INFECTION AND IMMUNITY, Feb. 1983, p. 817-822
0019-9567/83/020817-06$02.00/0

Vol. 39, No. 2

Activation of Peritoneal Macrophages by Concanavalin A or *Mycobacterium bovis* BCG for Fungicidal Activity Against *Blastomyces dermatitidis* and Effect of Specific Antibody and Complement

ELMER BRUMMER,* ALAN M. SUGAR, AND DAVID A. STEVENS

Division of Infectious Diseases, Department of Medicine, Santa Clara Valley Medical Center, San Jose, California 95128; Stanford University, Stanford, California 94305; and the Institute For Medical Research, San Jose, California 95128

Received 25 June 1982/Accepted 2 November 1982

With a new short-term assay, where the reduction of CFUs in the inoculum could be measured, we investigated the killing of the dimorphic fungal pathogen *Blastomyces dermatitidis* in its yeast phase by murine peritoneal macrophages. Peritoneal macrophages from concanavalin A- or *Mycobacterium bovis* BCG-treated mice, but not resident or thioglycolate-elicited macrophages, significantly reduced the CFUs of *B. dermatitidis* in the inoculum. The activation of peritoneal macrophages for fungicidal activity by concanavalin A treatment was shown to be dose dependent and transient, i.e., absent after 72 h. These results indicate that it is possible for murine peritoneal macrophages to kill *B. dermatitidis* in vitro. The addition of specific antibody or complement or both did not enhance the killing of *B. dermatitidis* by these nonspecifically activated macrophages.

Resident macrophages are important components in the host's early defense against pathogens. This is especially true for most extracellular bacterial pathogens, which are killed with facility by both resident alveolar and peritoneal macrophages (15, 19, 20, 27). However, specific antibody or complement or both may be required for the phagocytosis and killing of some bacterial pathogens, e.g., encapsulated forms (3, 32, 33). On the other hand, resident macrophages need to become activated before they can effectively kill obligate or facultative intracellular bacterial pathogens in vitro (2, 6, 12, 14, 17, 22).

In contrast to the killing of extracellular bacterial pathogens, resident macrophages are relatively inefficient in killing certain fungal pathogens, e.g., *Candida albicans* (21, 30) and *Cryptococcus neoformans* (11, 14, 23). However, activated macrophages are more effective in killing these pathogens (14, 30) and can kill facultative intracellular fungal pathogens, e.g., *Histoplasma capsulatum* (17, 18) or *Coccidioides immitis* (4, 5).

We studied another fungal pathogen, *Blastomyces dermatitidis*, which histologic studies have shown is commonly extracellular. We have reported that resident peritoneal macrophages are less efficient than activated macrophages in restricting the replication of this pathogen in vitro (7, 8). In these 24-h in vitro assays, we were able to document the inhibition of replication but not the killing of *B. dermatitidis*. With a new short-term (4-h) assay, in which the reduction of CFUs in the original inoculum could be measured, we investigated the killing of *B. dermatitidis* by macrophages in vitro. We report here on the ability of peritoneal macrophages (i) from untreated mice or (ii) from thioglycolate-, concanavalin A (ConA)-, or *Mycobacterium bovis* BCG-treated mice to kill *B. dermatitidis* in vitro. The effect of complement or immune serum or both on the ability of macrophages to kill *B. dermatitidis* is also reported.

MATERIALS AND METHODS

Animals. Male BALB/cByJ mice (Jackson Laboratories, Bar Harbor, Me.), 8 to 12 weeks of age, were used throughout this study.

Fungi. A strain of *Blastomyces dermatitidis* (ATCC 26199), which is virulent in mice (16), was used in these studies. Log-phase growth was harvested from 3-ml, 72-h liquid (16) cultures grown on a rotary shaker (200 rpm) at 37°C. The yeast cells were pelleted by centrifugation (900 × *g*, 10 min), washed twice with 4 ml of saline, and suspended in tissue culture medium. Individual fungi and multicellular fungal units were counted with a hemacytometer, and CFUs were determined by plating in triplicate 1-ml portions of an appropriate dilution on blood agar plates.

