ONSET OF COCCIDIOIDOMYCOSIS IN MOUSE LUNG AFTER INTRAVENOUS INJECTION

by

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(with 4 figs.)

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INTRODUCTION

Studies of the histologic responses of the host to infection by Coccidioides immitis have not been described for the very early stages of the disease. FORBUS & BESTEBREURTJE (1946) studied tissues obtained from patients at autopsy; TAGER & LIEBOW (1942) used mice that were sacrificed at various times after infection. The latter used intranasal and intraperitoneal methods of infection, but could not trace the development of the tissue response between 9 hours and 4 days. TARBET et al. (1952) used the intraperitoneal route of injection and found the first evidence of response at 20 hours after injection. In studies both of tissues from patients with coccidioidomycosis and of mice experimentally infected, a regular pattern of response was observed: Spherules attracted mononuclear cells; these accumulated and almost completely replaced the polymorphonuclear leukocytes that had dominated the earliest stages in the development of the spherule.

The present investigation was designed to observe in the mouse lung, the early stages of tissue response to large numbers of viable C. immitis arthrospores and hyphal fragments injected intravenously. The route and large dose used would allow very early accurate observations of the tissue reactions.

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2) In conducting the research reported here, the investigators adhered to "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.
MATERIALS AND METHODS

Mature, male, white, Swiss mice, Webster strain, born and raised at the Fort Detrick animal farm and weighing 25 to 30 grams each were used for this investigation. The inoculum, *C. immitis*, strain Silveira, grown on Sabouraud agar slants for 4 months at 34°C, was removed from the slants by scraping with a stiff wire after the addition of a 0.01% aqueous solution of triethanolamine oleate. The fungal elements were broken up by shaking with glass beads. Ten animals received an injection of 0.25 ml of the fungal suspension and the remaining seven received 0.1 ml, shown by plate counts to contain 11,600,000 and 2,900,000 viable particles respectively. Two animals receiving the higher dose died immediately after injection.

The animals that survived the challenge inoculation were sacrificed by an overdose of Nembutal, three each at 6, 24, 30, 48 and 54 hours after challenge. Animals receiving both dose levels were sacrificed at each time interval, except the 30-hour group, which consisted of three animals receiving the lower dose. One noninfected control animal was sacrificed at the 54-hour period. The lungs of all animals were fixed in 10% buffered formalin; tissue sections were stained by the Giemsa or Gomori technique.

A second series of 21 animals were similarly injected, but with dead arthrospores. One million dead arthrospores, as determined by direct microscopic count, in 0.5 ml of TEO solution, were administered to each animal intravenously. Three animals each were sacrificed at 0, 19, 25, 43, 50, 68 and 73 hours. Animals were sacrificed and tissue preserved as previously described.

RESULTS

In the two animals that died immediately after challenge, the pulmonary vessels were dilated and contained tangled masses of branching septate hyphae, some of which appeared to be swelling and assuming a spherical form (Fig. 1). It is assumed that this swelling occurred before injection and was not a result of interaction with the mouse tissue. These fungal elements, although visible with the Giemsa stain, were clearer and more prominent with the Gomori stain. No cellular reaction was elicited at this time. One mouse had pre-existing chronic murine pneumonia (CMP) in one lung. A few fungal elements were also seen in the large vessels of the heart and liver. The spleen and lymph nodes exhibited hyperplasia of the lymphocytic elements.

Six hours after challenge, the pulmonary vasculature again contained tangled masses of fungal elements. However, a moderate inflammatory response, characterized by small focal collections of lymphocytes with some neutrophils, was noted for the first time. In most instances the inflammatory cells surrounded the rounding arthrospores and hyphal elements.