INFECTION AND IMMUNITY, Sept. 1995, p. 3550–3554 Vol. 63, No. 9
0019-9567/95/$04.00+0
Copyright © 1995, American Society for Microbiology

Mechanisms of Inhibition of *Cryptococcus neoformans* by Human Lymphocytes

STUART M. LEVITZ,* ELEANOR A. NORTH, MICHELE P. DUPONT, AND THOMAS S. HARRISON

Evans Memorial Department of Clinical Research and Department of Medicine, Boston University Medical Center Hospital, Boston, Massachusetts

Received 17 March 1995/Returned for modification 29 April 1995/Accepted 14 June 1995

Recently, our laboratory and others have demonstrated that human peripheral blood T and NK lymphocytes directly inhibit the growth of *Cryptococcus neoformans*. In this study, we further define the conditions under which lymphocyte-mediated fungistasis against *C. neoformans* occurs and examine whether mechanisms implicated in lymphocyte-mediated activities against other target cells are also involved in anticryptococcal activity. The addition of whole or broken heat-killed *C. neoformans* modestly inhibited lymphocyte-mediated fungistasis, whereas other particulates had no effect. The hydroxyl radical scavenger catechin, but not diethyl urea or propyl gallate, profoundly inhibited fungistasis. Salicylic acid inhibited fungistasis in a dose-dependent fashion. However, two other cyclooxygenase inhibitors, piroxicam and indomethacin, had no effect, suggesting that the mechanism of inhibition by salicylic acid was cyclooxygenase independent. Reagent prostaglandin E_2, at concentrations shown by others to inhibit NK cell-mediated bactericidal and tumorlytic activities, had no effect on lymphocyte-mediated fungistasis. The addition of selected monoclonal antibodies or ligands reactive with receptors on human lymphocytes had no significant effect on lymphocyte-mediated fungistasis. Acapsular, small-capsuled, and large-capsuled *C. neoformans* organisms were inhibited by lymphocytes to an approximately equal extent. These data demonstrate that lymphocyte-mediated activity against *C. neoformans* proceeds regardless of the presence of capsule and by mechanisms at least in part dissimilar from those seen with other target cells.

The yeast *Cryptococcus neoformans* preferentially causes infections in persons with defective T-cell function, especially those with AIDS (17). The reason impaired T-cell function results in marked susceptibility to cryptococcosis remains incompletely understood. Experimental evidence suggests that when the normal host is challenged with *C. neoformans*, activated T cells secrete cytokines which empower other cells of the immune system (in particular, mononuclear phagocytes) to inhibit and kill *C. neoformans* (18). Recently, our laboratory and others have described a second general mechanism by which T cells can contribute to immunity against cryptococcosis. Resting and interleukin 2-activated human peripheral blood lymphocytes directly bind to and inhibit the growth of *C. neoformans* (16, 19, 20, 30). Fractionation of the lymphocytes into $CD4^+$ T cells, $CD8^+$ cells (a mixture of T and NK cells), and $CD56^+$ NK cells revealed that all three cell populations mediated fungistasis. The Jurkat T-cell leukemia line also inhibited cryptococcal growth (10).

The receptor(s) and cognate ligand(s) on the lymphocyte and *C. neoformans*, respectively, responsible for binding are unknown. Evidence for a receptor-mediated event can be found in studies in which fungistasis was inhibited when lymphocyte receptors were cleaved with the proteases trypsin and bromelain (20). Growth inhibition proceeds in the absence of opsonins, suggesting that lymphocytes recognize epitopes exposed on yeast cells. Support for this concept comes from microscopy studies showing an intimate association between T cells and the capsule of *C. neoformans* (19, 20, 30).

* Corresponding author. Mailing address: Room E336, Boston University Medical Center Hospital, 88 E. Newton St., Boston, MA 02118. Phone: (617) 638-7904. Fax: (617) 638-8070. Electronic mail address: slevitz@med-med1.bu.edu.

A myriad of mechanisms have been proposed to explain how T and NK cells mediate cytotoxicity against susceptible target cells. Available data suggest that different mechanisms are likely to be operative, depending on the natures of the effector and target cells (14, 32). In this study, we further define the conditions under which lymphocyte-mediated fungistasis against *C. neoformans* occurs and examine whether mechanisms implicated in lymphocyte-mediated activities against other target cells are also involved in anticryptococcal activity.

MATERIALS AND METHODS

Materials. Pooled human serum (PHS) was obtained by combining serum samples from at least 10 healthy donors under conditions preserving complement activity. PHS contained low levels of anticapsular (anti-glucuronoxylomannan) antibody of the immunoglobulin G, M, and A classes, as measured by a sensitive enzyme-linked immunosorbent assay in the laboratory of Thomas Kozel (Reno, Nev.) (11). PHS was heat inactivated by incubation at 56°C for 30 min. The cell culture medium was RPMI 1640 (Biowhittaker, Inc., Walkersville, Md.) supplemented with L-glutamine, penicillin, streptomycin, and 10% PHS (hereafter referred to as complete medium). Flat-bottom 96-well half-area polystyrene tissue culture plates (no. 3696; Costar, Cambridge, Mass.) were used for antifungal assays.

