and rapidity of development. The first reaction began on the 14th day, while the second one began within 48 hours. As is the case with BCG, therefore, a first injection of lepromin causes in the dog an alteration of the normal state of reactivity, provoking a state of hypersensitivity. In other words, lepromin is capable of sensitizing a dog in the same way that BCG does.

The course of evolution of the reinjection lesions in these animals is shown in Table 1.

For one thing, these results served to confirm the experiments of Wade on the sensitization of dogs to lepromin. They also suggested that the dog is a good animal for use in experiments of cross sensitization between lepromin and BCG. As has been said, we were not able to produce the Wade phenomenon using the guinea-pig as the experimental animal.

THIRD EXPERIMENT, CROSS SENSITIZATION

A. DOG SENSITIZED WITH BCG AND INJECTED WITH LEPROMIN

Dog No. 3 of the first experiment, which had been injected intradermally with 15 mgm. of BCG on December 8, 1954, was given an intradermal injection of 0.1 cc. of lepromin 28 days after the BCG injection (January 5, 1955). At that time the BCG-reaction lesion was in the stage of ulceration.

In 48 hours after the lepromin injection there was observed an edematous and erythematous infiltration approximately 10 mm. in diameter. With the passing of the days this became increasingly infiltrated and larger, until by the 7th day it had formed a nodule; that became ulcerated by the 15th day, and was healed 36 days after the injection.

B. DOG SENSITIZED WITH LEPROMIN AND INJECTED WITH BCG

Dog No. 5 of the first experiment, which had been injected intradermally with 0.1 cc. of lepromin on July 13, 1955, was given an injection of 0.1 cc. of the BCG suspension. This second injection was made 27 days after the lepromin injection (August 9), when the reaction to that antigen was in the nodular stage, in which it persisted without ulceration.

After 48 hours the site of the BCG injection presented an edematous, erythematous infiltration approximately 10 mm. in diameter. This rapidly became more infiltrated and increased in size, until by the 7th day it formed an erythematous nodule. This became ulcerated within another week, healing by the 31st day.

Comments on the third experiment.—The BCG-sensitized dog which was given an injection of lepromin reacted to the latter antigen in a precocious

and accelerated manner, giving a response essentially similar to that which would have resulted from a second injection of BCG. That is to say, sensitization of the dog with BCG creates a state of cross sensitization to lepromin.

The lepromin-sensitized dog, when later injected with BCG, exhibited—as in the preceding case—an accelerated local reaction. In other words, the sensitization of the dog with lepromin creates a state of cross sensitization to BCG.

Briefly, then, the present experiment shows that a state of cross sensitization between integral lepromin and BCG can be experimentally induced in the dog by single injections of either antigen.

CONCLUSIONS

1. A single intradermal injection of lepromin in the dog causes a local reaction which is similar to that produced by a single injection of BCG, with the difference that it is somewhat later in its appearance and somewhat more prolonged in its evolution.

2. An intradermal injection of lepromin is capable of creating in the dog a condition of sensitization, the existence of which is revealed by a further injection of lepromin. The reaction to the second injection is of earlier appearance than that to the first injection, like that which occurs with BCG.

3. A dog sensitized to lepromin also becomes sensitized to BCG, and *vice versa*, a dog sensitized with BCG becomes sensitized also to lepromin.

SUMMARY

It has been found that integral lepromin can sensitize dogs, confirming the observations of Wade (the Wade phenomenon), just as injections of BCG cause sensitization to that antigen. Using animals so sensitized it has been found, in both cases, that there exists a cross sensitization between BCG and lepromin.

CONCLUSIONES Y RESUMEN

1. La inyección única de lepromina en perro produce una reacción local, semejante a la producida con una inyección única de B.C.G. con la diferencia que es un poco tardía en su aparición y un poco mas prolongada en su evolución.

2. Una inyección de lepromina por vía intradérmica es capaz de crear un estado de sensibilización, que se revela frente a una neuva inyección de lepromina, dando una reacción de aparición precoz, a semejanza de lo que ocurre con B.C.G.

3. Un perro sensibilizado a la lepromina queda sensibilizado al B.C.G. y, *vice versa*, un perro sensibilizado con B.C.G. queda sensibilizado también a la lepromina.

Comprobamos que la lepromina integral es capaz de sensibilizar a perros, confirmando las investigaciones de Wade (fenómen de Wade) y utilizando estas esperiencias comprobamos la sensibilización cruzada entre B.C.G. y lepromina, y lepromina y B.C.G.

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