INFECTION AND IMMUNITY, Feb. 1991, p. 737–741
0019-9567/91/020737-05$02.00/0

Vol. 59, No. 2

Inhibition of Intracellular Growth of *Histoplasma capsulatum* Yeast Cells by Cytokine-Activated Human Monocytes and Macrophages

SIMON L. NEWMAN,* LISA GOOTEE, CHRISTINE BUCHER,† AND WARD E. BULLOCK

Division of Infectious Diseases, Department of Medicine, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267

Received 31 August 1990/Accepted 20 November 1990

Human monocytes/macrophages (Mϕ) were infected with *Histoplasma capsulatum* yeast cells, and intracellular growth was quantified after 24 h of incubation in medium alone or in medium containing cytokines. Yeast cells multiplied within freshly isolated monocytes, cultured Mϕ, and alveolar Mϕ with intracellular generation times of 14.2 ± 1.4, 18.5 ± 2.1, and 19.9 ± 1.9 h (mean ± standard error of the mean), respectively. Monocytes and Mϕ inhibited the intracellular growth of yeast cells in response to cytokine supernatant; maximum inhibition was obtained when cytokines were added to cell monolayers immediately after infection. Opsonization of yeast cells in normal serum or in *H. capsulatum*-immune serum did not affect the intracellular generation time of yeast cells in either control Mϕ or cytokine-activated Mϕ.

Histoplasma capsulatum infects the host via deposition of microconidia into the terminal bronchioles and alveoli of the lung. Inhaled microconidia convert into yeast cells and are phagocytized by alveolar macrophages (AM), within which they multiply (5). Presumably, the dividing yeast cells destroy the AM and then are ingested by other resident AM and by macrophages (Mϕ) recruited to the loci of infection. Maturation of specific cell-mediated immunity activates Mϕ to inhibit yeast cell proliferation; the disease process gradually resolves in most immunocompetent hosts (11, 21, 27).

In vitro studies on the interaction of *H. capsulatum* with Mϕ have demonstrated that peritoneal Mϕ (PM) from mice immune to *H. capsulatum*, but not from normal mice, restrict intracellular growth of yeast cells and that inhibition of growth is dependent on the presence of immune lymphocytes (13, 15, 16). Furthermore, lymphokines generated from immune splenic T cells stimulated with *H. capsulatum* antigens or recombinant murine gamma interferon (IFN-γ) activate resident mouse PM to inhibit intracellular growth of yeast cells (34, 35). Killing of yeast cells has not been observed.

We have shown that unopsonized *H. capsulatum* yeast cells and conidia bind to the surface of human monocytes, cultured monocyte-derived Mϕ, AM, and polymorphonuclear neutrophils (PMN) via the CD18 family of adhesion-promoting glycoproteins (1, 22, 30). Attachment of unopsonized yeast cells and conidia to cultured Mϕ and AM is followed rapidly by ingestion (22). In contrast, monocytes and PMN ingest few unopsonized yeast cells (22, 30). Phagocytosis of unopsonized yeast cells by Mϕ (1) and of opsonized yeast cells by PMN (30) is followed by activation of the respiratory burst and the production of toxic oxygen metabolites.

The current study was designed for the following reasons: (i) to quantify the intracellular growth of *H. capsulatum* yeast cells in human monocytes and Mϕ, (ii) to determine whether human Mϕ can be activated to inhibit the intracellular growth of these yeast cells and/or to kill them, and (iii) to determine whether opsonization of yeast cells affects their rate of growth within Mϕ.

H. capsulatum (strain G217B) was grown in HMM media (33) at 37°C for 2 to 3 days and was harvested by centrifugation. Yeast cells were washed in Hanks balanced salt solution containing 20 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) and 0.25% bovine serum albumin (HBSA) and resuspended to a volume of 30 ml in HBSA, and large aggregates were removed by centrifugation at 200 × *g* for 5 min. The single-cell suspension obtained was standardized to 1×10^6 cells per ml in HBSA.