Peritoneal macrophages. Peritoneal cells from treated or untreated mice were collected from the abdominal cavity of each mouse by repeated lavage using a

total of 10 ml of Eagle minimal medium (MEM) (GIBCO Laboratories, Grand Island, N.Y.) containing heparin (10 U/ml). The cells were centrifuged (225 × *g*, 10 min), and the cells from three to six mice were pooled in 10 ml of MEM without heparin. After centrifugation, the pooled cells were suspended in complete tissue culture medium (RPMI 1640; GIBCO) containing 10% (vol/vol) of heat-inactivated fetal calf serum, penicillin (100 U/ml), and streptomycin (100 μg/ml). The number of cells per milliliter was determined with a hemacytometer, and cell samples were dispensed (0.2 ml of 5×10^6/ml) into flat-bottom wells of Micro Test II tissue culture plates (no. 3040; Falcon Plastics, Oxnard, Calif.). After 2 h at 37°C in 5% CO_2–95% air, nonadherent cells were removed from the wells by aspiration and one wash with medium. When the number of nonadherent cells was subtracted from the number of incubated cells, the average number of adherent cells per well was 0.5×10^6. Adherent cells had several characteristics of macrophages as previously reported (7, 8), and 90% were esterase positive.

Treatment of mice. Mice were treated in one of the following ways: (i) 1 ml of thioglycolate medium (Difco Laboratories, Detroit, Mich.) intraperitoneally (i.p.) 4 days before peritoneal cell harvest (i.e., day −4); (ii) 20, 100, or 500 μg of concanavalin A (ConA) (Sigma Chemical Co., St. Louis, Mo.), i.p., day −1 or −3; (iii) 0.2 ml of 29×10^6 to 189×10^6 viable BCG per ml (Glaxo Laboratories, Middlesex, England), i.p., day −12; or (iv) infected subcutaneously at two dorsal sites with 20,000 CFUs of *B. dermatitidis* per site, week −4.

Measurement of reduction of CFUs of *B. dermatitidis*. Triplicate or quadruplicate wells without cells or containing adherent cells were challenged with 0.2 ml of complete tissue culture medium containing 1×10^3 to 2×10^3 CFUs of *B. dermatitidis*. The CFUs of the inoculum were determined by plating 1 ml of an appropriate dilution on blood agar plates in quadruplicate at 0 h. After 4 h at 37°C in 5% CO_2–95% air, each well was harvested with a Pasteur pipette, using distilled water to lyse macrophages and also to rinse the wells. The contents of each well were eventually diluted to 20 ml with distilled water. The number of CFUs per well was determined by plating 1 ml of the diluted well contents on a blood agar plate and counting the number of colonies after 4 days at 37°C. The percent reduction of CFUs was calculated by the formula (1 − [CFU experimental/CFU of inoculum]) × 100. When harvested control cultures and cocultures were examined microscopically as previously described (7, 8), there was no evidence of multicellular fungal units or single cell units clumping together compared with cultures at 0 h; therefore, the reduction of CFUs in cocultures was not due to clumping.

Serum, spleen, and lymph node cells. Spleen and lymph nodes were harvested from mice, and single cell suspensions were prepared as previously described (9). To obtain serum, mice were bled at sacrifice by severing the bracchial artery, and the blood was collected from the pouch formed between the skin and the torso. Blood was allowed to clot at room temperature for 2 h, and then the serum was collected and used promptly in in vitro assays. The complement activity in such sera was demonstrated in cytotoxicity assays as described elsewhere (E. Brummer, P. A. Morozumi, P. T. Vo, and D. A. Stevens, Cell. Immunol., in press). Serum antibody titer to *B. dermatitidis* antigens was determined by an enzyme-linked immunosorbent assay previously described (24). The absorption of immune serum (titer 1:40) with *B. dermatitidis* reduced the enzyme-linked immunosorbent assay titer to <1:10, which was equivalent to the titer of serum from untreated mice. The serum from mice recovered from subcutaneous *B. dermatitidis* infection (4 weeks) had enzyme-linked immunosorbent assay titers from 1:80 to >1:320.

Statistical analysis. Student's *t* test was used to determine the significance of differences between means.

