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Acute Cavitary Histoplasmosis in Rhesus Monkeys: Influence of Immunological Status

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Male rhesus monkeys were randomized into groups according to body weight, immunized with different *Histoplasma capsulatum* antigens, and two weeks later were infected intratracheally with 10^6 *H. capsulatum* yeast cells. Complement fixation antibody titers, skin tests, and chest X rays were performed at weekly intervals from immunization until autopsy, at which time the spleens were cultured and the lungs and other organs were dissected. Pulmonary cavities were found in 33% of the animals, and extrapulmonary dissemination was present in 85% of the animals. Delayed hypersensitivity and circulating antibody activity was detected in all animals at some time during the experimental period; however, animals which developed pulmonary cavities had a longer period before circulating antibodies were detected than animals which did not develop pulmonary cavities. Delayed hypersensitivity developed at approximately the same time in both cavitary and noncavitary animals. Early appearance of delayed hypersensitivity was associated with reduced amounts of extrapulmonary dissemination, in that animals with a later onset of skin test reactivity had more *H. capsulatum* cultured from the spleen. There was no correlation between the onset or titers of circulating antibodies and the spleen culture results.

Attempts have been made to correlate immunological responses with the extent of disease in human cases of chronic infectious respiratory diseases, notably tuberculosis and histoplasmosis (1, 3, 4, 5). However, these studies have failed to show clear-cut differences between individuals which have developed varying degrees of pulmonary and extra-pulmonary pathology.

In the present study immunized and nonimmunized monkeys were followed through immunization, infection, and autopsy by observing their complement fixation titers, skin test reactivity, and appearance of pulmonary cavities. Significant differences in immunological status were observed between cavitary and noncavitary animals early in the course of infection, but these differences disappeared soon after the appearance of pulmonary cavities.

MATERIALS AND METHODS

Animals. Conditioned male rhesus monkeys (*Macaca mulatta*) weighing from four to six pounds (1.8 to 2.7 kg) were used in all studies. After receipt the animals were held at this facility for a minimum of 2 weeks before use. During this period, serological tests, skin tests, and chest X rays were performed to insure freedom from respiratory infection. Any animal with preexisting infection was excluded from the experimental group.

Animal monitoring. All animals were monitored at weekly intervals by cardiac puncture for serological tests, skin tests with histoplasmin (HKC-43, performed in the eyelid until the first positive test, then discontinued), body weight, rectal temperature, and chest X ray (posterior-anterior projection only). For monitoring, the animals were immobilized with minimal amounts of phencyclidine (Sernalan). The serological tests were performed by the Serology Reference Laboratory of the Mycology Program.

Animal autopsy. At the end of the experiment all animals were killed with ether and autopsied. At autopsy, the spleens were removed aseptically, minced into sterile weighed tissue grinders, reweighed, homogenized with 5 ml of Hanks balanced salt solution (BSS) and plated on Sabouraud agar for colony counts.

The lungs were removed, examined for cavities by transverse section, and photographed. Other organs, including liver, spleen, kidneys, adrenals, and any obvious lymph nodes, were removed, sectioned, and photographed. Representative samples were cultured, and others were placed in buffered Formalin for histopathologic examination.

Immunization groups. The animals were placed into groups according to their body weight. The mean weight per group was 2.3 kg (±0.2 kg) with the whole

spectrum of body weights represented in each group.

The groups were immunized as follows. (i) The control group received only a placebo consisting of 1 ml of BSS. (ii). The heat-killed yeast group received 10^8 heat-killed (60 C, 2 h) *Histoplasma capsulatum* yeast cells. (iii) The live yeast group received 10^5 viable *H. capsulatum* yeast cells. (iv). The ultrafiltered cell wall polysaccharide group received 4 mg of mucopolysaccharide per kg extracted with dimethyl sulfoxide (DMSO) from the yeast cell walls of *H. capsulatum* and passed through an XM-100 (Amicon) ultrafiltration membrane but retained by a PM-10 membrane. (v) The precipitated cell wall polysaccharide group received 4 mg of mucopolysaccharide per kg extracted exactly as in (iv) above and then was precipitated from solution with 4 vol of cold absolute ethanol. The immunizing materials were contained in BSS and were injected subcutaneously into the volar surface of the forearms, 0.5 ml per injection site.

Animal challenge. Two weeks after immunization all animals were challenged by an intratracheal injection of 10^6 viable *H. capsulatum* yeast cells contained in 0.3 ml of BSS. This dose was 220 times the previously determined mean infective dose (ID_{50}) causing extrapulmonary dissemination in 50% of the animals (2).