Inhibitors and antibodies. Putative inhibitors were obtained from Sigma Chemical Co. (St. Louis, Mo.). Piroxicam and glucuronic acid were dissolved in dimethyl sulfoxide (DMSO) and then diluted in phosphate-buffered saline (PBS; pH 7.4) to give final concentrations of DMSO between 0.01 and 1%. These concentrations of DMSO had no independent effect on lymphocyte-mediated fungistasis or cryptococcal growth. Catechin was dissolved in either DMSO or hot water with similar results. Hyaluronic acid was dissolved in hot PBS (pH 5.3). The remaining inhibitors were dissolved in PBS or RPMI 1640. Mannan from *Saccharomyces cerevisiae* was prepared by the Cetavlon method.

Monoclonal antibodies (MAb) directed against CD11a (TS1/22), CD18 (TS1/18), and CD62E (ELAM-1-blocking MAb H18/7) were generously provided by F. W. Luscinskas (Brigham and Women's Hospital, Boston, Mass). MAb directed against CD3 and CD45 were from Caltag Laboratories (South San Francisco, Calif.), while anti-CD5 was from Olympus Immunochemicals (Lake Success, N.Y.). All MAb were used at greater than saturating concentrations, as determined by flow cytometry.

Preliminary experiments established that cryptococcal growth in complete medium without lymphocytes was not significantly affected by any of the inhibitors and MAb. Moreover, consistent with studies by others (7), none of the inhibitors affected lymphocyte viability as measured by propidium iodide staining.

Fungi. Serotype A strain 145, obtained from Thomas Mitchell (Duke University Medical Center, Durham, N.C.), was used for all studies unless otherwise indicated. This well-described encapsulated strain is virulent in mouse models of infection and has previously been shown by our laboratory to be inhibited by human T and NK cells (20). Yeast cells were harvested from 4-day-old cultures grown on Sabouraud dextrose agar medium at 30°C, vortexed, and washed three times in PBS before being used in antifungal assays (16, 19, 20). Following negative staining with India ink, average capsule thickness was estimated at 0.7 μm by using a microscope equipped with a calibrated ocular objective.

To study the effects of media and capsule size, as indicated, *C. neoformans* 145 was grown (i) on asparagine minimal agar medium at 30°C and (ii) in RPMI 1640 at 37°C in humidified air supplemented with 5% CO_2. Under such conditions, capsule thickness was estimated at 0.7 and 5.1 μm, respectively (20, 25). CAP67 (obtained from Eric Jacobson, Virginia Commonwealth University, Richmond, Va.), a UV-derived mutant acapsular strain (12), was grown on asparagine minimal agar medium at 30°C. Greater than 90% of cells of both *C. neoformans* strains were present as single yeast cells when grown under the conditions described above, and the clumping of organisms was not observed.

For the experiments depicted in Fig. 1, a well-defined isolate of *Candida albicans* (23) was grown in the yeast phase on Sabouraud dextrose agar. Hyphae were obtained by the incubation of yeasts in RPMI 1640 for 4 h at 37°C (23). *C. neoformans* (strain 145) and *C. albicans* were heat killed by immersion in a 60°C water bath for 30 min, washed, and resuspended at 10^8 (10^7 for hyphae) fungal cells per ml. As hyphae are difficult to accurately enumerate because of clumping, the concentration of hyphae is based on the starting number of yeast cells. *C. neoformans* was broken by vortexing 10^8 heat-killed yeast cells for 30 min with 425- to 600-μm-diameter glass beads. The broken cells were then separated from the beads by decanting. Under these conditions, greater than 80% of the organisms were fragmented when studied under phase-contrast microscopy. Following separation from the glass beads, half of the broken yeast cells were passed through a 0.4-μm-pore-size filter. Monodispersed latex beads (mean diameter, 3.0 ± 0.1 μm; Polysciences, Inc., Warrington, Pa.) were washed and resuspended in RPMI 1640 at 10^8 beads per ml. To each cell well, 10 μl of fungal or bead suspension was added.

Lymphocytes. Human peripheral blood samples were obtained by venipuncture from healthy volunteers. For each set of experiments, the same blood donor was not used more than once. Blood was anticoagulated with heparin, and peripheral blood mononuclear cells were purified by centrifugation on a Ficoll-Hypaque density gradient. Monocytes and B cells were then depleted by adherence to polystyrene tissue culture petri dishes for 1 h, with subsequent passage of nonadherent cells over a nylon wool column (13, 19, 20). The resulting cells, previously referred to as lymphocytes, were over 95% T and NK cells and had undetectable numbers of monocytes and B cells by flow cytometric analysis (20).

