PERSISTENCE OF SENSITIZATION AND CROSS-SENSITIZATION TO LEPROMIN AND BCG IN THE DOG[1]

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In a previous communication (1) we reported observations that confirmed the observations of Wade on the sensitization of the dog by integral lepromin. Furthermore, we demonstrated that cross sensitization between lepromin and BCG can be established in the dog by injections of either of these antigens. The time interval between the first, or sensitizing, injection and the second, or test, injection in these experiments was four weeks.

The purpose of the investigation here reported was to ascertain how persistent might be this phenomenon of sensitization to lepromin and to BCG, and the cross-sensitization between the two, so far as that could be determined by test injections of the antigens made eight months and more after the sensitizing injections.

For this purpose we carried out three experiments. (1) The first consisted of lepromin tests of two BCG-sensitized dogs, somewhat over 9 months and 16 months, respectively, after they had been vaccinated with BCG. (2) The second consisted of BCG tests of two lepromin-sensitized dogs, about 8 months and 9 months, respectively, after the injection of the sensitizing dose of lepromin. In these experiments three control dogs were tested with the homologous antigens. (3) The third experiment concerned the reactions to both lepromin and BCG in two dogs which had been vaccinated with both antigens, about 8 months and 16 months previously.

MATERIAL

The several dogs used in these experiments had been used in the previous work (1), during which they gained the sensitivities being tested. The antigens, as before, were a BCG vaccine containing 0.15 gm. of bacilli per cubic centimeter,[2] and integral lepromin prepared according to the Mitsuda-Hayashi technique. The dose of each was 0.1 cc., intradermally.

FIRST EXPERIMENT

A. LEPROMIN TESTS OF DOGS PREVIOUSLY SENSITIZED BY BCG

DOG NO. 1:—This animal had received two intradermal injections of BCG (15 mgm. each) a month apart, on November 24 and December 22,

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

1954. Some 16 months (498 days) after the second sensitizing injection, on May 3, 1956, an injection of lepromin was given to test for cross sensitization.

Within 48 hours there appeared a 5 mm. erythematous papule, but this had diminished in another 2 days to 3 mm., disappearing by the 7th day. No further local reaction was observed by the 21st day, but then there began to develop a nodular reaction which grew to a diameter of 14 mm.; this became ulcerated on the 40th day and healed by the 54th day.

DOG NO. 7:—Sensitized by a single intradermal injection of the suspension done on July 19, 1955. Over nine months (289 days) later, on May 3, 1956, this animal was given an injection of lepromin intradermally to test for cross sensitization.

After 48 hours there was observed an erythematous papule 5 mm. in diameter, but in 2 more days it had decreased in size, and by the 7th day it had disappeared. Within 9 days after the injection, however, there was seen a small erythematous nodule which increased in size in later days, ulcerating on the 23rd day and healing about the 60th day.

B. LATE BCG TEST OF A BCG-SENSITIZED DOG (CONTROL)

DOG. NO. 6:—Sensitized by a single injection of BCG on July 19, 1955. Reinjected with BCG about 9 months (289 days) later, on May 3, 1956.

Within 48 hours there was observed a 7 mm. erythematous wheal with central vesiculation. This remained unchanged for another two days, after which it developed into an erythematous nodule by the 7th day. It ulcerated on the 11th day, and healed within 23 days after the injection.

SECOND EXPERIMENT

A. BCG TESTS OF DOGS PREVIOUSLY SENSITIZED BY LEPROMIN

DOG. NO. 12:—Sensitized with lepromin by an injection given on September 6, 1955. About 9 months (266 days) later, on May 29, 1956, the animal was given a cross-sensitization test injection of BCG.

In 48 hours there was a 5 mm. papule which disappeared after another 2 days. On the 7th day a small, 3 mm. papule was seen which increased in size to become a nodule that ulcerated on the 10th day after injection and healed by the 29th day.

DOG NO. 13:—Sensitized with a single dose of lepromin injected on September 6, 1955. Tested for cross sensitization with an injection of BCG about 8 months (240 days) later, May 3, 1956.

