TECHNICAL MANUSCRIPT 113

CANINE VACCINATION WITH VIABLE *Coccidioides immitis*: CONTROL OF TISSUE REACTION WITH ANTIBIOTIC THERAPY

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CANINE VACCINATION WITH VIABLE *COCCIDIOIDES IMMITIS*: CONTROL OF TISSUE REACTION WITH ANTIBIOTIC THERAPY

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ABSTRACT

Evidence has been presented that the subcutaneous vaccination of dogs with viable *Coccidioides immitis* arthrospores offers good protection against subsequent respiratory challenge with a large dose of live arthrospores. The administration of oral presolubilized Amphotericin B (Fungizone) following vaccination eliminated the undesirable side effects of the vaccine but did not interfere with the development of immunity. No physiologic or histologic evidence of renal damage attributable to Amphotericin B was demonstrated.

I. INTRODUCTION

The immunogenesis of various antigenic components of the saprophytic and parasitic stages of Coccidioides immitis has been studied by Negroni, Vivoli, and Bonfiglioli,[1] Friedman and Smith,[2] Pappagianis et al,[3] Levine, Cobb, and Smith,[4] Converse et al,[5] Kong, Levine, and Smith,[6] 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 following respiratory or intraperitoneal challenge, however, has never been attained with a killed vaccine.

Pappagianis et al[7] and Converse, Castleberry, and Snyder[8] reported the resistance of monkeys to a second infection (respiratory) with C. immitis following the subcutaneous administration of viable C. immitis arthrospores. Converse's investigations demonstrated that, even in very low doses (10 arthrospores), the viable vaccine protected against subsequent extremely heavy aerosol challenge. However, ulceration at the site of vaccination and/or regional lymphadenopathy 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 preclubilized Amphotericin B (Fungizone)* at the time of the vaccination would alleviate the adverse reaction to the vaccination. The reports of Campbell and Hill[9] and of Castleberry et al[10] have described the use of orally administered Fungizone in C. immitis-exposed animals. These investigators ascribed no adverse physiological reaction to its use in this manner.

This 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.

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* Fungizone: E.R. Squibb and Sons, New York 22, New York.

II. MATERIALS AND METHODS

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

The vaccine was prepared by suspending viable arthrospores of C. immitis, strain D-76** (highly virulent for dogs), in normal saline (260 spores per milliliter). The high dose and virulence of this strain insured somatic reaction to the subcutaneous deposition of the vaccine.

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

Fourteen of the 16 dogs were vaccinated subcutaneously in the medial surface of the right thigh with one milliliter of the vaccine. Administration of Amphotericin B (150 mg/day) to six of these vaccinees was initiated immediately and continued for 21 days. The remaining eight dogs were untreated. Fifty-four days following their vaccination, 12 of the 14 vaccinated dogs and two nonvaccinated, nontreated controls were exposed via the respiratory route in the manner described by Converse et al.[11] 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 sacrificed at this time, rather than exposed, to evaluate the gross and histopathologic responses of the tissue to the vaccine. Seventy-seven days after aerosol challenge, the remaining 12 vaccinated and the two unvaccinated, untreated control animals were also sacrificed. Intravenously administered pentobarbital was used for this purpose. The dogs were necropsied and the tissues fixed in ten per cent buffered formalin, embedded in paraffin, sectioned and stained. Hematoxylin eosin and the Gomori methenamine silver stains were used routinely. Lung material from all animals was cultured on GPY (2 per cent glucose, 1 per cent peptone, 0.1 per cent yeast autolysate) agar slants.

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* In conducting the research reported herein, the investigators adhered to "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.

** Kindly supplied by Dr. Raymond E. Reed, The University of Arizona.

III. RESULTS

By the seventeenth day, the vaccinated dogs that had not received the Amphotericin B had developed an induration at the vaccination site. Without exception, these eventually ulcerated (Figure 1, a 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 plasma 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 millimeters) greyish-tan nodules scattered over the pleural and cut surfaces of the lungs of three of the treated and one of the nontreated animals (Table I). Histopathological examination of these pulmonary lesions revealed small isolated granulomatous lesions that occasionally contained a spherule (Figure 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 these lesions. The remaining five animals demonstrated no gross or microscopic evidence of coccidioidomycosis. All animals with the exception of the two nonvaccinated and challenged control dogs were negative to culture for C. immitis.

The pleural and cut surfaces of the lungs of the two nonvaccinated and challenged control dogs were liberally covered with relatively large (0.2 to 1 centimeter) firm, greyish-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 (Figure 2). C. immitis was cultured from the lung lesions of each of these two dogs.

