The Role of Delayed Hypersensitivity and Toxicity of BCG in the Development of Lesions at Sensitization Sites (33379)

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In earlier studies we noted the formation of subcutaneous granulomatous lesions (nodules) induced with heat-killed *Mycobacterium bovis* (BCG) (1) at sensitization sites. This type of lesion reported previously has been assumed to be due largely to delayed hypersensitivity (2–7). However, the bacillary bodies themselves are known to be toxic and to cause lesions (7, 8). Inasmuch as some of the doses of BCG we used were very large, it became important to clarify the separate role of toxicity and of hypersensitivity and to define conditions that influence the induction of subcutaneous nodules by BCG. In the present studies we have quantitated the size and rate of development of the nodules and assessed the role of the ingredients in the sensitizing preparations on the formation of lesions. Toxicity of the organisms caused the induction of early small nodular reactions that remained small when delayed hypersensitivity was suppressed but enlarged when not suppressed.

Materials and Methods. Sensitization of guinea pigs. Female guinea pigs of the Hartley strain weighing about 500 g obtained from the NIH Animal Production Section were used. Each experimental group consisted of eight animals that were sensitized subcutaneously in the nuchal area with a single injection of heat-killed BCG incorporated in 0.2 ml of either saline or a water-in-oil emulsion (9). The emulsion was composed of equal volumes of saline and a mixture of Arlacel A: Drakeol 6-VR (35:65) containing the BCG organisms. Some animals were sensitized intraperitoneally with 5 mg BCG in 1.0 ml of saline before receiving the sensitizing dose in the nuchal area.

Lesions and skin tests. The subcutaneous nodule that developed in each animal in the nuchal area was measured with Vernier calipers (Fig. 1). Skin tests were performed on plucked and clipped surfaces of the guinea pig skin by injecting 0.1 ml of tuberculin D, prepared from culture filtrates of BCG (9). Dermal reactions were measured at 24 hr after injection of antigen and reported as the product of the lesser and greater diameters.

Results. Effects of suspending medium and dose of BCG on the development of subcutaneous lesions. Saline-suspended BCG when injected subcutaneously caused the formation of smaller and more slowly developing subcutaneous lesions than did water-in-oil emulsions containing comparable amounts of BCG (Fig. 2). Although the rate of development was slower with the 1-mg dose, the maximum size of nodule induced by a 1-mg or a 10-mg dose of BCG suspended in saline was the same and did not exceed 10 mm in diameter.

In animals sensitized with 1 mg of BCG in saline, the mean area of reaction after challenge with 1 μg of tuberculin 4 weeks later was lower (112 mm^2) than that (307 mm^2) in animals sensitized with the same dose of organisms in water-in-oil emulsion.

Water-in-oil emulsions containing the 1-mg dose induced no lesions by day 2, but the 10-mg and 100-mg doses had induced palpable lesions of 5-mm diameter (Fig. 2). On day 7, however, the nodules were all about the same size (10 mm) regardless of the dose. The 10-mg and the 100-mg doses induced peak reactions of 14 mm and 12 mm in diameter respectively by day 28. Nodules in animals that received 10 mg remained relatively constant in size for 190 days while those in animals that received 100 mg decreased to 7 mm by day 80 when the animals were sacrificed. On the other hand, the nodules in animals sensitized with the 1-mg dose increased progressively until day 128 at which time they averaged 23 mm in diameter and then remained unchanged through day 190.

