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Interaction of Nonhuman Primate Peripheral Blood Leukocytes and *Coccidioides immitis* In Vitro

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The leukocytes from rhesus macaques could not kill either endospores or arthrospores of *Coccidioides immitis* even in the presence of immune serum and complement, although these leukocytes were able to kill *Candida* and *Listeria* organisms.

In experimental animals infected intranasally with arthrospores of *Coccidioides immitis*, there is an initial migration of polymorphonuclear leukocytes into lungs (5), indicating that the leukocytes may be important in the development of coccidioidomycosis. Phagocytosis of mycelial fragments of *C. immitis* by human peripheral blood leukocytes was first observed in vitro by Baker and Braude (1). Pappagianis and Kobayashi (4) observed phagocytosis of arthrospores by polymorphonuclear cells in the alveoli of a mouse infected intranasally with *C. immitis*. Other investigators (6) found that neutrophil leukocytes obtained from vaccinated dogs phagocytized arthrospores more avidly than did neutrophils from normal dogs. Phagocytosis of killed endospores by human monocytes was observed by Deresinski et al. (3). However, attempts to quantitate the survival of *C. immitis* after phagocytosis by peripheral blood leukocytes have not been made. The following investigation quantitatively evaluates the interaction between either arthrospores or endospores and peripheral blood leukocytes from normal rhesus macaques.

Peripheral blood was collected from normal rhesus macaques (*Macaca mulatta*), using ethylenediaminetetraacetic acid to prevent coagulation. The blood was centrifuged at $800 \times g$, and the leukoplasma was removed and centrifuged at $800 \times g$. The cells were suspended in 0.87% NH_4Cl for 10 min to facilitate lysis of erythrocytes. The cells were washed once in phosphate-buffered saline and suspended in 25% fetal calf serum plus HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid)-buffered minimal essential medium to a cell concentration of 2×10^6 per ml. In 11 different blood samples, 59.5 ± 1% (standard error of the mean) of the cells were polymorphonuclear leukocytes, 9.6 ± 1% were monocytes, and 29.9 ± 2% were lymphocytes.

Arthrospores and endospores of *C. immitis* were obtained from D. Pappagianis. As controls for the bactericidal and fungicidal capabilities of the leukocytes from rhesus macaques, *Candida albicans* (human isolate obtained from D. Pappagianis) and *Listeria monocytogenes* were also used. Both organisms were grown in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) for 18 h, washed, and suspended in phosphate-buffered saline. *L. monocytogenes* was diluted on the basis of optical density. *C. albicans* and *C. immitis* samples were stained with lactophenol cotton blue and counted to determine the number of organisms per milliliter. Leukocytes were mixed with endospores, arthrospores, or *C. albicans* or *L. monocytogenes* organisms at a ratio of one organism per 10 leukocytes. The cell mixtures were added to 96-well, flat-bottomed tissue culture plates (Costar, Cambridge, Mass.) and rocked 20 times per min on a Labquake Rocker (Lab Industries, Berkeley, Calif.) at 37°C. The inoculum was diluted in medium and incubated in the same manner as were the infected leukocytes. At the time of infection and at 2 and 4 h postinfection, some of the cultures were diluted in distilled water for 10 min at 37°C to lyse the leukocytes and then diluted and streaked on Trypticase soy agar to determine the recovery of the inoculum from the leukocytes. Duplicate plates of two samples per time period were streaked. The mean value of the total colony units per sample was determined. The percent killing was calculated by dividing the total colony-forming units obtained at a particular time period from the leukocytes by the total colony-forming units found at that time in the cultures containing inoculum in medium without leukocytes.

As shown in Table 1, a total of 53% of the *C. albicans* organisms added to the leukocytes were killed by 2 h postinfection. The macaque leukocytes were more efficient in killing *L. monocytogenes* organisms, with 97% of the inoculum killed within 1 h. In contrast, only 13% of the *C. immitis* endospores were killed by 4 h postinfection. Apparently, normal rhesus macaque leukocytes cannot kill endospores as easily as the

TABLE 1. *Percent killing of C. albicans, L. monocytogenes, and C. immitis endospores in peripheral blood leukocyte cultures*

Inoculum	Percent killing[a] at following time postinfection:		
	1 h	2 h	4 h
C. albicans	33 ± 4	53 ± 5	45 ± 5
L. monocytogenes	97 ± 2	ND[b]	97 ± 1
C. immitis endospores	0	0	13 ± 3

[a] The values were obtained from duplicate plates of two samples in two different experiments, with the mean value of the percent killing ± the standard error of the mean given.

[b] ND, Not determined.

leukocytes kill *L. monocytogenes* or *C. albicans* organisms.

For studying the effect of immune serum or complement on the fate of *C. immitis* in leukocyte cultures, we obtained serum from an infected rhesus macaque. The complement-fixing antibody titer specific for coccidioidin of the serum was 1:128. Complement was obtained from serum samples from normal rhesus macaques, which facilitated lysis of antibody-coated erythrocytes. *C. immitis* endospores or arthrospores were mixed with immune serum or complement and held for 30 min before infecting the leukocytes. As shown in Table 2, even after the spores were mixed with immune serum and complement, at least 87% of the endospores and 95% of the arthrospores could be recovered from the leukocytes. The macaque leukocytes could kill *L. monocytogenes* and *C. albicans* organisms in the absence of specific antibody, but could not kill endospores or arthrospores in the presence of coccidioidin-specific antibody and complement. An examination of stained preparations of the leukocyte cultures demonstrated that phagocytosis of arthrospores and endospores had occurred. However, it is not clear how *C. immitis* avoids intracellular destruction within leukocytes. Collins and Pappagianis (2) found that hen egg lysozyme affected the viability of spherules at levels of the enzyme normally present in tissues. Perhaps there were insufficient levels of lysozyme present in the leukocyte cultures, or *C. immitis* does not readily trigger the release of the leukocytic lysosomal enzymes. From our studies, it is apparent that peripheral blood leukocytes from normal rhesus macaques cannot eliminate *C. immitis* organisms in vitro as easily as these same leukocytes kill *C. albicans* or *L. monocytogenes* organisms.

TABLE 2. *Percent killing of C. immitis endospores and arthrospores in the presence of immune serum and complement in peripheral blood leukocyte cultures*

Inoculum	Serum[a]	Percent killing[b] at following time postinfection:		
		90 min	3 h	4 h
Endospores	IMS + C	0	13 ± 3	ND[c]
Endospores	C	0	9 ± 1	ND
Arthrospores	IMS + C	ND	ND	5
Arthrospores	C	ND	ND	8 ± 2

[a] The immune serum (IMS) was obtained from an infected rhesus macaque. The complement-fixing antibody titer specific for coccidioidin was 1:128. The complement (C) was obtained from normal rhesus macaque serum, which facilitated the lysis of antibody-coated erythrocytes.

[b] The values were obtained from duplicate plates of two samples in two different experiments, with the mean value of the percent killing ± the standard error of the mean given.

[c] ND, Not determined.

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