The Jurkat human T-cell leukemia line (clone E6-1; American Type Culture Collection Tumor Immunology Bank, Rockville, Md.) was maintained by passage in RPMI 1640 containing 10% fetal calf serum. This cell line expresses CD3 and CD4. Jurkat cells were freshly passaged 1 day prior to use in antifungal assays.

Lymphocyte-mediated fungistasis. Antifungal activities were determined as in previous assays in our laboratory (10, 16, 19–22). Briefly, cell wells contained 10^6 lymphocytes and 10^4 CFU of *C. neoformans* in a final volume of 100 to 150 μl of complete medium. For experiments with MAb depicted in Table 1, heat-inactivated PHS was substituted for PHS to avoid complement-mediated lysis of antibody-coated lymphocytes. Plates were incubated for 18 h at 37°C in humidified air supplemented with 5% CO_2. Then the number of CFU of *C. neoformans* per well was determined by lysing the effector cells with 0.1% Triton X-100 in distilled water, diluting, and spreading on Sabouraud dextrose agar plates.

For each experiment, two sets of cell wells containing *C. neoformans*, medium, and PHS, but no effector cells, were included. The first set was treated with detergent, diluted, and plated immediately. These CFU counts were used to calculate the inoculum of live organisms added per well. The second set was incubated at 37°C for 18 h before being processed and plated. These CFU counts were used to calculate fungal replication in medium containing no effector cells. The results are expressed as percent growth according to the following formula: [(CFU experimental/CFU inoculum) − 1] × 100. Thus, a value of 0 indicates that the number of CFU at the conclusion of incubation was the same as that at the start and that no net fungal growth occurred. Positive values denote fungal growth, with values of 100, 300, and 700% indicating averages of one, two, and three replications per fungal cell, respectively. Negative values mean a decrement in CFU occurred during the course of incubation; therefore, fungal killing took place. The term lymphocyte-mediated fungistasis is used to denote a significant reduction in percent growth in wells containing lymphocytes compared with that in wells lacking lymphocytes. It must be recognized, however, that during the incubation of fungi with effectors, some fungi may be killed while others replicate. Therefore, fungal killing could still take place even if a positive value for percent growth is obtained.

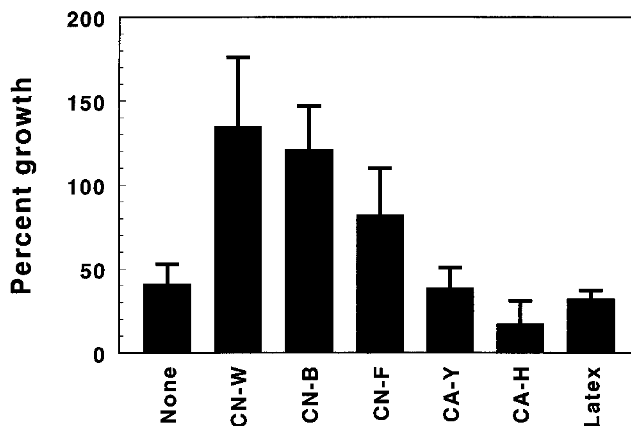

FIG. 1. Effect of heat-killed *C. neoformans*, *C. albicans*, and latex beads on lymphocyte-mediated fungistasis. Lymphocytes and live *C. neoformans* were incubated for 18 h, and the percent growth of *C. neoformans* was determined as described in Materials and Methods. Wells contained no inhibitor (None), whole heat-killed *C. neoformans* (CN-W), heat-killed *C. neoformans* broken with glass beads (CN-B), heat-killed *C. neoformans* broken with glass beads and filtered (CN-F), heat-killed *C. albicans* yeast cells (CA-Y), heat-killed *C. albicans* hyphae (CA-H), or latex beads (Latex). Data are the means ± SEM of three (CN-W, CN-B, and CN-F) or two (CA-Y, CA-H, and Latex) experiments, each performed in triplicate. The fungal growth in wells containing no lymphocytes was (468 ± 71)% ($P < 0.05$, for the results of the None group compared with those for the CN-W and CN-B groups).

Clumping of strain 145 did not occur under these assay conditions. As for strain CAP67, after 18 h of incubation with and without lymphocytes, 90 and 78%, respectively, of fungi were present as solitary or single-budded yeast cells.

Statistics. For sample groups of two, the means and standard errors of the means (SEM) were compared by the unpaired, two-tailed *t* test with a statistical software program (SigmaStat; Jandel Corporation, San Rafael, Calif.). For multiple comparisons, one-way analysis of variance was performed. Bonferroni's method was used to adjust the *P* values to determine significance.