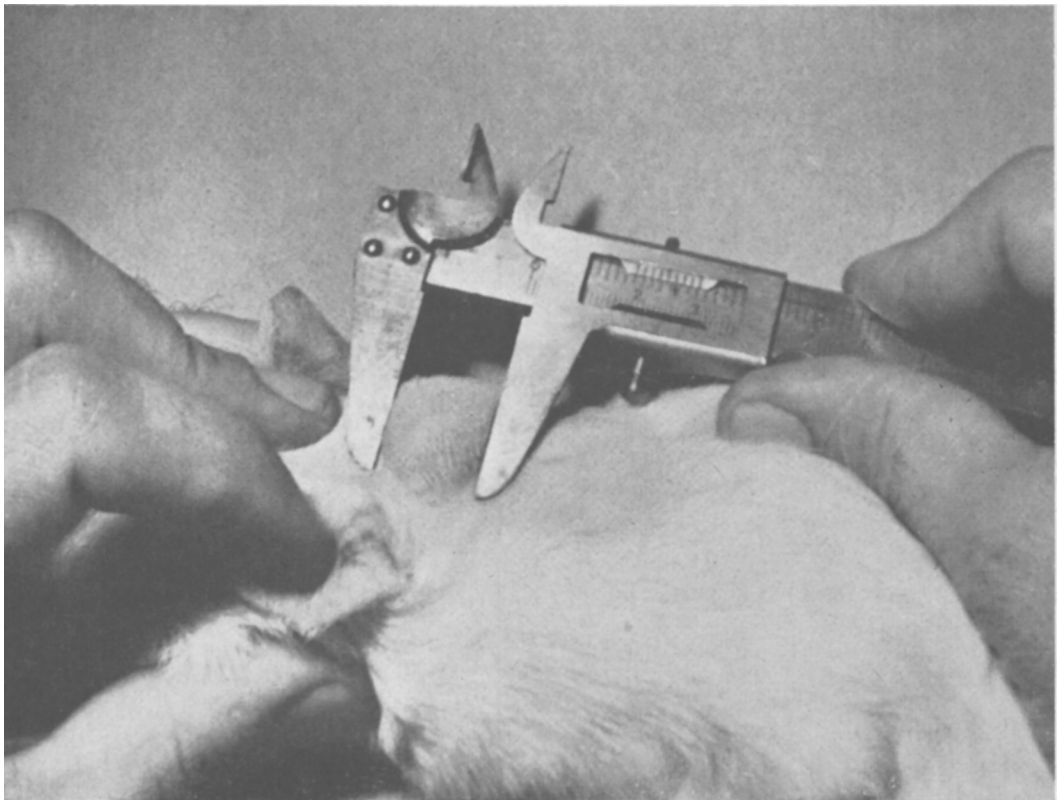

FIG. 1. Measurement of subcutaneous nodules.

Thus, for the 1-mg, 10-mg, and 100-mg doses, there was a reciprocal relationship between the size of the subcutaneous nodule and the dose of BCG.

Effect of presensitization on the rate of development of subcutaneous lesions. In the following studies we compared the rate of development of the subcutaneous lesions in normal and sensitized animals. The guinea pigs in the sensitized group received 5 mg of BCG in water-in-oil emulsion intraperitoneally. Three weeks later a saline suspension or an emulsion of BCG organisms was introduced subcutaneously in the nuchal region. No measurable lesions were induced by 1 mg of BCG in water-in-oil emulsion in normal animals for the first four days, while in the presensitized animals well-developed palpable masses had formed by the second day (Fig. 3). When this dose was administered in saline, the nonpresensitized animals did not develop measurable lesions (1–2 mm in diameter) until day 14, while the presensitized animals had firm nodules by the second day. Differences were less striking with the 10-mg dose: by day 2, nodules about 5 mm in diameter were present in presensitized animals that received BCG in saline and in nonpresensitized animals that received the BCG suspended either in saline or emulsified in oil. The presensitized group that received 10 mg of BCG in oil developed lesions of about 10 mm by day 2.

Suppression of nodules. Ten- and 100-mg doses of BCG, whether administered in water-in-oil emulsion or in saline, invariably induced nodules of significant size by the second day (Figs. 2 and 3). Since both toxicity and delayed hypersensitivity might contribute to the production of such lesions, we attempted to evaluate the roles of each by suppressing delayed hypersensitivity and determining whether reactions due to toxicity might be retained. Methotrexate has been shown not to interfere with nonspecific inflammation, but to be effective in reducing