TABLE 1. Reduction of CFUs by macrophages from ConA-treated mice

Experimental group	CFUs/well	Reduction of CFUs (%)	$P <$ [a]
B. dermatitidis inoculum per well	660 ± 60[b]		
+ Medium	780 ± 100[c]	0	NS
+ Resident macrophages	660 ± 40[c]	0	NS
+ Thioglycolate macrophages[d]	720 ± 60[c]	0	NS
+ ConA macrophages[e]	520 ± 60[c]	21	0.001

[a] NS, Not significant ($P > 0.05$) for the reduction of inoculum CFUs.
[b] CFUs ± standard deviation at 0 h.
[c] CFUs ± standard deviation of quintuplicate wells at 4 h.
[d] Macrophages elicited by thioglycolate.
[e] Macrophages elicited by 100 μg of ConA, day −1.

RESULTS

Reduction of CFUs of *B. dermatitidis* by macrophages from ConA-treated mice. Peritoneal cells were harvested from untreated, thioglycolate-treated or ConA (100 μg)-treated mice. Peritoneal cells adherent to Micro Test II plate wells were challenged with 660 ± 60 (standard deviation) CFUs of *B. dermatitidis*. After 4 h at 37°C in 5% CO_2–95% air, all cultures were harvested, and the CFUs per well were determined (Table 1). Neither resident macrophages nor thioglycolate-elicited macrophages reduced the number of CFUs in the inoculum. Only macrophages from ConA-treated mice significantly ($P < 0.001$) reduced the number of CFUs of *B. dermatitidis* in the inoculum. This was not enhanced by the addition of fresh mouse serum (5% of total culture volume) as a source of complement (data not shown).

Reduction of CFUs of *B. dermatitidis* by macrophages from BCG-treated mice. Peritoneal cells were harvested from groups (five mice per group) of untreated, ConA (100 μg, day −1)-, or BCG-treated mice. Peritoneal cells adherent to

TABLE 2. Reduction of CFUs by macrophages from BCG-treated mice

Experimental group	CFUs/well	Reduction of CFUs (%)	$P <$
B. dermatitidis inoculum per well	2,160 ± 180[a]		
+ Medium	2,033 ± 283[b]	6	NS[c]
+ Resident macrophages	2,313 ± 60	0	NS[c]
+ ConA macrophages[d]	1,573 ± 120	27	0.01
+ BCG macrophages	1,600 ± 180	26	0.02

[a] CFUs ± standard deviation at 0 h.
[b] CFUs ± standard deviation of triplicate wells at 4 h.
[c] NS, Not significant ($P > 0.05$) compared with the inoculum.
[d] Macrophages elicited by 100 μg of ConA, day −1.

Micro Test II plate wells were challenged with a larger inoculum of 2,160 ± 180 CFUs of *B. dermatitidis*. After 4 h at 37°C in 5% CO_2–95% air, all cultures were harvested, and the CFUs per well were determined. Resident macrophages again were not able to reduce the inoculum CFUs of *B. dermatitidis* (Table 2); however, macrophages from both ConA- and BCG-treated mice significantly ($P < 0.02$) reduced (27 and 26%, respectively) the CFUs in the inoculum.

Effect of the dose of ConA and time after administration on macrophage activation. In one experiment, groups of mice were treated with 20, 100, or 500 μg of ConA, i.p.; then, 1 and 3 days later, adherent peritoneal cells were tested. In a second experiment, groups of mice were treated at days −3 and −1 with 20, 100, or 500 μg of ConA i.p., and adherent peritoneal cells were tested simultaneously on day 0. The combined results from these two experiments are given in Fig. 1. Only macrophages from mice given 100 or 500 μg of ConA 1 day previously significantly ($P < 0.05$) reduced (30%) the CFUs of *B. dermatitidis* in the inoculum. These results indicate that 20 μg of ConA was not a sufficient dose to activate macrophages for the reduction of CFUs, and no advantage was achieved by increasing the dose of ConA from 100 to 500 μg. Furthermore, the activation of macrophages by ConA, even at 500 μg/mouse, was transient and disappeared by 3 days posttreatment.