RESULTS

Effect of heat-killed whole and broken yeast cells. Initial experiments examined the effect of the addition of heat-killed yeast cells on lymphocyte-mediated fungistasis (Fig. 1). Heat-killed *C. neoformans* was studied under the following three conditions: (i) whole, (ii) broken with glass beads, and (iii) broken with glass beads and then filtered. In addition, heat-killed *C. albicans* (yeast cells and hyphae) and latex beads were tested. The fungal growth in wells containing either whole or broken heat-killed *C. neoformans* was significantly greater than growth in wells containing no heat-killed yeast cells. In contrast, heat-killed *C. albicans* yeast cells, hyphae, and latex beads had no significant effect on lymphocyte-mediated fungistasis.

Hydroxyl radical scavengers. The abilities of hydroxyl radical scavengers to inhibit lymphocyte-mediated fungistasis were studied next (Fig. 2). At both concentrations tested, catechin profoundly inhibited fungistasis. However, two other hydroxyl radical scavengers, diethyl urea and propyl gallate, at concentrations known to scavenge hydroxyl radicals, had no significant effect. Unfortunately, attempts to test the hydroxyl radical scavengers quinacrine (100 mM) and thiourea (80 mM) for the ability to inhibit lymphocyte-mediated fungistasis were unsuccessful because these compounds proved to be toxic to *C. neoformans* in complete medium without lymphocytes.

Prostaglandins. Cyclooxygenase-catalyzed metabolites of arachidonic acid, particularly prostaglandin E_2 (PGE_2), inhibit NK and LAK activities against tumor and bacterial targets (8, 26). Two approaches, one with cyclooxygenase inhibitors and

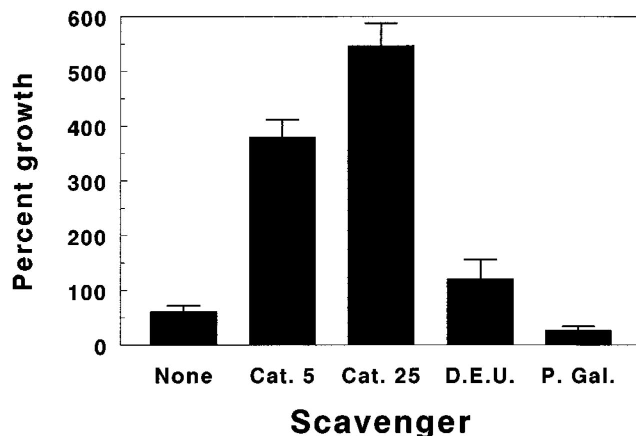

FIG. 2. Effect of hydroxyl radical scavengers on lymphocyte-mediated fungistasis. The percent growth of *C. neoformans* was determined as described in Materials and Methods. Wells contained no scavengers (None), 5 mM catechin (Cat. 5), 25 mM catechin (Cat. 25), 40 mM diethyl urea (D.E.U.), or 500 μM propyl gallate (P. Gal.). Data are the means ± SEM of three to five experiments, each performed in triplicate. The fungal growth in wells containing no lymphocytes was (660 ± 40)% ($P < 10^{-4}$, for the results of the None group compared with those for catechin at either concentration).

the other with reagent PGE_2, were taken to assess the effects of prostaglandins and related compounds on lymphocyte-mediated fungistasis (Fig. 3). Salicylic acid inhibited fungistasis in a dose-dependent fashion. However, two other cyclooxygenase inhibitors, piroxicam and indomethacin, had no effect. Reagent PGE_2, at concentrations shown by others to inhibit NK cell-mediated bactericidal and tumorlytic activities (8, 26), had no effect on lymphocyte-mediated fungistasis. Salicylic acid also significantly inhibited Jurkat T-cell-mediated fungistasis [mean growth ± SEM in two triplicate experiments was (49 ± 12)%, (119 ± 65)%, and (260 ± 59)% in wells containing no inhibitor and 20 and 100 mM salicylic acid, respectively; $P < 0.02$, comparing the results for no inhibitor with those for either concentration of salicylic acid].