In 48 hours there appeared an erythematous infiltration 10 mm. in diameter which had transformed into a nodule by the 7th day. It became ulcerated on the 12th day, and healed by the 21st day.

B. LATE LEPROMIN TESTS OF LEPROMIN-SENSITIZED DOGS (CONTROL)

DOG. NO. 10:—Sensitized by an injection of lepromin given on August 4, 1955. About 9 months (273 days) later, on May 3, 1956, it was reinjected with the same antigen.

In 48 hours there appeared an erythematous wheal, 10 mm. in diameter, which remained of that size for 4 days but subsequently transformed into a nodule that became ulcerated on the 9th day.

DOG NO. 11:—Sensitized by lepromin injected on August 4, 1955. Nearly 10 months (299 days) later another lepromin injection was given.

Within 48 hours a 10 mm. erythematous wheal appeared which on the 4th day assumed a nodular aspect, measuring 6 mm., and became ulcerated on the 10th day.

THIRD EXPERIMENT

REACTIONS TO BOTH ANTIGENS IN DOGS PREVIOUSLY SENSITIZED WITH BOTH

DOG NO. 4:—This dog had received an injection of lepromin on January 5, 1955, followed by one of BCG on January 26th.[3] About 16 months (489 days) later, on May 29, 1956, both antigens were injected simultaneously.

(*a*) Reaction to lepromin: Within 48 hours there appeared an erythematous wheal measuring 10 x 12 mm. which in another 2 days assumed the aspect of an erythematous nodule 10 mm. in diameter. On the 7th day it was still of the same size, but after 2 more days it measured a little less and showed central softening. By the 15th day it had ulcerated and decreased further in size, to 5 mm.; on the 28th day it had healed.

(*b*) Reaction to BCG: Within 48 hours there was an erythematous wheal 10 mm. in diameter, which by the 4th day had become a 5 mm. nodule. On the 9th day it measured 7 mm. and had undergone central softening, and 2 days later it had ulcerated. On the 19th day it was healed.

DOG. NO. 5:—This animal was given an injection of lepromin on July 13, 1955, and on August 9 it was vaccinated with BCG. Nearly 9 months (263 days) later, on May 29, 1956, both antigens were again injected, simultaneously.

(*a*) Reaction to lepromin: After 48 hours there was a 10 mm. erythematous wheal, which by the 7th day had become a 6 mm. nodule. Later it ulcerated, and by the 28th day it was healed.

(*b*) Reaction to BCG: In 48 hours a 10 mm. erythematous wheal had appeared, which by the 7th day had developed into a 5 mm. erythematous nodule. Subsequently it increased in size and ulcerated, healing by the 28th day.

The data on the dogs involved in these experiments are summarized in Table 1.

DISCUSSION

Two dogs (Nos. 1 and 7), sensitized with BCG and tested with integral lepromin after 498 and 289 days, respectively, did not respond to the test injection with a frank early reaction, but only a papulation which soon disappeared. In both animals the subsequent formation of the nodular re-

[3] This dog was among those injected for the previous study but which escaped and could not be included in that report, but he returned later.

action lesion, and its ulceration and healing, followed a relatively slow course much as it would in normal dogs.

It is noteworthy that the No. 1 animal had received two sensitizing doses of BCG a month apart, yet it still failed when tested to show the cross

TABLE 1.—*Reactions to test injections made after prolonged intervals after sensitization.*

Dog No.[a]	Previous injection (s)	Interval (days)	Final injection (s)	Time of observation of each stage (days)			
				Onset[b]	Nodule	Ulceration	Healing
1	BCG (2)	498	Lepromin	(Temp.)	21+	40	54
7	BCG	289	Lepromin	(Temp.)	9	23	60
6 (C)	BCG	289	BCG	2W	7	11	23
12	Lepromin	266	BCG	(Temp.)	7+	10	29
13	Lepromin	240	BCG	2	7	12	21
10 (C)	Lepromin	273	Lepromin	2W	4+	9	(?)
11 (C)	Lepromin	299	Lepromin	2W	4	10	(?)
4	Lepromin BCG	489	Lepromin BCG	2W 2W	4 4	15 11	28 19
5	Lepromin BCG	263	Lepromin BCG	2W 2W	7 7	(?) (?)	28 28

a Dogs indicated by (C) served as homologous-antigen controls for those immediately preceding them in the list.

b Observations after 48 hours. Manifestations that proved temporary are so indicated. W = wheal.

effect with lepromin that was seen in dogs tested after short intervals in the previous study. However, in this instance the interval between sensitization and final test was extraordinarily long, about 16 months.