It was of interest that in concurrent experiments in these mice (data not shown), we found the ConA and BCG treatments described also significantly increased spleen weight, number of spleen cells, and peritoneal cell yields (but not the number of lymph node cells) compared with untreated mice at the time when fungicidal activity was demonstrated in peritoneal cells. However, the increased spleen weight and the number of spleen and peritoneal cells persisted to 72 h after ConA treatment, when fungicidal activity could no longer be shown.

Effect of complement or immune serum on reduction of CFUs by macrophages. We noted above that complement failed to augment the activity of macrophages from ConA-treated mice. Furthermore, the presence of complement had no effect on the inability of resident or thioglycolate-elicited macrophages to reduce the CFUs in the inoculum (data not shown). We also tested the effect of adding fresh immune serum to the tissue culture medium (Table 3). In this and in a second experiment of this type, it was found that fresh immune serum lessened the ability of ConA-activated macrophages to reduce inoculum CFUs. Moreover, the addition of fresh immune serum (10%) had no effect on the inability of resident macrophages or macrophages from mice that had resolved subcutaneously *B. dermatitidis* infections to reduce the CFUs in the inoculum (data not shown). These findings suggest that complement and serum antibody to *B. dermatitidis* are not necessary and do not augment the reduction of CFUs in the inoculum by ConA-activated macrophages nor enable nonactivated macrophages to do so.

Effect of immune serum on macrophage inhibi-

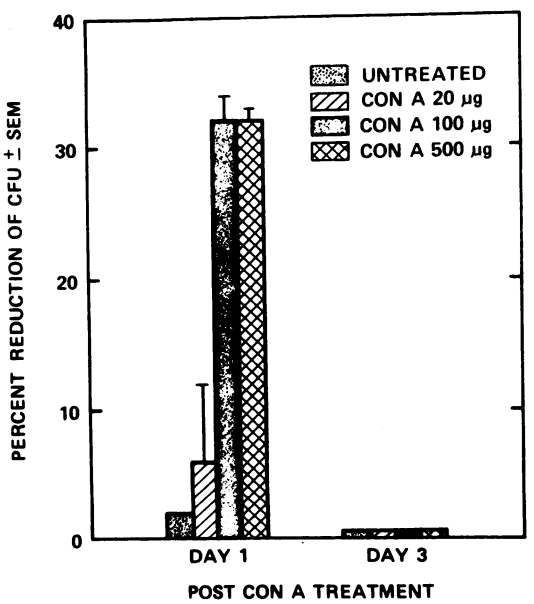

FIG. 1. Effect of ConA dose and time after treatment on macrophage activation. The reduction in the CFUs in the inoculum of *B. dermatitidis* at 4 h by macrophages from untreated (⊡), 20 μg (▨), 100 μg (■), or 500 μg (▩) is shown for ConA-treated mice 1 or 3 days posttreatment. The combined results from two separate experiments are given.

TABLE 3. Effect of complement or immune serum on reduction of CFUs by ConA-activated macrophages

Experimental group	CFUs/well	Reduction of CFUs (%)	$P <$
B. dermatitidis inoculum per well	1,350 ± 65[a]		
+ Medium	1,805 ± 230[b]	0	NS[c]
+ ConA macrophages	810 ± 218[b]	40	0.01
+ Con A macrophages + immune serum[d]	1,065 ± 123[b]	21	0.02

[a] CFUs ± standard deviation at 0 h.
[b] CFUs ± standard deviation of triplicate wells at 4 h.
[c] NS, Not significant ($P > 0.05$) for the reduction of inoculum CFUs.
[d] Ten percent of the total culture volume.

tion of *B. dermatitidis* replication. We have reported previously that peritoneal macrophages from mice treated wtih ConA or thioglycolate, but not those from untreated mice, significantly inhibited the replication of *B. dermatitidis* in a 24-h in vitro assay (7, 8). In conjunction with measuring the effect of immune serum on the reduction of inoculum CFUs by macrophages (see above), we also measured the effect of immune serum on the macrophage inhibition of *B. dermatitidis* replication (Table 4). Although macrophages from ConA- or thioglycolate-treated mice significantly ($P < 0.01$) inhibited (40 to 60%) the replication of *B. dermatitidis* (Table 4), it was not enhanced by the presence of specific antibody and complement (Table 4).

