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ANTIBIOTIC CONTROL OF TISSUE REACTIONS IN DOGS VACCINATED WITH VIABLE CELLS OF *COCCIDIOIDES IMMITIS*[1]

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ABSTRACT

CASTLEBERRY, MERIDA W. (U.S. Army Biological Laboratories, Fort Detrick, Frederick, Md.), JOHN L. CONVERSE, AND PETER J. SOTO, JR. Antibiotic control of tissue reactions in dogs vaccinated with viable cells of *Coccidioides immitis*. J. Bacteriol. **87**:1216-1220. 1964.—A total of 12 dogs (15 to 25 lb each), vaccinated with viable *Coccidioides immitis* (subcutaneous injection of 260 viable arthrospores in the medial surface of the hind leg), resisted a respiratory challenge (aerosol) with the same organism (13,000 viable arthrospores) administered (aerosol) 2 months after vaccination. Oral amphotericin B therapy (150 mg of Fungizone per day for 21 days) of 6 of the 12 dogs, initiated immediately after vaccination, eliminated the undesirable side reactions of the viable vaccine (ulcerated vaccination site and inguinal lymphadenopathy exhibited by the 6 untreated dogs) without affecting the immunogenicity of the vaccine. Clinical observation (blood-urea nitrogen levels) during and after therapy and histological examination approximately 3 months after respiratory challenge failed to disclose any evidence of nephrotoxicity or renal damage due to the oral antibiotic therapy (total doses of more than 3 g of amphotericin B).

The immunogenesis of various antigenic components of the saprophytic and parasitic stages of *Coccidioides immitis* was studied by Negroni, Vivoli, and Bonfiglioli (1949), Friedman and Smith (1956), Pappagianis et al. (1961), Levine, Cobb, and Smith (1960), Converse et al. (1962*b*), Kong, Levine, and Smith (1962), and others. A fair degree of immunity was developed by several of these preparations, if death was used as the unit of measure. Absolute prevention of coccidioidomycosis lesions after respiratory or intraperitoneal challenge, however, has never been attained with a killed vaccine.

Pappagianis et al. (1960) and Converse, Castleberry, and Snyder (1961) reported the resistance of monkeys to a second infection (respiratory) with *C. immitis* after the subcutaneous administration of viable *C. immitis* arthrospores. The investigations of Converse et al. (1961) demonstrated that, even in very low doses (ten arthrospores), the viable vaccine protected against subsequent, extremely heavy aerosol challenge. However, ulceration at the site of vaccination or regional lymphadenopathy or at both sites was occasionally encountered.

Several methods to circumvent these undesirable tissue reactions to the viable vaccine are under study. One of these studies was based on the hope that concomitant oral administration of amphotericin B (Fungizone; E. R. Squibb & Sons, New York, N.Y.) at the time of the vaccination would alleviate the adverse reaction to the vaccination. Campbell and Hill (1959) and Castleberry et al. (1963) described the use of orally administered amphotericin B in *C. immitis*-exposed animals. These investigators ascribed no adverse physiological reaction to its use in this manner.

The present report concerns an evaluation of the effects of amphotericin B on the untoward local reactions of a viable vaccine against coccidioidomycosis, and a determination of the effectiveness of such a vaccine in dogs.

MATERIALS AND METHODS

A total of 16 healthy mixed-breed dogs of both sexes weighing between 15 and 25 lb were employed in this study.

The vaccine was prepared by suspending viable arthrospores of *C. immitis*, strain D-76 (highly

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virulent for dogs), in normal saline (260 spores per ml). The high dose and virulence of this strain insured somatic reaction to the subcutaneous deposition of the vaccine.

Amphotericin B was dissolved in distilled water (15 mg/ml). Each dog was fed twice daily with a split dose of 5 ml of the amphotericin B solution mixed in his food. It was accepted readily by all dogs.