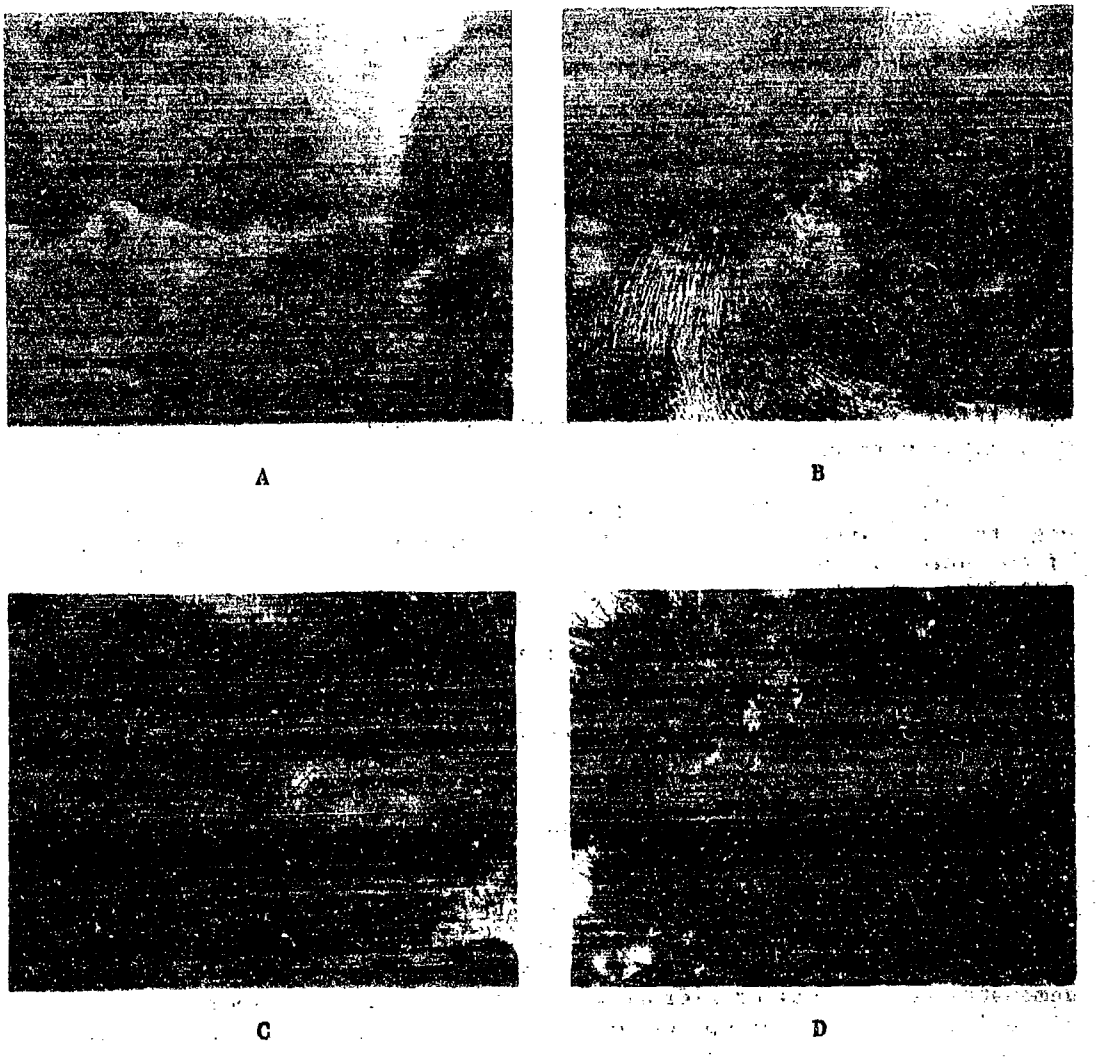

Figure 1. A,B. Ulcerated Vaccination Sites Developing by the Seventeenth Day Postvaccination in Dogs not Receiving Oral Presolubilized Amphotericin B (Fungizone) Therapy at the Time of Vaccination.
C. Dissection, Showing Enlarged Inguinal Lymph Nodes.
D. Dissection, Showing Involvement of the Subcutis. Note Penetration of the Gracilis Muscle.

TABLE 1. RESPONSE OF DOGS TO SUBCUTANEOUS VACCINATION AND AEROSOL CHALLENGE WITH VIABLE *COCCIDIOIDES IMMITIS* ARTHROSPORES

Viable Vaccine	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 spores	3 gm	13,000	-	-	+	-	-	+
			-	-	-	-	-	-
			-	-	-	-	-	-
			-	-	+	-	-	+[c]
			-	-	+	-	-	+
			-	-	-	-	-	-
260 spores	None[d]	13,000	+	+	-	+	+	-
			+	+	+	+	+	+
			+	+	-	+	+	+[c]
			+	+	-	+	+	+[c]
			+	+	-	+	+	-
			+	+	-	+	+	+
260 spores	None	None (Vaccine controls)	+	+	-	+	+	-
			+	+	-	+	+	-
None (Disease controls)	None	13,000	-	-	+++	-	-	+++
			-	-	+++	-	-	+++

a. + indicates ulcerated vaccination site or inguinal lymphadenopathy.
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 and the inguinal lymph nodes of animals in this group were healed at 130 days postvaccination.