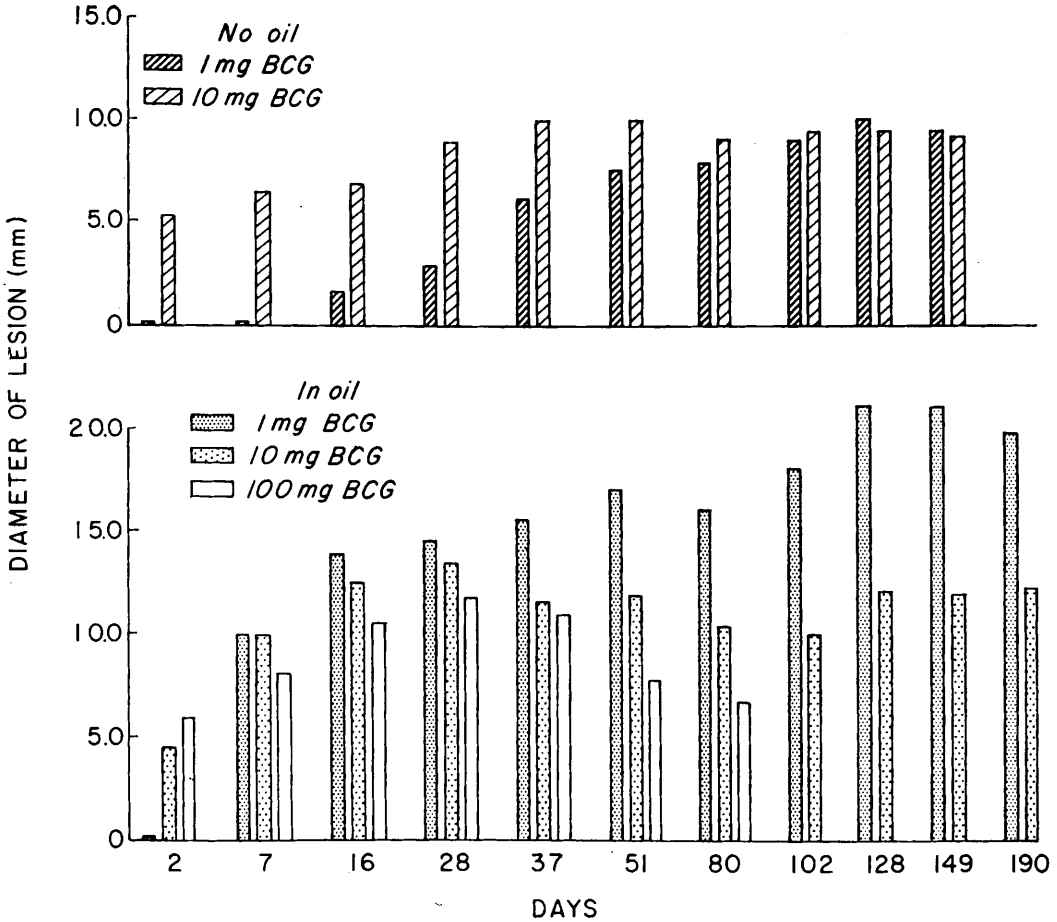

FIG. 2. Effects of suspending medium and dose of BCG on the development of subcutaneous lesions. Oil refers to organisms suspended in water-in-oil emulsion. No oil refers to organisms suspended in saline. Results are average values for eight animals.

delayed as well as immediate hypersensitivity (10). We therefore placed one group of four guinea pigs on Methotrexate for seven days before subcutaneous sensitization with 10 mg of BCG in Freund's adjuvant. Another group of four animals was similarly sensitized but not given Methotrexate. Both of these groups had nodules of equivalent size (5 mm) by the second day (Table I). In the untreated group the nodules enlarged with time, while in the treated group they remained the same size. Fifteen days after sensitization the size of the lesions remained small and tuberculin hypersensitivity was relatively low in the Methotrexate-treated animals. However, 16 days after cessation of the treatment the lesions had increased in size and tuberculin reactivity was equal to that in the controls (Table I). Both the subcutaneous nodules and tuberculin reactivity continued to increase with time up to the end of the observation period at 71 days. When Methotrexate treatment was started after the nodules were 5–10 mm in size, there was neither a regression nor a significant growth of the nodules during three weeks of treatment.

Histological observations. The nodules induced by subcutaneous sensitization were examined at different periods after injection. During the first week the material in the lesion was of liquid consistency and yellowish white. With time the nodules became firmer and the material that could be extruded by

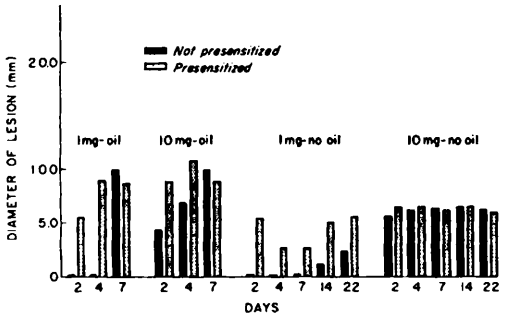

FIG. 3. Effect of presensitization on the rate of development of subcutaneous lesions. Oil refers to organisms suspended in water-in-oil emulsion. No oil refers to organisms suspended in saline. Results are average values for eight animals.

incision had a creamy appearance. Microscopically the 3-, 7-, and 14-day lesions contained predominantly lymphocytes and plasma cells around a central core of polymorphonuclear leukocytes. Older lesions taken months after sensitization contained giant cells and debris of degenerated cells. Most of the viable cells, primarily lymphocytes and plasma cells, were found in the periphery of the lesion.