Of the 16 dogs, 14 were vaccinated subcutaneously in the medial surface of the right thigh with 1 ml of the vaccine. Administration of amphotericin B (150 mg per day) to six of these vaccinees was initiated immediately and continued for 21 days. The remaining eight dogs were untreated. At 54 days after vaccination, 12 of the 14 vaccinated dogs and 2 nonvaccinated, nontreated controls were exposed via the respiratory route in the manner described by Converse et al. (1962*a*). These dogs received an average inhaled dose of approximately 13,000 viable arthrospores of the Cash strain of *C. immitis.* Two vaccinated, untreated control dogs were killed at this time, rather than exposed, to evaluate the gross and histopathological responses of the tissue to the vaccine. The remaining 12 vaccinated and the 2 unvaccinated, untreated control animals were also killed 77 days after aerosol challenge. Intravenously administered pentobarbital was used for this purpose. The dogs were necropsied, and the tissues were fixed in 10% buffered formalin, embedded in paraffin, sectioned, and stained. Hemotoxylin-eosin and the Gomori methenamine silver stains were used routinely. Lung material from all animals was cultured on GPY (2% glucose, 1% peptone, 0.1% yeast autolysate) agar slants.

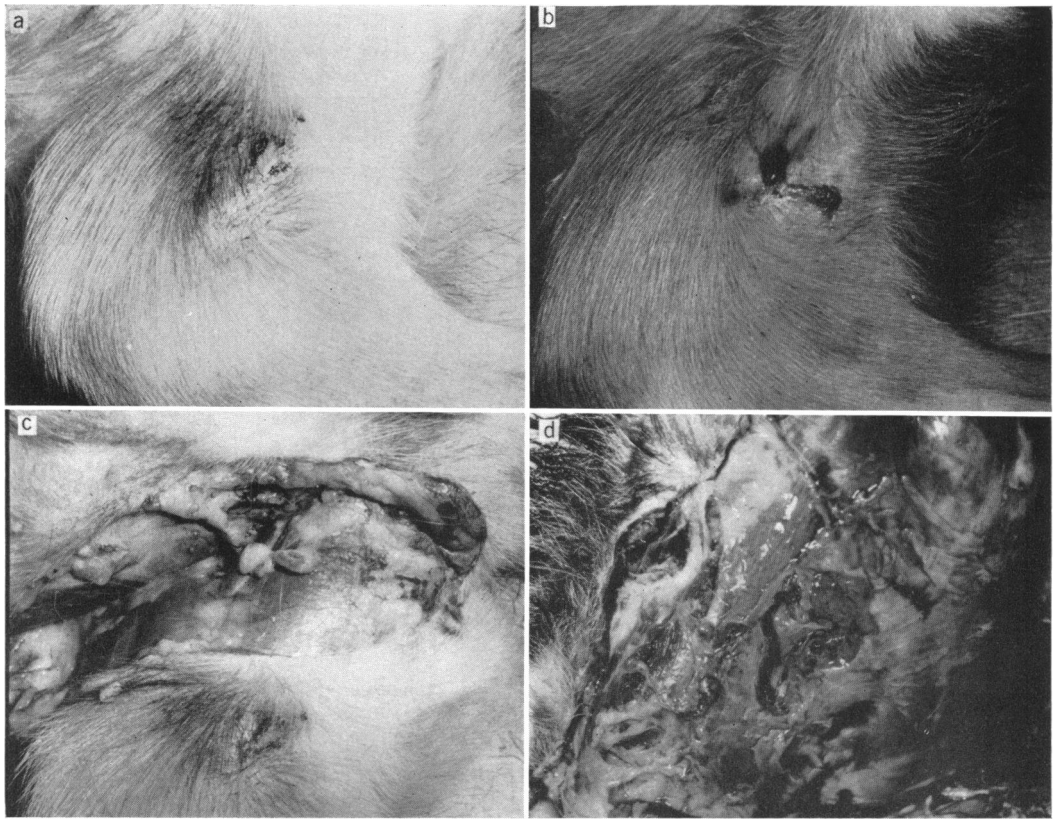

FIG. 1. *Ulcerated vaccination sites developing by the 17th day postvaccination in dogs not receiving oral amphotericin B therapy at the time of vaccination (a and b). Dissection, showing enlarged inguinal lymph nodes (c). Dissection, showing involvement of the subcutis (d). Note penetration of the gracilis muscle.*

RESULTS

By the 17th day, the vaccinated dogs that did not receive the amphotericin B had developed an induration at the vaccination site. Without exception, these eventually ulcerated (Fig. 1a and b). The lesions had healed, however, at sacrifice 130 days after vaccination. Histopathological examination of these areas showed a fibroblastic response extending rather deeply into the subcutis. An increased number of lymphocytes and plasms cells accompanied the scarring, but *C. immitis* was not seen. A mild reactive hyperplasia of the regional (right inguinal) lymph nodes was noted in several of the untreated vaccinees. These changes were attributed to the lesion produced by the subcutaneous deposition of viable *C. immitis* arthrospores; however, no spherules of *C. immitis* were noted here.