The dog (No. 6) which served as a control for the two just discussed, both sensitized and finally tested with BCG, had retained sensitization to the homologous antigen during the nine-month interval and showed an accelerated response. There was a definite early reaction, and the nodule formation, ulceration, and healing all occurred in about one-half the time that they take in normal dogs (see Table 1 of the preceding study).

Two dogs (Nos. 12 and 13) that had been sensitized with lepromin and were tested with BCG after 266 and 240 days, respectively, showed persistence of cross sensitivity. They both responded to the vaccine with an accelerated course of nodule formation, ulceration and healing. In one of them (No. 13) there was also a frank early reaction, but in the other one that effect was doubtful.

In this instance two dogs (Nos. 10 and 11) were used as the control to ascertain whether sensitivity to the homologous antigen had persisted. The intervals between the sensitizing and test injections of lepromin were 273 and 299 days, respectively. Both animals responded with frank early reactions and accelerated nodulation and ulceration.

Two dogs (Nos. 4 and 5) that had been sensitized with both lepromin

and BCG given four weeks apart were tested with both antigens given simultaneously, after intervals of 489 and 263 days, respectively. Both of these animals responded to both test injections with frank early reactions and acceleration of the further course of the reaction lesions. Here there was persistence of both direct and cross sensitivities for a full 16 months.

SUMMARY AND CONCLUSIONS

A study has been made of the persistence of the phenomena of specific and nonspecific (cross) sensitization to lepromin and BCG in the dog. The time intervals between the sensitizing injection (or the second such injection when there had been two) and most of the test injections was around 9 to 10 months, but in two instances the interval was about 16 months.

Three experiments were performed: (1) Of three dogs sensitized with BCG, two were tested with lepromin and the other with the homologous antigen (control). (2) Of four dogs sensitized with lepromin, two were tested with BCG and two with the homologous antigen (control). (3) Two dogs had been sensitized with both antigens, and were tested simultaneously with the two.

From the results obtained it is concluded that the nonspecific (cross) sensitization to lepromin induced in the dog by BCG appears to be a phenomenon of transitory character which may disappear within nine months, whereas the nonspecific sensitization to BCG induced by lepromin is a more stable phenomenon, causing accelerated reactions after nine months and presumably longer. The specific sensitization induced by either lepromin or BCG is equally stable.

RESUMEN

Los autores estudian el factor tiempo en el fenómeno de sensibilización inespecífica (consensibilización cruzada), y el de sensibilización específica, con lepromina y B.C.G.

Realizan 3 experiencias: en la primera, sensibilizan a 3 perros con B.C.G. y despues de 289 y 526 días, efectuan lepromina a 2 perros y al tercero, revacunan con B.C.G., despues de 289 días; en la segunda experiencia sensibilizan 4 perros con lepromina, y despues de 240 a 299 días inyectan lepromina a 2 perros y B.C.G. a los otros 2 perros; en la tercera experiencia sensibilizan a 2 perros con lepromina y B.C.G. y despues de 321 y 510 días repiten simultaneamente ambos antígenos.

De los resultados obtenidos concluyen: que la sensibilización inespecífica (cosensibilización cruzada), entre lepromina y B.C.G. parece ser un fenómeno de carácter transitorio, mientras que la sensibilización específica para la lepromina y B.C.G. en el perro, parece ser un fenómeno más estable.

REFERENCE

1. Olmos Castro, N. and Arcuri, P. B. Sensitization of the dog with lepromin and BCG, and evidence of cross sensitization. Internat. J. Leprosy **25** (1957) 238-241.