DISCUSSION

We have presented data in this report which indicate that macrophages from ConA- or BCG-treated mice, but not from untreated or thioglycolate-treated mice, were able to significantly reduce the number of CFUs in an inoculum of *B. dermatitidis* in a 4-h assay. Since *B. dermatitidis* does not replicate rapidly (doubling time in vitro 5 to 10 h), the reduction of CFUs by macrophages in 4-h assays represented killing. The high ratio of macrophages to inoculum CFUs (250:1 to 500:1) used in these studies gave consistent results, and the microscopic observation of phagocytosis indicated that all of the multicellular fungal units in the inoculum were surrounded and covered by the macrophages. Because of this, other macrophage/fungus ratios were not studied.

Specific antibody and complement were not required for the killing of *B. dermatitidis* by these activated macrophages. Furthermore, the killing of *B. dermatitidis* by ConA-activated macrophages was not augmented when fresh immune serum was present. Although these findings indicate that *B. dermatitidis* can be killed by appropriately activated macrophages without the aid of specific antibody and complement, they do not rule out a role for these factors under other conditions.

The fungicidal activity of non-specifically activated murine macrophages (ConA, BCG) for *C. neoformans*, similar to that reported here for *B. dermatitidis*, has been described (31). Complement (fresh rabbit serum), but not specific antibody, was required in that system. The enhanced fungicidal activity of non-specifically activated macrophages (lipopolysaccharide, BCG) has also been reported for *C. albicans* (30). Whether non-specifically activated macrophages are fungicidal for dimorphic pathogenic fungi other than *B. dermatitidis*, e.g., *H. capsulatum*, *C. immitis*, or *Paracoccidioides brasiliensis*, is not known at this time.

In the present study, we demonstrated that the activation of resident peritoneal macrophages for fungicidal activity by ConA was dose dependent up to a point and was transient, for example, present at day 1 but absent by day 3. These results are consistent with ConA activation of macrophages for tumoricidal activity reported to be present at day 1 by Ruco and Meltzer (28) but absent at day 3 by Adams (1). Both ConA and BCG treatment increased spleen weight and cellularity, but the activation of peritoneal macrophages by ConA, in contrast to BCG, was transient. This suggests two types of

TABLE 4. Effect of immune serum on macrophage inhibition of *B. dermatitidis* replication in 24-h cocultures

B. dermatitidis +	CFUs/well[a]	% Inhibition of replication	$P <$
1 Medium	18,000 ± 2,200		
Resident macrophages	15,800 ± 900	12	NS[b]
ConA macrophages	10,700 ± 700	40	0.01
Thioglycolate macrophages	7,300 ± 300	60	0.01
2 Medium + IMS + C'[c]	14,500 ± 1,200	—	—
Resident macrophages	14,000 ± 1,200	4	NS[b]
ConA macrophages	11,700 ± 600	20	0.02
Thioglycolate macrophages	10,700 ± 1,000	27	0.02

[a] CFUs ± the standard deviation of triplicate cultures at 24 h; at 0, 2,660 ± 240.
[b] NS, Not significant, $P > 0.05$.
[c] Immune mouse serum (5%) + 5% fresh normal mouse serum in all cultures in 2.

activation, a short intense transient stimulation (ConA) and the continual stimulation of an i.p. BCG infection. Possible mechanisms by which ConA stimulation or BCG infection activates macrophages, for example, by lymphokines, have been described by others (28, 29).