Visible reaction to the vaccination did not develop in any of the dogs that received amphotericin B. Subsequent histological examination of the skin and subcutis in the area of the original vaccination site failed to reveal any changes. Histological examination of the right inguinal lymph glands of these dogs revealed no significant changes, except for minimal reactive hyperplasia.

In addition to the vaccination-site scars of the untreated dogs, necropsy also revealed a few small (1 to 3 mm), grayish-tan nodules scattered over the pleural and cut surfaces of the lungs of three of the treated and one of the nontreated ahimals (Table 1). Histopathological examination of these pulmonary lesions revealed small isolated granulomatous lesions that occasionally contained a spherule (Fig. 2). Similar pulmonary lesions were encountered in three other animals that had demonstrated no gross lesions. Interestingly enough, giant cells, which are generally present in coccidioidal granulomata, were not noted in

TABLE 1. *Response of dogs to subcutaneous vaccination and aerosol challenge with viable Coccidioides immitis arthrospores*

Viable vaccine (spores)	Amphotericin B therapy	Respiratory challenge (spores)	Pathology					
			Gross			Histopathology		
			Vaccination site[a]	Inguinal lymph node[a]	Lung[b]	Vaccination site[a]	Inguinal lymph node[a]	Lung[b]
260	3 g	13,000	−	−	−	−	−	+
			−	−	−	−	−	−
			−	−	−	−	−	−
			−	−	−	−	−	+[c]
			−	−	+	−	−	+
			−	−	−	−	−	−
260	None[d]	13,000	+	+	−	+	+	−
			+	+	+	+	+	+
			+	+	−	+	+	+[c]
			+	+	−	+	+	+[c]
			+	+	−	+	+	−
			+	+	−	+	+	+
260	None	None (vaccine controls)	+	+	−	+	+	−
			+	+	−	+	+	−
None (disease controls)	None	13,000	−	−	+++	−	−	+++
			−	−	+++	−	−	+++

[a] Ulcerated vaccination site or inguinal lymphadenopathy indicated by +.

[b] Degrees of pathological involvement: −, negative; +, minimal; ++, moderate; +++, severe.

[c] Histological changes compatible with, but not diagnostic of, coccidioidomycosis. No spherules seen.

[d] Lesions noted at the vaccination site; the inguinal lymph nodes of animals in this group were healed at 130 days postvaccination.

these lesions. The remaining five animals demonstrated no gross or microscopic evidence of coccidioidomycosis. All animals with the exception of the two nonvaccinated, challenged, control dogs were negative to culture for *C. immitis*.

The pleural and cut surfaces of the lungs of the two nonvaccinated, challenged, control dogs were liberally covered with relatively large (0.2 to 1 cm), firm, grayish-yellow nodules. Histopathological examination of these lesions revealed essentially an amalgamation of smaller, early, and well-developed granulomata. These confluent lesions were in turn surrounded by a restraining collar of young connective tissue and lymphocytes. No histological differences were noted between the lesions of the control dogs and those of the 12 vaccinees, except for the larger size and greater number of lesions found in the two controls (Fig. 2). *C. immitis* was cultured from the lung lesions of each of these two dogs.

Except at the vaccination site, no lesions attributable to coccidioidomycosis were noted in the two vaccinated, untreated, unchallenged control dogs killed at 54 days. The vaccination ulcer of each dog was still oozing pus at the time of sacrifice (Fig. 1a and b). This exudate was not cultured; however, a smear was made from each dog, stained, and examined. No spherules were seen here. The histopathology of the affected dermal layers was characterized by the replacement of the epithelial layer with necrotic debris. Underlying this were proliferative collagenous elements, which were liberally interspersed with lymphocytes and plasma cells. This reaction had penetrated, in one dog, to the underlying gracilis