Human monocytes were separated from buffy coats via sequential centrifugation on Ficoll-Hypaque and Percoll gradients and were cultured in Teflon beakers at 1×10^6 cells per ml in RPMI 1640 (Grand Island Biological Co., Grand Island, N.Y.) containing 15% human serum and 10 μg of gentamicin per ml (Sigma Chemical Co., St. Louis, Mo.) (22). After 4 to 7 days of culture, Mϕ were washed and suspended to 2.5×10^5/ml in HBSA containing 0.3 units of aprotinin per ml (22). When freshly isolated monocytes were studied, mononuclear cells were obtained by dextran sedimentation and Ficoll-Hypaque centrifugation (23). Mononuclear cells were suspended to 3×10^6/ml to 4×10^6/ml in HBSS containing 20 mM HEPES and 0.1% autologous plasma-serum.

Human AM were obtained by bronchoalveolar lavage from healthy volunteers, who had given informed consent to an approved protocol (7). The lavage fluid contained >90% AM on the basis of differential counts of Wright-Giemsa-stained cytocentrifuged preparations, and >95% of AM were viable on the basis of trypan blue dye exclusion. The AM were washed twice in RPMI 1640 containing 2% fetal bovine serum and resuspended to 2.5×10^5/ml.

One-milliliter aliquots of monocytes, cultured Mϕ, or AM were adhered to 12-mm-diameter glass coverslips in 24-well culture plates (Costar, Cambridge, Mass.) for 1 h at 37°C in 5% CO_2–95% air. The cells were washed, 1×10^6 viable yeast cells were added, and the cells were incubated for 30 min at 37°C to allow phagocytosis to proceed. The cells were washed twice in HBSA and incubated for an additional 30 min to ensure that all yeast cells were internalized. Two of the cell monolayers were fixed in 2% glutaraldehyde–1% sucrose in 0.01 M phosphate buffer–0.15 M NaCl, pH 7.2

* Corresponding author.
† Present address: 712 N.W. Avenue, Akron, OH 44278.

(phosphate-buffered saline). These cells provided a baseline for the percentage of Mϕ initially infected and for determining the average number of yeast cells per infected Mϕ and were designated 1 h infection.

The remaining Mϕ were incubated for 24 to 72 h at 37°C in M199 (Grand Island Biological Co.) containing 10% human serum and 10 μg of gentamicin per ml or in medium containing cytokine supernatant. After incubation, medium was removed, the cells were washed, and the monolayers were fixed in glutaraldehyde. Coverslips were mounted cell side down onto microscope slides, and the number of yeast cells per 100 infected Mϕ was determined via phase-contrast microscopy at a magnification of ×1,000.

Cytokine supernatant was prepared from mononuclear cells suspended to 1×10^6/ml in RPMI 1640 containing 10% fetal bovine serum and 10 μg of gentamicin per ml. The cells were stimulated with 3 to 5 μg of phytohemagglutinin per ml, and the supernatant was harvested after overnight incubation at 37°C in 5% CO_2–95% air.

Intracellular growth of *H. capsulatum* yeast cells in monocytes and Mϕ. After 1 h of infection, 20 to 30% of the monocytes and Mϕ were infected with yeast cells. Infected monocytes contained 2.2 ± 0.2 yeast cells per cell (mean ± standard error of the mean [SEM], $n = 4$), whereas infected Mϕ cultured for 4 to 7 days contained 4.0 ± 0.4 yeast cells per cell (range, 3.3 to 5.2 yeast cells per Mϕ). Infected AM contained 3.2 ± 0.3 ($n = 5$) yeast cells per cell.

During 24 h of incubation in medium, yeast cells grew in human monocytes with an average intracellular generation time (IGT) of 14.2 ± 1.4 h. In contrast, the average IGT in Mϕ cultured for 4 to 7 days was 18.5 ± 2.1 h (range, 14.2 to 23.8 h). The IGT of yeast cells in infected human AM was 19.9 ± 1.9 h. Although yeast cells multiplied at a consistently lower rate within cultured Mϕ than in freshly isolated monocytes, these differences were not statistically significant ($P > 0.05$, two-tailed Wilcoxon rank sum test [2]).

No yeast cells were observed outside of Mϕ at any time during culture, and the IGT was consistently 16 to 19 h through 72 h of incubation. Lysis of Mϕ was not observed until 96 h of incubation.