As reported here and previously (8, 9), macrophages from mice treated wih ConA or thioglycolate, but not those from untreated mice, significantly inhibited the replication of *B. dermatitidis* in 24-h cocultures. In these previous studies, the macrophages from ConA-treated mice were selected by a 24-h incubation period, compared with that described here. The present studies suggest that two mechanisms could participate in the inhibition of replication. ConA-activated macrophages kill a part of the inoculum, but surviving fungi replicate and exceed the original inoculum (Table 4). Macrophages from thioglycolate-treated mice, on the other hand, did not kill *B. dermatitidis*, yet they significantly inhibited replication in the 24-h assay. This indicates that the mechanism of inhibition does not have to involve killing. In addition to enlarging our appreciation of the complexity of effector cell-fungus interactions by the agents studied here, the observations of enhanced inhibition and killing could possibly also have implications for immunomodulation in general as an approach to therapy. Other immunomodulating agents whose actions include the activation of macrophages (26) can also activate macrophages for antifungal activity (10, 25), and one such class of agents has been shown to enhance in vivo resistance against fungal infections (13), including blastomycosis (P. A. Morozumi, E. Brummer, and D. A. Stevens, Mycopathologia, in press).

LITERATURE CITED

1. **Adams, D. O.** 1980. Effector mechanisms of cytolytically activated macrophages. I. Secretion of neutral proteases and effect of protease inhibitors. J. Immunol. **124**:286–292.
2. **Alexander, J., and C. C. Smith.** 1978. Growth of *Mycobacterium lepraemurium* in non-stimulated and stimulated mouse peritoneal and bone marrow derived macrophages in vitro. Infect. Immun. **22**:631–636.
3. **Bartell, P. F., and A. Krikszens.** 1980. Influence of anti-slime glycoprotein serum on the interaction between *Pseudomonas aeruginosa* and macrophages. Infect. Immun. **27**:777–783.
4. **Beaman, L., and C. A. Holmberg.** 1980. In vitro response of alveolar macrophages to infection with *Coccidioides immitis*. Infect. Immun. **28**:594–600.
5. **Beaman, L., E. Benjamini, and D. Pappagianis.** 1981. Role of lymphocytes in macrophage-induced killing of *Coccidioides immitis* in vitro. Infect. Immun. **34**:347–353.
6. **Blanden, R. V., G. B. Mackaness, and F. M. Collins.** 1966. Mechanisms of acquired resistance in mouse typhoid. J. Exp. Med. **124**:585–600.
7. **Brummer, E., P. A. Morozumi, D. E. Philpott, and D. A. Stevens.** 1981. Virulence of fungi: correlation of virulence of *Blastomyces dermatitidis* in vivo with escape from macrophage inhibition or replication in vitro. Infect. Immun. **32**:864–871.
8. **Brummer, E., P. A. Morozumi, and D. A. Stevens.** 1980. Macrophages and fungi: in vitro effects of method of macrophage induction, activation by different stimuli, and soluble factors on blastomyces. RES J. Reticuloendothel. Soc. **28**:507–518.
9. **Brummer, E., T. W. Vris, and H. S. Lawrence.** 1977. A microculture system for measurement of antigen-induced murine lymphocyte proliferation: advantages of 5% horse serum and 5×10^{-5} M mercaptoethanol. J. Immunol. Methods **17**:319–327.
10. **Cummings, N. P., M. J. Pabst, and R. B. Johnston.** 1980. Activation of macrophages for enhanced release of superoxide anion and greater killing of *Candida albicans* by injection of muramyl dipeptide. J. Exp. Med. **152**:1659–1669.
11. **Diamond, R. D., and J. E. Bennett.** 1973. Growth of *Cryptococcus neoformans* within human macrophages in vitro. Infect. Immun. **7**:231–236.
12. **Filice, G., B. L. Beaman, and J. S. Remington.** 1980. Effects of activated macrophages on *Nocardia asteroides*. Infect. Immun. **27**:643–649.
13. **Fraser-Smith, E. B., R. V. Waters, and T. R. Matthews.** 1982. Correlation between in vivo anti-*Pseudomonas* and anti-*Candida* activities and clearance of carbon by the reticuloendothelial system for various muramyl dipeptide analogs, using normal and immunosuppressed mice. Infect. Immun. **35**:105–110.
14. **Gentry, L. O., and J. S. Remington.** 1971. Resistance against *Cryptococcus* conferred by intracellular bacteria and protozoa. J. Infect. Dis. **123**:22–31.
15. **Goldstein, E., W. Lippert, and D. Warshauer.** 1974. Pulmonary alveolar macrophages: defender against bacterial infection of the lung. J. Clin. Invest. **54**:519–528.
16. **Harvey, R. P., E. S. Schmid, C. C. Carrington, and D. A. Stevens.** 1978. Mouse model of pulmonary blastomycosis: utility, simplicity and quantitative parameters. Am. Rev. Respir. Dis. **17**:695–703.
17. **Howard, D. H.** 1965. Intracellular growth of *Histoplasma capsulatum*. J. Bacteriol. **89**:518–523.
18. **Howard, D. H., and V. Otto.** 1977. Experiments on lymphocyte mediated cellular immunity in murine histoplasmosis. Infect. Immun. **16**:226–231.
19. **Jakab, G. J., and G. M. Green.** 1976. Defect in intracellular killing of *Staphylococcus aureus* within alveolar macrophages in Sendai virus infected murine lung. J. Clin. Invest. **57**:1533–1539.
20. **Kim, M., E. Goldstein, J. P. Lewis, W. Lippert, and D. Warshauer.** 1976. Murine pulmonary alveolar macrophages: rates of bacterial ingestion, inactivation and destruction. J. Infect. Dis. **133**:310–320.
21. **Lehrer, R. I., L. G. Ferrari, J. Patterson-Delafield, and T. Sorrell.** 1980. Fungicidal activity of rabbit alveolar and peritoneal macrophages against *Candida albicans*. Infect. Immun. **28**:1001–1008.
22. **Melendez, M., M. C. Gonzalez, M. Reid, C. Fuentes, and D. Castillo.** 1978. Immunity to antigenically related *Salmonella*: effects of humoral factors on the bactericidal activity of normal and immune peritoneal exudate cells. Infect. Immun. **22**:640–643.
23. **Mitchell, T. G., and L. Friedman.** 1972. In vitro phagocytosis and intracellular fate of variously encapsulated strains of *Cryptococcus neoformans*. Infect. Immun. **5**:491–498.
24. **Morozumi, P. A., J. W. Halpern, and D. A. Stevens.** 1981. Susceptibility differences of inbred strains of mice to blastomycosis. Infect. Immun. **32**:160–168.
25. **Nozawa, R. T., R. Sekiguchi, and T. Yokota.** 1980. Stimulation by conditioned medium of L-929 fibroblasts, *E. coli* lipopolysaccharide, and muramyl dipeptide of candidacidal activity of mouse macrophages. Cell. Immunol. **53**:116–124.
26. **Parant, M.** 1979. Biologic properties of a new synthetic