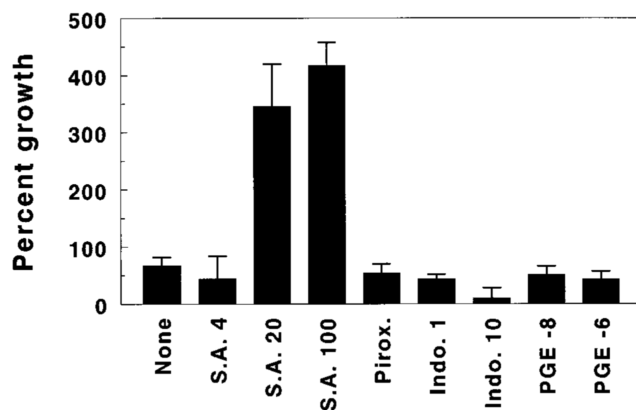

FIG. 3. Effect of cyclooxygenase inhibitors and PGE_2 on lymphocyte-mediated fungistasis. The percent growth of *C. neoformans* was determined as described in Materials and Methods. Wells contained no inhibitors (None); salicylic acid at 4 (S.A. 4), 20 (S.A. 20), or 100 mM (S.A. 100); piroxicam at 10 μg/ml (Pirox.); indomethacin at 1 (Indo. 1) or 10 μM (Indo. 10); or PGE_2 at 10^{-8} (PGE −8) or 10^{-6} M (PGE −6). Data are the means ± SEM of nine triplicate experiments. Each inhibitor was tested in at least three of those experiments. The percent growth in wells containing *C. neoformans* but no lymphocytes was (491 ± 42)% ($P < 10^{-4}$, for the results of the None group compared with those for salicylic acid at 20 and 100 mM).

TABLE 1. Lymphocyte-mediated fungistasis in the presence of MAb against specific cell surface receptors[a]

Target receptor	Mean growth ± SEM (%)
None	128 ± 23
CD11a (LFA-1)	77 ± 26
CD45	56 ± 25
CD3	148 ± 30
CD5	101 ± 23
CD18	123 ± 30
CD62E (ELAM-1)	91 ± 16

[a] Lymphocytes were incubated with *C. neoformans* in the absence (None) or presence of MAb directed against the indicated leukocyte receptor, and the percent growth was determined as described in Materials and Methods. Data are the means ± SEM of three experiments, each performed in triplicate. Fungal growth in wells containing no lymphocytes was (513 ± 44)%. There are no significant differences when the results for the None group are compared with those for any other group.

Receptor blockade. A panel of MAb reactive with receptors on human lymphocytes was tested for its ability to inhibit lymphocyte-mediated fungistasis. Candidate receptors were chosen for blockade primarily on the basis of previous investigations suggesting their involvement in NK- or cytotoxic-T-lymphocyte-mediated activity against tumor or microbial targets (1, 27, 31, 33). Antibody against CD62E (ELAM-1) was included as a negative control as CD62E is not known to be present on lymphocytes. No MAb tested significantly affected lymphocyte-mediated fungistasis (Table 1).

An alternative approach to receptor blockade that used putative ligands for leukocyte receptors was tried next. Such compounds were selected on the basis of similarity to the structural and physicochemical features of cryptococcal capsular polysaccharide, including the repeating mannose backbone, glucuronic acid side chains, and anionic nature (4). Thus, mannan and methyl α-D-mannopyranoside were selected to bind mannose receptors, while glucuronic acid and hyaluronic acid were chosen to bind hyaluronate receptors (CD44). Hyaluronic acid is a mucopolysaccharide with alternating β-1-3 glucuronidic and β-1-4 glucosaminidic bonds. The anionic carbohydrate polymer dextran sulfate, which binds to multiple lymphocytic receptors, including the extracellular matrix protein receptor 4-1BB and the sheep erythrocyte receptor CD2 (5, 34), was used to control for charge interactions. The growth of *C. neoformans* in wells containing putative ligands was similar to that in control wells (Fig. 4).

Effect of capsule. The data discussed above provide indirect evidence that lymphocyte binding to cryptococcal capsular polysaccharide is not a prerequisite for lymphocyte-mediated fungistasis. To test this hypothesis further, we examined lymphocyte-mediated fungistasis of the acapsular strain CAP67 and encapsulated strain 145 grown under conditions promoting either small or large capsules (Fig. 5). Neither the presence of capsule nor the size of the capsule had any significant influence on lymphocyte-mediated fungistasis.

DISCUSSION

The data presented here further define the conditions and mechanisms under which lymphocyte-mediated fungistasis against *C. neoformans* occurs. We found that compounds which serve as effective inhibitors of non-major histocompatibility complex-restricted lymphocyte activity against tumor and bacterial targets do not necessarily affect activity against *C. neoformans*, suggesting that a unique sequence of events leads to lymphocyte-mediated anticryptococcal activity.

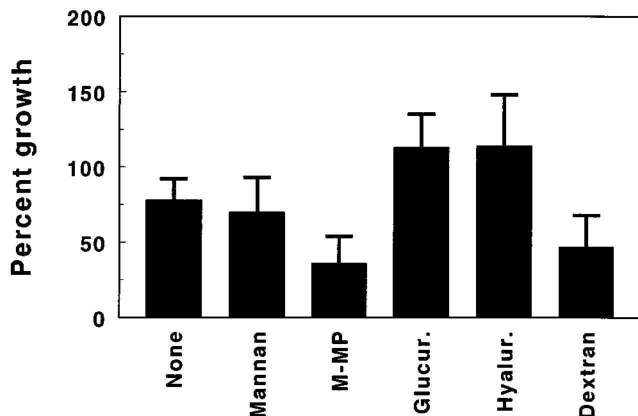

FIG. 4. Effect of soluble putative ligands on lymphocyte-mediated fungistasis. The percent growth of *C. neoformans* was determined as described in Materials and Methods. Wells contained no inhibitors (None), mannan at 10 mg/ml, methyl α-D-mannopyranoside at 100 mM (M-MP), glucuronic acid at 100 mM (Glucur.), hyaluronic acid at 1 mg/ml (Hyalur.), or dextran sulfate at 10 mg/ml (Dextran). Data are the means ± SEM of three to five triplicate experiments. The percent growth in wells containing *C. neoformans* but no lymphocytes was (376 ± 32)%. There are no significant differences when the results for the None group are compared with those for any other group.