Activation of Mϕ by cytokines. We next determined whether human Mϕ could be activated by cytokines to restrict the intracellular growth of yeast cells or to kill them. Mϕ were infected with yeast cells and then were incubated for 24 to 48 h at 37°C in medium alone or in medium containing various concentrations of a phytohemagglutinin-generated cytokine supernatant. Cytokine supernatant activated Mϕ to inhibit the intracellular growth of yeast cells in a concentration-dependent fashion. Maximum inhibition was obtained with 40% supernatant. Control medium containing phytohemagglutinin did not activate Mϕ to inhibit the intracellular growth of yeast cells. Therefore, 40% supernatant was used in subsequent experiments to quantify the ability of monocytes and cultured Mϕ to be activated by cytokines.

Freshly isolated monocytes, or Mϕ cultured for 4, 5, or 7 days, were infected with yeast cells and then were incubated for 24 to 48 h in medium alone or in medium containing 40% cytokine supernatant. The data in Fig. 1 show that monocytes and Mϕ cultured for various periods responded equally well to cytokines, as evidenced by similar inhibition of the intracellular growth of yeast cells. Inhibition of intracellular growth was maintained through 48 h of culture and did not require additional cytokines (data not shown).

We next sought to determine whether preincubation of Mϕ with cytokines would enhance the ability of Mϕ to restrict the intracellular growth of yeast cells. Mϕ cultured

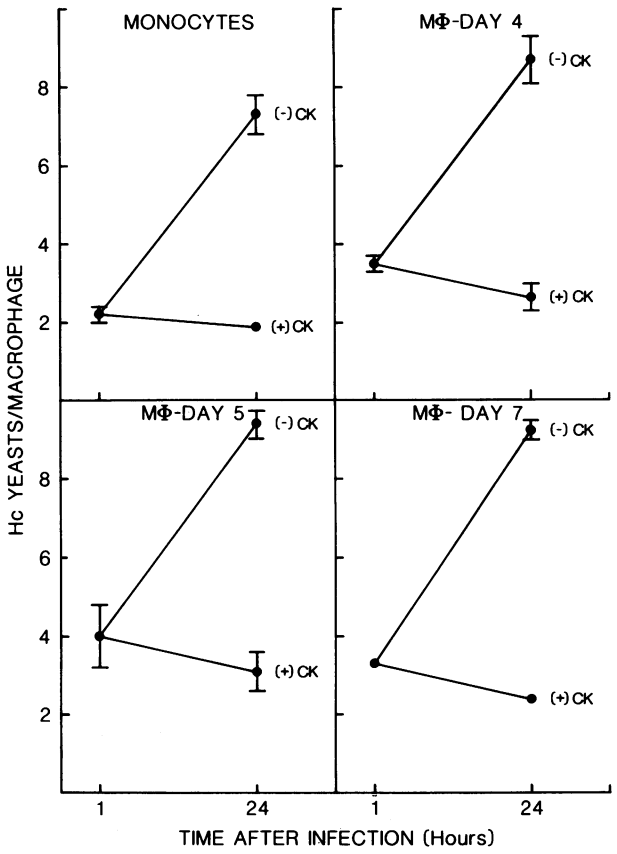

FIG. 1. Cytokines activate both monocytes and cultured Mϕ to inhibit the intracellular growth of yeast cells. Freshly isolated monocytes and Mϕ cultured for 4, 5, and 7 days were adhered to glass coverslips and infected with yeast cells. Cells were cultured for 24 h in medium alone or in medium containing 40% cytokine supernatant (CK), and the number of yeast cells per infected Mϕ was determined via phase-contrast microscopy. Results are the means ± SEM of four experiments performed in duplicate.

for 4 to 5 days were adhered to coverslips and incubated in medium or in 40% cytokine supernatant. After 24 h, Mϕ were washed, infected with yeast cells, and then incubated for a further 24 h in medium alone or in medium containing 40% cytokine supernatant. Preincubation of Mϕ with cytokines resulted in considerably less inhibition of intracellular yeast cell growth (Fig. 2) than when Mϕ were infected with yeast cells and cytokines were added immediately thereafter (Fig. 1). In fact, preincubation of Mϕ with cytokines did not decrease the intracellular generation time at all when medium alone was added to the cells postinfection (Fig. 2b). When Mϕ were preincubated in medium alone and then incubated with cytokines after infection with yeast cells, they inhibited the intracellular growth of yeast cells as well as Mϕ that had been incubated with cytokines both before and after infection.