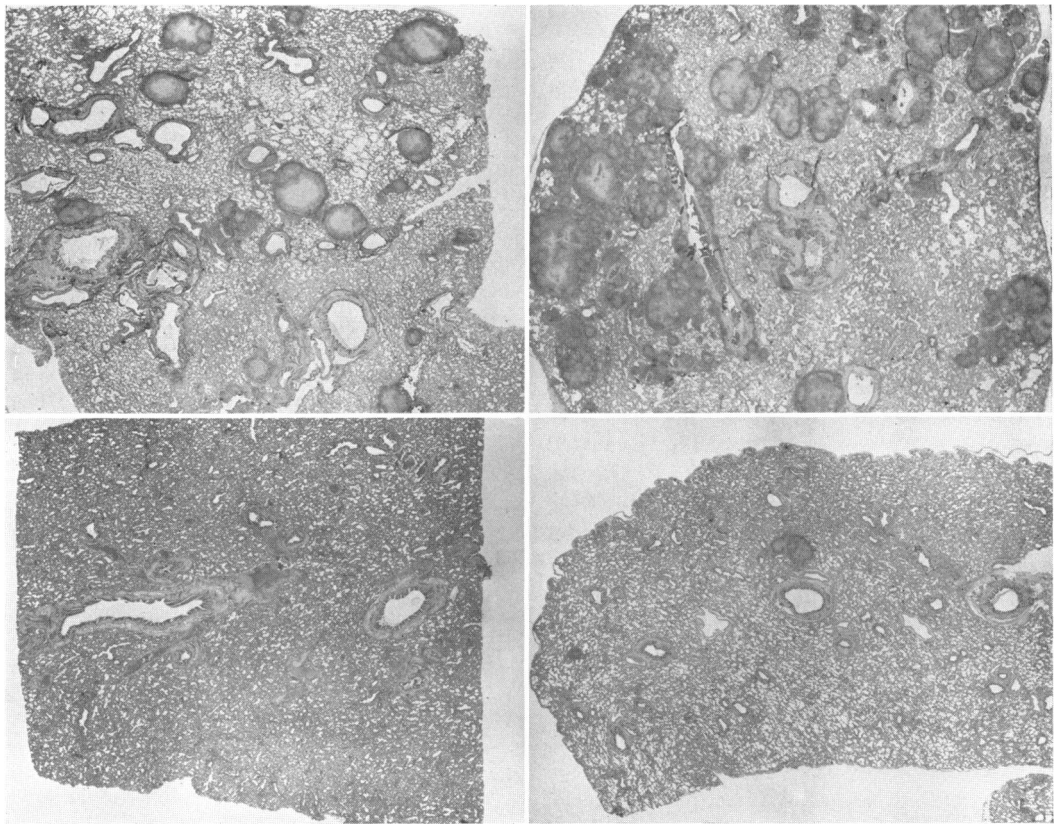

FIG. 2. *Comparison of histological lung sections of vaccinated and nonvaccinated dogs 77 days after respiratory challenge. Top, nonvaccinated control dogs; bottom, vaccinated dogs (diagnosis: left, negative; right, minimal). Hematoxylin and eosin. 7 ×.*

muscle (Fig. 1d). No spherules of *C. immitis* were seen.

DISCUSSION

Three very important observations may be made from the data presented. First, oral treatment with amphotericin B immediately after vaccination blocked the undesirable side effects of the viable vaccine. This was evidenced by the lack of ulceration at the site of vaccination, the lack of inguinal lymphodenopathy, and the lack of histological changes in these areas.

Secondly, therapy at the time of vaccination did not interfere with the development of immunity. This was shown by the fact that the resistance to the subsequent respiratory challenge was essentially the same in the vaccinated, untreated animals and the vaccinated, treated animals.

Thirdly, clinical and histological examination of all dogs receiving the amphotericin B (total doses of more than 3 g) failed to disclose any evidence of renal damage. Previous study (Castleberry et al., 1963) showed that the blood-urea nitrogen values in dogs remained well within normal limits at this dosage level.

It is also evident that the viable vaccine was as effective in dogs as it was in monkeys (Converse et al., 1961). Of the 12 vaccinated dogs, 5 remained free of infection. Of the remaining 7 dogs, 4 exhibited only very minimal lung changes; 3 were in the doubtful category (few focal granulomata; no spherules seen). This was in contrast to the massive involvement of the vaccinated control dogs. Moreover, all 12 of the vaccinated animals showed negative cultures for *C. immitis*. The fact that *C. immitis* could not be seen at the site of vaccination or in the inguinal lymph nodes indicated that the vaccine strain was probably cleared from the tissues at the time of autopsy.

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