The ability of heat-killed whole or broken yeast cells to partially inhibit lymphocyte-mediated fungistasis of live organisms suggests that heat-killed and live organisms compete for the same receptor. Alternatively, heat-killed organisms may deplete the lymphocytes of agents of fungistasis. While the inhibition was modest, it appeared to be specific as inhibition was not seen with yeast phase and hyphal phase *C. albicans* and latex beads.

The potent oxidant hydroxyl radical has been implicated in phagocyte microbicidal activity (29), but it remains controversial as to whether the hydroxyl radical is generated by lymphocytes. Hydroxyl radical scavengers inhibit NK cell-mediated lysis of tumor targets (7, 9). However, by electron spin resonance spectroscopy, hydroxyl radical production was not demonstrated in activated NK cells (9). While our data demonstrate that catechin profoundly inhibits lymphocyte-mediated fungistasis, it remains to be determined whether the mechanism is via the scavenging of hydroxyl radicals. The promiscuous nature of hydroxyl radical reactivity toward biomolecules necessitates that it be generated in close proximity to targets for it to generate maximal cell damage (29). In this regard, we and others have demonstrated that intimate contact between lymphocytes and *C. neoformans* does occur (19, 20, 30). Nevertheless, the inability of propyl gallate and diethyl urea to inhibit lymphocyte-mediated fungistasis argues against a role for hydroxyl radicals.

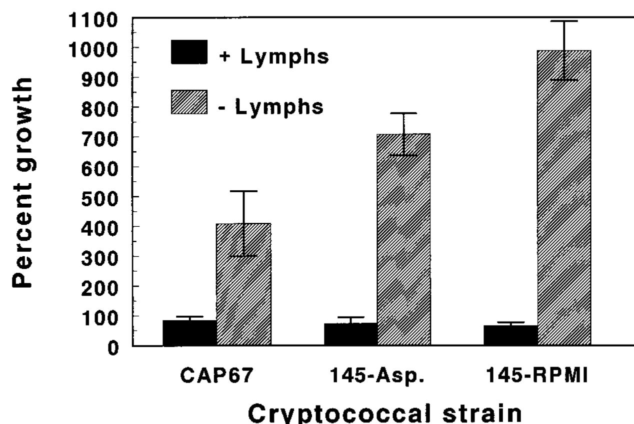

FIG. 5. Effect of capsule and growth conditions on lymphocyte-mediated fungistasis. Wells were incubated with *C. neoformans* in the presence (+ Lymphs) or absence (− Lymphs) of lymphocytes, and the percent growth was determined as described in Materials and Methods. The cryptococcal strains tested included acapsular strain CAP67 and encapsulated strain 145 grown on asparagine agar (145-Asp.) or in RPMI 1640 (145-RPMI). These two growth conditions result in small- and large-sized capsules, respectively. Data are the means ± SEM of two to four triplicate experiments. For all three conditions, $P < 0.01$ for comparisons of growth in the presence and absence of lymphocytes. The mean capsule thickness of 145-Asp. increased from 0.7 to 1.1 μm during the 18-h incubation with lymphocytes.

Our data showing inhibition of lymphocyte-mediated fungistasis by salicylic acid, not by other cyclooxygenase inhibitors or PGE_2, suggest that salicylic acid inhibits by a mechanism independent of PGE_2. Recently, salicylic acid, not other cyclooxygenase inhibitors, was shown to inhibit the activation of the transcription factor NF-κB in Jurkat T cells stimulated by phorbol myristate acetate and phytohemagglutin (15). Future studies will address whether *C. neoformans* induces translocation of NF-κB and/or other transcription factors, and if so, whether such translocation is a prerequisite for lymphocyte-mediated fungistasis.

Of the MAb and ligands reactive with leukocyte receptors that were tested, none inhibited fungistasis. While the data suggest a lack of involvement of such receptors in lymphocyte-mediated fungistasis, these results must be interpreted with caution as the MAb may not block the relevant epitope on the receptor. Moreover, cross-linking of receptors by MAb may result in lymphocyte activation and/or receptor upregulation. The lack of effect of the anionic carbohydrate polymer dextran sulfate suggests that charge interactions between lymphocytes and the negatively charged cryptococcal capsule are not a prerequisite for fungistasis.