Effect of opsonization of yeast cells on intracellular growth. Phagocytosis of *H. capsulatum* yeast cells by Mϕ proceeds rapidly in the absence of serum opsonins (22). However, opsonization could affect the intracellular fate of yeast cells by ligating additional receptors on the Mϕ membrane (i.e., CR_1 and FcR), thereby more efficiently activating Mϕ fungicidal or fungistatic activity. We tested this hypothesis by opsonizing yeast cells in 10% pooled human serum (PHS),

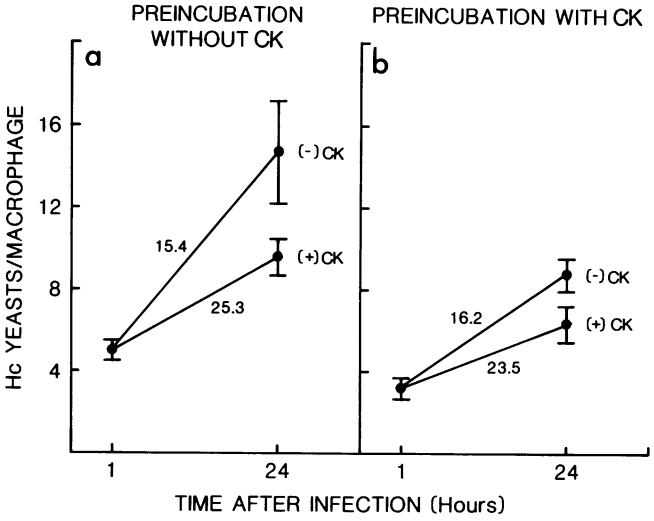

FIG. 2. Mϕ preincubated with cytokines (CK) for 24 h minimally restrict the intracellular growth of yeast cells. Cultured Mϕ were adhered to glass coverslips and then incubated for 24 h in medium only (a) or in medium containing 40% cytokine supernatant (b). The monolayers were washed, infected with yeast cells, and then incubated for an additional 24 h in medium alone [(−) CK] or in medium containing 40% cytokine supernatant [(+) CK]. Results are the means ± SEM of four experiments performed in duplicate. The numbers adjacent to each line on the graphs are the IGTs of yeast cells.

10% heat-inactivated PHS (56°C, 30 min), and 10% heat-inactivated human immune serum and then quantified their intracellular growth in Mϕ incubated in medium alone or in medium containing cytokines. PHS was prepared from eight individual donors and contained no complement-fixing antibody against *H. capsulatum*. The immune serum was from a histoplasmosis patient with a complement fixation titer of 1:128 (provided by L. J. Wheat, Indiana University, Indianapolis).

Opsonized and unopsonized yeast cells were added to Mϕ monolayers to achieve an average of 2.3 to 2.4 yeast cells per infected Mϕ. As shown in Table 1, opsonization of yeast cells with immune or nonimmune serum did not affect their rate of intracellular growth in control Mϕ or in Mϕ stimulated with cytokines.

These experiments demonstrate that *H. capsulatum* yeast cells multiply within human monocytes, cultured Mϕ, and AM and that cytokines activate human monocytes/Mϕ to inhibit the intracellular growth of yeast cells. The pattern of intracellular multiplication of yeast cells in human monocytes/Mϕ and the ability of cytokines to activate monocytes/Mϕ to inhibit the intracellular growth of yeast cells are different in several respects from those reported for most other intracellular pathogens.

First, *H. capsulatum* yeast cells grow better in freshly isolated monocytes than in cultured Mϕ or in AM. In contrast, previous studies have found that viral (4), fungal (6, 28), bacterial (3, 12, 26), and protozoan (19, 20) intracellular pathogens multiply more rapidly in cultured Mϕ than in monocytes. This loss in Mϕ antimicrobial activity is temporally concomitant with the loss during culture of the enzyme myeloperoxidase (28) and the decline in the ability of cultured Mϕ to be stimulated to release O_2^- (25, 28), H_2O_2 (19, 28), and ·OH (28).