Discussion. The lesions induced with heat-killed BCG in saline or in water-in-oil emulsions at the injection sites of guinea pigs are reminiscent of the phenomenon first described in 1891 by Koch (11). The roles of both delayed hypersensitivity to and toxicity of the organisms in the production of lesions to tubercle bacilli have long been held important and were reviewed by Rich (8). The findings reported here quantitate and extend the evidence that hypersensitivity plays a major role in the production of nodules at the injection site. Toxicity with larger doses of organisms, however, plays a role in the early reactions.

The 1-mg dose of BCG in saline induced no measurable nodules in the normal animals for approximately one to two weeks. However, if the animals were presensitized by the intraperitoneal route several weeks before the intranuchal challenge, definite nodules of greater than 5-mm diameter could be measured by the second day. These observations are akin to those of Olcott (12) who noted that when heat-killed tubercle bacilli were injected into the peritoneal cavities of sensitive rabbits, tubercles were produced more rapidly than in normal animals. Thus, with the small dose of 1 mg of BCG, no evidence of toxicity was noted and hypersensitivity appeared to play the major role in the induction of nodules.

Nodule formation due to toxicity could be distinguished when delayed hypersensitivity was suppressed with Methotrexate. Thus, when normal and treated animals received intranuchal injections of 10 mg of BCG in saline or emulsion, nodules of equivalent size developed by the second day (Table I). Both nodule enlargement and delayed skin reactivity remained suppressed until Methotrexate administration was halted. Inasmuch as nonspecific inflammation is not affected by the drug (10), we concluded that the early lesions in this case were due to toxicity of the organisms and the later development to

TABLE I. The Effect of Methotrexate on the Development of Nodules and Tuberculin Sensitivity.*

	Days post sensitization						
	2	3	6	9	15	31	67
Nodule size (mm)							
Treated	5	5	5	5	5	8	13
Control	5	8	13	14	15	16	16
Skin reaction area (mm^2)							
Treated					46 (+)	165(+++)	224(+++)
Control					167(+++)	173(+++)	225(+++)

* Methotrexate was started seven days before sensitization with 10 mg of BCG in oil adjuvant and stopped on postsensitization day 16. Skin tested with 1 μg tuberculin/0.1 ml. Reactions read at 24 hr. (+ to +++) indicates intensity of erythema.

delayed hypersensitivity. The lesions induced on day 2 and day 4 in the presensitized group were larger than in the nonpresensitized group for the 10-mg dose in emulsion (Fig. 2). In this instance, both toxicity and hypersensitivity appeared to be involved in the early reaction.

There was, within the limits of test doses used here, a reciprocal effect between size of nodules induced and the dose of organisms given in water-in-oil emulsions: the 100-mg dose of BCG induced the smallest nodules and the 1-mg dose induced the largest (Fig. 2). This observation emphasizes the greater importance of hypersensitivity than toxicity in the induction of the nodules. If toxicity of the organisms were of greater importance, larger nodules should have been induced with the larger dose. It was previously noted (1) that a 10- to 25-mg sensitizing dose of BCG induced an optimal degree of skin sensitivity and that larger doses resulted in lowered hypersensitivity. Thus, the smaller nodules induced by the largest dose of BCG correlated with a lowered degree of delayed skin hypersensitivity. These observations might be explained on the basis of a tolerance or desensitization mechanism. In general, large doses of protein antigens or simple chemical sensitizers have been reported to cause tolerance to antigens in guinea pigs (13, 14). With the large doses of BCG it is possible that partial tolerance may have been induced. It is also possible that antigens from the largest sensitizing dose were liberated in a manner to effect a continuing partial desensitization resulting in smaller subcutaneous lesions as well as lessened subcutaneous skin reactivity. Graded desensitization of established hypersensitivity has been shown to occur in a stepwise fashion with graded doses of antigen injected parenterally prior to skin testing (15, 16).

Summary. Subcutaneous nodules were induced at sensitization sites in guinea pigs injected subcutaneously with BCG organisms. When the organisms were suspended in water-in-oil emulsions, the size of the nodule was inversely related to the dose: 1.0 mg induced a larger lesion than 10 mg or 100 mg. Lesions induced by 1.0 mg of BCG were due primarily to delayed hypersensitivity, while lesions induced by 10 or more mg were due initially to toxicity and later to delayed sensitivity.

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