Assuming that a specific receptor-ligand interaction occurs between lymphocyte and fungus, the finding that the capsule does not impact upon fungistasis implies that either more than one ligand is involved or a common ligand is present on encapsulated and acapsular isolates. Alternatively, lymphocytes may not recognize a specific ligand on the fungus but respond to a physicochemical property of the fungal surface or to a chemotactic factor released by yeast cells. Other microorganisms, including *C. albicans*, also are inhibited by lymphocytes via mechanisms that remain largely undefined (2, 24). Future studies are needed to determine what the mediators of microbistasis are and whether (as it appears from these studies) disparate mechanisms are operative, depending upon the microbial target.

The experiments reported here used a mixed population of T and NK cells. Previously, we and others have shown that lymphocytes highly enriched for $CD4^+$, $CD8^+$, and $CD56^+$ subsets mediate fungistasis (19, 20, 30). Although clearly there are profound differences between T and NK cells, there are some similarities that may help to explain why *C. neoformans* is susceptible to both cell types. For example, both T and NK cells mediate the lysis of tumor cells through a similar series of steps, which include binding and signaling events, and delivery of the lethal hit by lymphocytes (3, 24). Moreover, both NK cells and non-major histocompatibility complex-restricted T lymphocytes express surface proteins that are required for target cell recognition and signaling during the lysis of tumor targets (6, 24, 28). Nevertheless, it remains entirely possible that lymphocyte subsets have disparate mechanisms by which they mediate fungistasis. This could explain why for those inhibitors that were active, only partial inhibition was seen.

ACKNOWLEDGMENT

This work was supported by grant AI25780 from the National Institutes of Health.