A second difference between the interaction of *H. capsulatum* yeast cells with human Mϕ and that of other intracellular parasites is that optimal activation of Mϕ requires that cytokines be added to the cultures immediately after infection of Mϕ by yeast cells. These results suggest that cytokine(s) very rapidly activate Mϕ to inhibit the intracellular growth of *H. capsulatum* yeast cells and that the effect is transient. In contrast, preincubation of cultured Mϕ with cytokine-containing supernatants or with IFN-γ for 24 to 48 h prior to infection does activate Mϕ to inhibit the intracellular growth of *Trypanosoma cruzi* (24), *Leishmania donovani* (19), *Toxoplasma gondii* (20), and *Chlamydia psittaci* (26). Although studies with these organisms indicate that cytokine activation of Mϕ microbicidal and microbistatic activity is concomitant with an increase in the production of respiratory burst products (19, 20), they also demonstrate clearly that cytokines activate Mϕ oxygen-independent activity as well (19, 20, 26, 28).

A third difference between the interaction of *H. capsulatum* yeast cells with human Mϕ and that of other intracellular parasites is that recombinant human IFN-γ does not activate cultured Mϕ to inhibit the intracellular growth of yeast cells (9, 21a). Likewise, IFN-γ does not activate cultured Mϕ to kill *Cryptococcus neoformans* (18). Thus, the specific cytokine(s) required to activate human Mϕ antifungal activity may be different from those that activate antibacterial and antiprotozoan activity.

H. capsulatum yeast cells activate the respiratory burst of phagocytes upon phagocytosis (1, 29, 30) but are not killed (9, 29). Furthermore, the intracellular growth of *H. capsulatum* yeast cells proceeds at a similar rate in freshly isolated monocytes and in cultured Mϕ that have no myeloperoxidase and a decreased ability to produce toxic oxygen metabolites. In addition, monocytes and cultured Mϕ that have been stimulated by cytokines inhibit the intracellular growth of yeast cells equally well. Therefore, these data suggest that yeast cells are resistant to toxic oxygen metabolites and that inhibition of the intracellular growth of yeast

TABLE 1. Effect of opsonization of *H. capsulatum* yeast cells on growth within Mϕ

Condition[a]	No. of yeast cells/Mϕ (mean ± SEM; n = 4)			IGT (h) in:	
	1 h	Medium, 24 h	Cytokine supernatant, 24 h	Medium, 24 h	Cytokine supernatant, 24 h
Unopsonized	2.3 ± 0.1	6.7 ± 0.6	4.8 ± 0.2	15.6	22.7
PHS	2.3 ± 0.0	6.7 ± 0.8	5.1 ± 0.3	15.8	21.1
ΔPHS	2.4 ± 0.1	6.3 ± 0.3	4.4 ± 0.1	16.9	26.9
ΔHuImS	2.4 ± 0.1	6.0 ± 0.4	4.4 ± 0.2	18.3	27.6

[a] Unopsonized, Unopsonized yeast cells; PHS, yeast cells opsonized in 10% pooled human serum; ΔPHS, yeast cells opsonized in 10% heat-inactivated pooled human serum; ΔHuImS, yeast cells opsonized in 10% heat-inactivated human immune serum.

cells by cytokine-activated monocytes/Mϕ probably is mediated by a non-oxygen-dependent pathway.

Although opsonization of *H. capsulatum* yeast cells in serum is not required for phagocytosis by human Mϕ (22), opsonization could influence the rate of intracellular growth of yeast cells. However, yeast cells opsonized in normal or immune serum multiplied within control and cytokine-activated Mϕ at similar rates. Thus, opsonization of yeast cells does not enhance Mϕ fungistatic activity regardless of whether the Mϕ are activated. Opsonization of *H. capsulatum* yeast cells in immune serum also does not alter their rate of intracellular growth in PM from mice or guinea pigs immunized by sublethal infection with viable yeast cells or immunized with killed yeast cells (14).

Studies of *H. capsulatum* reveal two interesting differences in the way that this yeast interacts with human Mϕ and mouse PM. First, unopsonized yeast cells stimulate the respiratory burst in human Mϕ (1) but not in mouse PM (8, 31, 32). Second, IFN-γ activates mouse PM to restrict the intracellular growth of yeast cells (35), but it does not activate human Mϕ to do so (9, 21a). Interestingly, IFN-γ also activates mouse PM (10, 17), but not cultured human Mϕ (18), to kill *C. neoformans*. Killing of *H. capsulatum* yeast cells has not been demonstrated in either human Mϕ or mouse Mϕ.