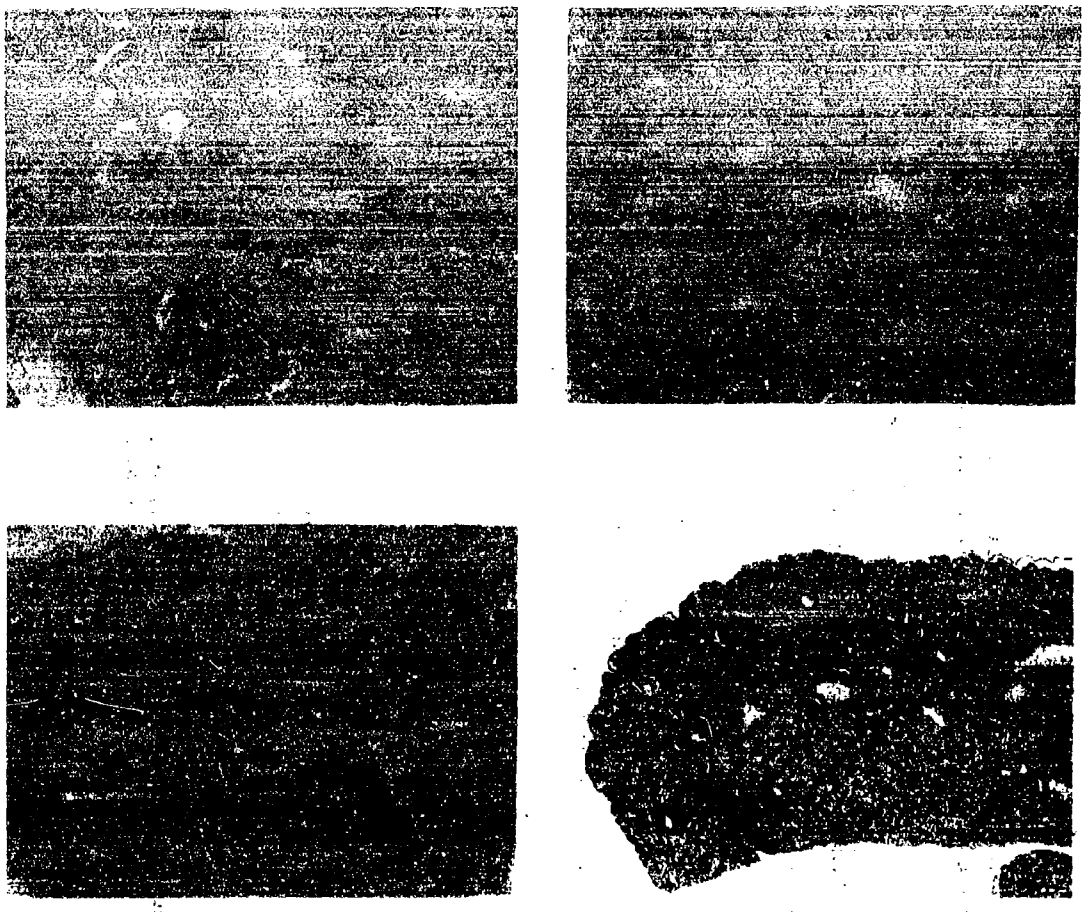

Figure 2. Comparison of Histological Lung Sections of Vaccinated and Nonvaccinated Dogs, 77 Days After Respiratory Challenge. Top: Nonvaccinated Control Dogs. Bottom: Vaccinated Dogs (Diagnosis: Left, Negative; Right, Minimal). Stain: Hematoxylin and Eosin.

Except at the vaccination site, no lesions attributable to coccidioidomycosis were noted in the two vaccinated, untreated, unchallenged control dogs sacrificed at 54 days. The vaccination ulcer of each dog was still oozing pus at the time of sacrifice (Figure 1, a and b). This exudate was not cultured; however, a smear was made from the vaccination site of 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 that were liberally interspersed with lymphocytes and plasma cells. This reaction had penetrated, in one dog, to the underlying gracilis muscle (Figure 1, d). No spherules of C. immitis were seen.

IV. DISCUSSION

Three very important observations may be made from the data presented. First, oral treatment with Fungizone immediately following 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 lymphodanopathy, and 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 three grams) failed to disclose any evidence of renal damage. Previous study[10] showed that the blood urea nitrogen (BUN) 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.[8] As shown in Table I, five of the 12 vaccinated dogs remained free of infection. Of the remaining seven dogs, four exhibited only very minimal lung changes; three were in the doubtful category (few focal granulomata, no spherules seen). This was in contrast to the massive involvement of the nonvaccinated 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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