adjuvant, muramyl dipeptide. Semin. Immunopathol. **2**:101–118.

27. **Pavillard, E. R.** 1963. In vitro phagocytic and bactericidal ability of alveolar and peritoneal macrophages of normal rats. Aust. J. Exp. Biol. **41**:265–274.
28. **Ruco, L. P., and M. S. Meltzer.** 1978. Defective tumoricidal capacity of macrophages from C3H/HeJ mice. J. Immunol. **120**:329–334.
29. **Ruco, L. P., and M. S. Meltzer.** 1977. Macrophage activation for tumor cytotoxicity: induction of tumoricidal macrophages by supernatants of PPD-stimulated Bacillus Calmette-Guerin immune spleen cell cultures. J. Immunol. **119**:889–896.
30. **Sasada, M., and R. B. Johnston.** 1980. Macrophage microbicidal activity: correlation between phagocytosis associated oxidative metabolism and killing of *Candida* by macrophages. J. Exp. Med. **152**:85–98.
31. **Sethi, K. K., and B. Pelster.** 1977. Destruction of *Cryptococcus neoformans* growth by non-specifically activated murine macrophages, p. 269. *In* Wagner and Hahn (ed.), Excerpta Medica International Congress. Elsevier/North-Holland Publishing Co., New York.
32. **Whitnack, E., A. L. Bisno, and E. M. Beachey.** 1981. Hyaluronate capsule prevents attachment of group A streptococci to mouse peritoneal macrophages. Infect. Immun. **31**:985–991.
33. **Wood, W. G.** 1960. Phagocytosis with particular reference to encapsulated bacteria. Bacteriol. Rev. **24**:41–49.