Current efforts are directed towards identifying the cytokine(s) that activates human Mϕ to inhibit the intracellular growth of *H. capsulatum* yeast cells and characterizing the mechanism(s) by which inhibition of growth is mediated.

We thank George S. Deepe for critical review of the manuscript.
This work was supported by Public Health Service grants AI-17339, AI-23985, and AI-28392 from the National Institute of Allergy and Infectious Diseases.

REFERENCES

1. **Bullock, W. E., and S. D. Wright.** 1987. Role of adherence-promoting receptors, CR3, LFA-1, and p150,95, in binding of *Histoplasma capsulatum* by human macrophages. J. Exp. Med. **165**:195–210.
2. **Colton, T.** 1974. Statistics in medicine. Little, Brown and Co., Boston.
3. **Czuprynski, C. J., P. A. Campbell, and P. M. Henson.** 1983. Killing of *Listeria monocytogenes* by human neutrophils and monocytes, but not by monocyte-derived macrophages. RES J. Reticuloendothel. Soc. **34**:29–44.
4. **Daniels, C. A., E. S. Kleinerman, and R. Snyderman.** 1978. Abortive and productive infections of human mononuclear phagocytes by type 1 herpes simplex. Am. J. Pathol. **91**:119–129.
5. **Deepe, G. S., Jr., and W. B. Bullock.** 1988. Histoplasmosis: a granulomatous inflammatory response, p. 733–749. *In* J. I. Gallin, I. M. Goldstein, and R. Snyderman (ed.), Inflammation: basic principles and clinical correlates. Raven Press, Ltd., N.Y.
6. **Diamond, R. D., and J. E. Bennett.** 1973. Growth of *Cryptococcus neoformans* within human macrophages *in vitro*. Infect. Immun. **7**:231–236.
7. **Dohn, M. N., and R. P. Baughman.** 1985. Effect of changing instilled volume for bronchoalveolar lavage in patients with interstitial lung disease. Am. Rev. Respir. Dis. **132**:390–392.
8. **Eissenberg, L. G., and W. E. Goldman.** 1987. *Histoplasma capsulatum* fails to trigger release of superoxide from macrophages. Infect. Immun. **55**:29–34.
9. **Fleischmann, J., B. Wu-Hsieh, and D. H. Howard.** 1990. The intracellular fate of *Histoplasma capsulatum* in human macrophages is unaffected by recombinant human interferon-γ. J. Infect. Dis. **161**:143–145.
10. **Flesch, I. E. A., G. Schwamberger, and S. H. E. Kaufmann.** 1989. Fungicidal activity of IFN-gamma-activated macrophages: extracellular killing of *Cryptococcus neoformans*. J. Immunol. **142**:3219–3224.
11. **Hill, G. A., and S. Marcus.** 1957. Bacteriol. Proc., p. 81.
12. **Horwitz, M. A., and S. C. Silverstein.** 1981. Activated human monocytes inhibit the intracellular multiplication of Legionnaires' disease bacteria. J. Exp. Med. **154**:1618–1635.
13. **Howard, D. H.** 1964. Intracellular behavior of *Histoplasma capsulatum*. J. Bacteriol. **87**:33–38.
14. **Howard, D. H.** 1965. Intracellular growth of *Histoplasma capsulatum*. J. Bacteriol. **89**:518–523.
15. **Howard, D. H.** 1973. Further studies on the inhibition of *Histoplasma capsulatum* within macrophages from immunized animals. Infect. Immun. **8**:577–581.
16. **Howard, D. H., V. Otto, and R. K. Gupta.** 1971. Lymphocyte-mediated cellular immunity in histoplasmosis. Infect. Immun. **4**:605–610.
17. **Levitz, S. M., and D. J. DiBenedetto.** 1988. Differential stimulation of murine resident peritoneal cells by selectively opsonized encapsulated and acapsular *Cryptococcus neoformans*. Infect. Immun. **56**:2544–2551.
18. **Levitz, S. M., and T. P. Farrell.** 1990. Growth inhibition of *Cryptococcus neoformans* by cultured human monocytes: role of the capsule, opsonins, the culture surface, and cytokines. Infect. Immun. **58**:1201–1209.