REFERENCES

1. **Bajpai, A., and Z. Brahmi.** 1994. Target cell-induced inactivation of cytolytic lymphocytes. Role and regulation of CD45 and calyculin A-inhibited phosphatase in response to interleukin-2. J. Biol. Chem. **269:**18864–18869.
2. **Beno, D. W. A., and H. L. Mathews.** 1993. Quantitative measurement of lymphocyte mediated growth inhibition of *Candida albicans*. J. Immunol. Methods **164:**155–164.
3. **Berke, G.** 1994. The binding and lysis of target cells by cytotoxic lymphocytes: molecular and cellular aspects. Annu. Rev. Immunol. **12:**735–773.
4. **Bhattacharjee, A. K., J. E. Bennett, and C. P. J. Glaudemans.** 1984. Capsular polysaccharides of Cryptococcus neoformans. Rev. Infect. Dis. **6:**619–624.
5. **Chalupny, N. J., R. Peach, D. Hollenbaugh, J. A. Ledbetter, A. G. Farr, and A. Aruffo.** 1992. T-cell activation molecule 4-1BB binds to extracellular matrix proteins. Proc. Natl. Acad. Sci. USA **89:**10360–10364.
6. **Chambers, C. A., S. Galliger, S. K. Anderson, S. Giardina, J. R. Ortaldo, N. Hozumi, and J. Roder.** 1994. Expression of the NK-TR gene is required for NK-like activity in human T cells. J. Immunol. **152:**2669–2674.
7. **Duwe, A. K., J. Werkmeister, J. C. Roder, R. Lauzon, and U. Payne.** 1985. Natural killer cell-mediated lysis involves an hydroxyl radical-dependent step. J. Immunol. **134:**2637–2644.
8. **Garcia-Penarrubia, P., A. D. Bankhurst, and F. T. Koster.** 1989. Prostaglandins from human T suppressor/cytotoxic cells modulate natural killer antibacterial activity. J. Exp. Med. **170:**601–606.
9. **Gibboney, J. J., R. A. Haak, F. W. Kleinhans, and Z. Brahmi.** 1988. Electron spin resonance spectroscopy does not reveal hydroxyl radical production in activated natural killer lymphocytes. J. Leukocyte Biol. **44:**545–550.
10. **Harrison, T. S., H. Kornfeld, and S. M. Levitz.** The effect of infection with human immunodeficiency virus on the anticryptococcal activity of lymphocytes and monocytes. J. Infect. Dis., in press.
11. **Houpt, D. C., G. S. T. Pfrommer, B. J. Young, T. A. Larson, and T. R. Kozel.** 1994. Occurrences, immunoglobulin classes, and biological activities of antibodies in normal human serum that are reactive with *Cryptococcus neoformans* glucuronoxylomannan. Infect. Immun. **62:**2857–2864.
12. **Jacobson, E. S., D. J. Ayers, A. C. Harrell, and C. C. Nicholas.** 1982. Genetic and phenotypic characterization of capsule mutants of *Cryptococcus neoformans*. J. Bacteriol. **150:**1292–1296.
13. **Julius, M. H., E. Simpson, and L. A. Herzenberg.** 1973. A rapid method for the isolation of functional thymus-derived murine lymphocytes. Eur. J. Immunol. **3:**645–649.
14. **Kägi, D., B. Lederman, K. Bürki, P. Seiler, B. Odermatt, K. J. Olsen, E. R. Podack, R. M. Zinkernagel, and H. Hengartner.** 1994. Cytotoxicity mediated by T cells and natural killer cells is greatly impaired in perforin-deficient mice. Nature (London) **369:**31–37.
15. **Kopp, E., and S. Ghosh.** 1994. Inhibition of NF-KB by sodium salicylate and aspirin. Science **265:**956–959.
16. **Levitz, S. M.** 1991. Activation of human peripheral blood mononuclear cells by interleukin-2 and granulocyte-macrophage colony-stimulating factor to inhibit *Cryptococcus neoformans*. Infect. Immun. **59:**3393–3397.
17. **Levitz, S. M.** 1991. The ecology of *Cryptococcus neoformans* and the epidemiology of cryptococcosis. Rev. Infect. Dis. **13:**1163–1169.
18. **Levitz, S. M.** 1994. Macrophage *Cryptococcus* interactions, p. 533–543. *In* B. S. Zwilling and T. K. Eisenstein (ed.), Macrophage pathogen interactions. Marcell Dekker, New York.
19. **Levitz, S. M., and M. P. Dupont.** 1993. Phenotypic and functional characterization of human lymphocytes activated by interleukin-2 to directly inhibit growth of *Cryptococcus neoformans* in vitro. J. Clin. Invest. **91:**1490–1498.
20. **Levitz, S. M., M. P. Dupont, and E. H. Smail.** 1994. Direct activity of human T lymphocytes and natural killer cells against *Cryptococcus neoformans*. Infect. Immun. **62:**194–202.
21. **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.
22. **Levitz, S. M., T. P. Farrell, and R. T. Maziarz.** 1991. Killing of *Cryptococcus neoformans* by human peripheral blood mononuclear cells stimulated in culture. J. Infect. Dis. **163:**1108–1113.
23. **Levitz, S. M., C. A. Lyman, T. Murata, J. A. Sullivan, G. L. Mandell, and R. D. Diamond.** 1987. Cytosolic calcium changes in individual neutrophils stimulated by opsonized and unopsonized *Candida albicans* hyphae. Infect. Immun. **55:**2783–2788.
24. **Levitz, S. M., H. L. Mathews, and J. W. Murphy.** Direct antimicrobial activity of T cells. Immunol. Today, in press.
25. **Levitz, S. M., A. Tabuni, H. Kornfeld, C. C. Reardon, and D. T. Golenbock.** 1994. Production of tumor necrosis factor alpha in human leukocytes stimulated by *Cryptococcus neoformans*. Infect. Immun. **62:**1975–1981.
26. **Linnemeyer, P. A., and S. B. Pollack.** 1993. Prostaglandin E2-induced changes in the phenotype, morphology, and lytic activity of IL-2-activated natural killer cells. J. Immunol. **150:**3747–3754.
27. **Liu, J. H., S. Wei, D. K. Blanchard, and J. Y. Djeu.** 1994. Restoration of lytic function in a human natural killer cell line by gene transfection. Cell. Immunol. **156:**24–35.
28. **Mathew, P. A., B. A. Garni-Wagner, K. Land, A. Takashima, E. Stoneman, M. Bennett, and V. Kumar.** 1993. Cloning and characterization of the 2B4 gene encoding a molecule associated with non-MHC-restricted killing mediated by activated natural killer cells and T cells. J. Immunol. **151:**5328–5337.
29. **McCormick, M. L., T. L. Roeder, M. A. Railsback, and B. E. Britigan.** 1994. Eosinophil peroxidase-dependent hydroxyl radical generation by human eosinophils. J. Biol. Chem. **269:**27914–27919.
30. **Murphy, J. W., M. R. Hidore, and S. C. Wong.** 1993. Direct interactions of human lymphocytes with the yeast-like organism, *Cryptococcus neoformans*. J. Clin. Invest. **91:**1553–1566.
31. **Ramos, O. F., M. Patarroyo, E. Yefenof, and E. Klein.** 1989. Requirement of leukocytic cell adhesion molecules (CD11a-c/CD18) in the enhanced NK lysis of iC3b-opsonized targets. J. Immunol. **142:**4100–4104.
32. **Ratner, A., and W. R. Clark.** 1991. Lack of target cell participation in cytotoxic T lymphocyte-mediated lysis. J. Immunol. **147:**55–59.
33. **Robertson, M. J., M. A. Caligiuri, T. J. Manley, H. Levine, and J. Ritz.** 1990. Human natural killer cell adhesion molecules. Differential expression after activation and participation in cytolysis. J. Immunol. **145:**3194–3201.
34. **Warren, H. S., and C. R. Parish.** 1990. Mapping the dextran sulfate binding site on CD2. Immunol. Cell Biol. **68:**199–205.