RESULTS

All animals developed delayed hypersensitivity to histoplasmin and complement-fixing (CF) antibodies to the yeast phase antigens of *H. capsulatum* at some time during the experimental period. The earliest recorded positive skin test was at 7 days prior to intratracheal challenge in an animal from the live yeast group (7 days after subcutaneous injection of 10^5 yeast cells). The earliest recorded positive CF test was also in an animal from the live yeast group and occurred at 4 days prior to challenge (10 days after subcutaneous injection).

Animals with CF titers of 1:8 or greater were considered to have positive tests. The titers ranged from 1:8 to 1:256, with the majority peaking at 1:64.

Table 1 shows the cavitation and spleen colonization data for the groups. The highest rate of cavitation (64%) and splenic isolations of *H. capsulatum* (87.9 colonies/g of spleen) occurred in the group of animals which had been preimmunized with the ultrafiltered *H. capsulatum* yeast cell wall polysaccharide. The lowest cavitation rate (0%) occurred in animals which had received 10^5 live *H. capsulatum* yeast cells subcutaneously 2 weeks prior to challenge. Local skin ulcers (approximately 20 mm in diameter) developed at the subcutaneous injection sites within the first 2-week period in the live yeast group. However, most of the ulcers had healed before the autopsy date. Lymphadenopathy proximal to the injection sites was still apparent in the animals at autopsy.

A retrospective examination of the chest X rays showed that all of the pulmonary cavities found at autopsy were in locations where pulmonary infiltrates were apparent on X ray. However, even after the locations of the cavities were known, many could not be visualized as frank cavities on the PA films. The details of the radiological aspects will be reported in a later communication.

In Tables 2 and 3, the time interval between the intratracheal challenge and the first appearance of CF antibody activity with yeast antigen (CF-Y) and delayed hypersensitivity to histoplasmin are compared for cavitary and noncavitary animals. Table 2 gives the average values for all animals, regardless of their immunization groups. The onset interval for skin test reactivity was not significantly different for either cavitary or noncavitary animals; however, CF-Y activity developed in animals which did not have pulmonary cavities approximately 14 days earlier than in cavitary animals. Table 3 shows that, with the exception of the live yeast group, the time intervals for the individual immunization groups were not significantly different from the overall values. In each group, noncavitary animals consistently developed CF-Y antibody activity earlier than did cavitary animals.

In Table 4, the animals in the control group are compared by their pulmonary classification and by whether or not the animals developed CF-Y activity during the first 14 days after intratracheal challenge. Of the animals which developed CF-Y activity during the first 14 days, none had pulmonary cavities at autopsy, whereas 58% of the animals which were still CF-Y negative by 14 days developed cavities. By 21 days after infection, there was no longer a significant difference in CF-Y activity between cavitary and noncavitary animals.

Table 5 shows that control animals which developed positive skin tests within the first 14 days after challenge had lower amounts of *H. capsulatum* recovered from the spleen than animals with negative skin tests during this period. When these data were statistically analyzed by the Mann-Whitney "U" test, they were significantly different at the 5% level excluding the six animals with negative cultures (6). If these animals are included, the results are significant at the 1% level. Also, when the skin test results by 14 days were compared with the proportion of animals with positive spleen cultures, it was found that a significantly greater number of animals with positive skin tests had negative spleen cultures (6/22; $x^2 = 5.3$; $P = 0.02$).

TABLE 1. *Pulmonary cavitation and spleen culture results*

Immunization group[a]	Pulmonary cavities	Positive spleen culture	Avg colonies per gram of spleen
Controls	19/46 (41%)	40/46 (89%)	28.6
Heat-killed yeast	1/12 (8%)	7/12 (58%)	2.3
Live yeast	0/16 (0%)	12/16 (75%)	2.9
Ultrafiltered cell wall polysaccharide	9/14 (64%)	14/14 (100%)	87.9
Precipitated cell wall polysaccharide	5/16 (31%)	16/16 (100%)	13.5

[a] Each animal received the appropriate immunizing antigen, and 2 weeks later all animals were challenged intratracheally with 10^8 *H. capsulatum* yeast cells. All animals were killed and autopsied 4 weeks after challenge.

TABLE 2. *Comparison of onset times for complement fixation and skin test activity in cavitary and noncavitary animals*

Pulmonary classification	No. of animals	Avg time after infection for the onset of:	
		CF-Y[a] (days)	Skin test (days)
Cavitary	34	24.4	14.8
Noncavitary	70	9.8	11.2

[a] CF-Y indicates the complement fixation test using *H. capsulatum* yeast cells for antigen.

DISCUSSION

One of the most serious consequences of chronic infectious pulmonary diseases is that a significant proportion of the persons who become infected develop pulmonary cavities. Most notable of these diseases are tuberculosis, histoplasmosis, and coccidioidomycosis.