19. **Murray, H. W., and D. M. Cartelli.** 1983. Killing of intracellular *Leishmania donovani* by human mononuclear phagocytes. Evidence for oxygen-dependent and -independent leishmanicidal activity. J. Clin. Invest. **72**:32–44.
20. **Murray, H. W., B. Y. Rubin, S. M. Carriero, A. M. Harris, and E. A. Jaffe.** 1985. Human mononuclear phagocyte antiprotozoal mechanisms: oxygen-dependent vs oxygen-independent activity against intracellular *Toxoplasma gondii*. J. Immunol. **134**:1982–1988.
21. **Newberry, W. M., Jr., J. W. Chandler, Jr., T. D. Y. Chin, and C. H. Kirkpatrick.** 1968. Immunology of the mycoses. I. Depressed lymphocyte transformation in chronic histoplasmosis. J. Immunol. **100**:436–443.
21a. **Newman, S. L.** Unpublished data.
22. **Newman, S. L., C. Bucher, J. Rhodes, and W. E. Bullock.** 1990. Phagocytosis of *Histoplasma capsulatum* yeasts and microconidia by human cultured macrophages and alveolar macrophages. Cellular cytoskeleton requirement for attachment and ingestion. J. Clin. Invest. **85**:223–230.
23. **Newman, S. L., R. A. Musson, and P. M. Henson.** 1980. Development of functional complement receptors during *in vitro* maturation of human monocytes into macrophages. J. Immunol. **125**:2236–2244.
24. **Nogueira, N., S. Chaplan, M. Reesink, J. Tydings, and Z. A. Cohn.** 1982. *Trypanosoma cruzi*: induction of microbicidal activity in human mononuclear phagocytes. J. Immunol. **128**: 2142–2146.
25. **Pabst, M. J., H. B. Hedegaard, and R. B. Johnston, Jr.** 1982. Cultured monocytes require exposure to bacterial products to maintain an optimal oxygen radical response. J. Immunol. **128**:123–128.
26. **Rothermel, C. D., B. Y. Rubin, E. A. Jaffe, and H. W. Murray.** 1986. Oxygen-independent inhibition of intracellular *Chlamydia psittaci* growth by human monocytes and interferon-γ-activated macrophages. J. Immunol. **137**:689–692.
27. **Salvin, S. B.** 1955/56. Acquired resistance in experimental histoplasmosis. Trans. N.Y. Acad. Sci. **18**:462–468.
28. **Sasada, M., A. Kubo, T. Nishimura, T. Kakita, T. Moriguchi, K. Yamamoto, and H. Uchino.** 1987. Candidacidal activity of monocyte-derived human macrophages: relationship between candida killing and oxygen radical generation by human macrophages. J. Leukocyte Biol. **41**:289–294.
29. **Schaffner, A., C. E. Davis, T. Schaffner, M. Markert, H. Douglas, and A. I. Braude.** 1986. *In vitro* susceptibility of fungi to killing by neutrophil granulocytes discriminates between primary pathogenicity and opportunism. J. Clin. Invest. **78**:511–524.
30. **Schnur, R. A., and S. L. Newman.** 1990. The respiratory burst response to *Histoplasma capsulatum* by human neutrophils. Evidence for intracellular trapping of superoxide anion. J.

Immunol. **144**:4765–4772.

31. **Wolf, J. E., A. L. Abegg, S. J. Travis, G. S. Kobayashi, and J. R. Little.** 1989. Effects of *Histoplasma capsulatum* on murine macrophage functions: inhibition of macrophage priming, oxidative burst, and antifungal activities. Infect. Immun. **57**:513–519.
32. **Wolf, J. E., V. Kerchberger, G. S. Kobayashi, and J. R. Little.** 1987. Modulation of the macrophage oxidative burst by *Histoplasma capsulatum*. J. Immunol. **138**:582–586.
33. **Worsham, P., and W. E. Goldman.** 1988. Quantitative plating of *Histoplasma capsulatum* without addition of conditioned medium or siderophores. J. Med. Vet. Mycol. **26**:137–143.
34. **Wu-Hsieh, B., and D. H. Howard.** 1984. Inhibition of growth of *Histoplasma capsulatum* by lymphokine stimulated macrophages. J. Immunol. **132**:2593–2597.
35. **Wu-Hsieh, B. A., and D. H. Howard.** 1987. Inhibition of the intracellular growth of *Histoplasma capsulatum* by recombinant murine gamma interferon. Infect. Immun. **55**:1014–1016.