Crowle (4), in 1966, suggested a two-phase "cellular-humoral" mechanism for pulmonary cavitation in tuberculosis. In his hypothesis, Crowle proposed that both pulmonary cavitation and immunological protection from progressive disease were manifestations of cellular immunity but directed against different antigens, and that the end point of this encounter between antigen and cellular components could be modified or blocked by the presence of circulating antibody.

In previous studies of histoplasmosis in rhesus monkeys, extrapulmonary dissemination and pulmonary cavitation rates were shown to be dependent upon the size of the infecting dose of organisms (2). Further, Chick et al. (Chest, in press) have shown that pulmonary cavitation in dogs is influenced by the immunological status of the animals. Experimental histoplasma infection of dogs with positive skin tests and negative serologies resulted in higher cavitation rates than infection of animals with either positive skin tests and serologies or negative skin tests and serologies.

In the present studies, delayed hypersensitivity was demonstrable in the animals between 7 and 21 days after infection. Even the animals immunized with live yeast cells developed skin

TABLE 3. *Onset times for animals preimmunized with different materials*

Immunization group	Pulmonary classification	No. of animals	Avg time (days) after infection for onset of:[a]	
			CF-Y	Skin test
Control	Cavitary	19	26.3	16.4
	Noncavitary	27	16.0	17.1
Heat-killed yeast	Cavitary	1	27.0	12.0
	Noncavitary	11	4.5	11.1
Live yeast	Cavitary	0	—	—
	Noncavitary	16	2.9	1.2
Ultrafiltered cell wall polysaccharide	Cavitary	9	27.3	13.0
	Noncavitary	5	14.2	13.5
Precipitated cell wall polysaccharide	Cavitary	5	22.4	12.5
	Noncavitary	11	8.1	11.8

[a] For convenience, all times are measured from the day of intratracheal challenge with 10^8 *H. capsulatum* yeast cells.

TABLE 4. *Pulmonary classification versus development of CF-Y activity*

CF-Y results within 14 days after infection[a]	Pulmonary classification	
	Cavitary	Noncavitary
+	0	13
−	19	14

[a] Control group animal only.

TABLE 5. *Spleen culture results versus skin test reactivity*

Skin test result by 14 days after infection[a]	Avg colonies per gram of spleen[b]	Proportion positive spleen culture
+	15.9	16/22
−	37.1	24/24

[a] Control group animals.

[b] Animals with negative spleen cultures were excluded from the average values.

test reactivity during the same period when the original subcutaneous injection was considered.

Unlike skin test reactivity, the development of CF antibody activity was consistently delayed in animals which sustained cavitary lesions. CF-Y activity appeared an average of 14 days later in cavitary animals than in noncavitary animals (Table 2). During the first 2 weeks after infection, the outcome of the pulmonary disease process can be predicted with fair accuracy in that none of the animals with positive CF-Y tests and positive skin tests developed pulmonary cavities; whereas, of the animals with positive skin tests but negative CF-Y tests during this period, 58% developed pulmonary cavities (Table 4). By 21 days after infection, there was not a significant difference between cavitary and noncavitary animals.

The presence or absence of CF-Y activity did not correlate with whether or not *H. capsulatum* was isolated from the spleens of the animals, possibly because the infecting dose was over 200 times greater than the previously determined ID_{50} and only 16 of the 104 animals had negative spleen cultures. In general, the animals with higher spleen culture colony counts had higher CF-Y titers. However, of the control animals that had *H. capsulatum* cultured from the spleen, those animals which developed delayed hypersensitivity during the first 14 days after infection had less than half as many colonies develop as animals with negative skin tests during this period. In addition, 27% of the skin test-positive control animals had negative spleen cultures, whereas all of the skin test-negative animals had *H. capsulatum* in the spleen (Table 5).

Preimmunization of the animals with either heat-killed or live *H. capsulatum* yeast cells resulted in a decrease in both pulmonary cavitation and extrapulmonary colonization (Table 1). Preimmunization with an ultrafiltered *H. capsulatum* yeast cell wall polysaccharide preparation resulted in an increase in both cavitation and colonization, possibly because of the induction of some degree of immunological tolerance.

These experiments do not establish the exact mechanism of pulmonary cavitation. However, they do strongly support the concept of an immunological mechanism and suggest several areas where future research should be directed. Some of these areas are the role T-lymphocytes and macrophages play in the local tissue destruction and necrosis, the possible role of IgE antibodies in these local phenomena, the immunoglobulin class(es) responsible for the observed suppression of cellular immunological expression, and the application of these observations to the control or modification of human disease.

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