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Interactions between Human Natural Killer (NK) Lymphocytes and Yeast Cells: Human NK Cells Do Not Kill *Candida albicans*, although *C. albicans* Blocks NK Lysis of K562 Cells

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Rodent natural killer (NK) lymphocytes are cytotoxic to certain fungi. We investigated whether human NK cells are cytotoxic to the yeast *Candida albicans*. We found that human peripheral blood lymphocytes possessing NK cell activity had little or no effect on the viability of the yeast. Unopsonized *C. albicans*, however, were able to block NK cell-mediated cytotoxicity at a ratio of 100 yeast to one K562 erythroleukemia cell. *C. albicans* was not toxic to the lymphocytes nor did it take up isotope released by the K562 cells. Furthermore, *C. albicans* that was pretreated with human serum blocked NK cell activity more than did untreated *C. albicans*. Binding of the yeasts to NK cells could account for the blocking effect of serum-treated yeasts, but not for that of the untreated yeasts. Flow cytometry indicated that there was preferential binding of *C. albicans* to NK lymphocytes but not to T cells when the yeasts were pretreated with human serum. In this report we affirm the results of the study by Vecchiarelli et al. (A. Vecchiarelli, F. Bistoni, E. Cenci, S. Perito, and A. Cassone, Sabouraudia 23:377–387, 1985), that the first report of rodent NK cell activity against the yeast *Cryptococcus neoformans* (J. W. Murphy and D. O. McDaniel, J. Immunol. 128:1577–1583, 1982) cannot be extrapolated to a general phenomenon of unprimed lymphocyte-mediated destruction of all species of yeast. Our data extend the observations to humans and also suggest that in vivo interactions between NK lymphocytes and opportunistic fungal pathogens may affect NK cell function.

Candida albicans is an opportunistic fungal pathogen. The nature of the immune resistance is not clear, with both humoral and cell-mediated components attributed to protection (18). The clinical condition of oral candidial thrush, in which pathogenic infections interfere with ingestion of food, occurs in immunocompromised patients, such as those with acquired immune deficiency syndrome. These infections are not readily explicable in the presence of normal humoral and granulocyte responses of these patients. This study constitutes an effort to extend our knowledge on the interactions of *C. albicans* with other cells of the human immune system.

Human natural killer (NK) cells are nonphagocytic (9) large granular lymphocytes (24) which are cytotoxic in vitro to certain tumor cell lines (11) and to virally infected cells (23). Mouse NK cells can also inhibit the in vitro growth of the yeasts *Cryptococcus neoformans* (16) and *Paracoccidioides brasiliensis* (10). However, mouse NK cells must not be inhibitory to all yeast species. Vecchiarelli et al. (26) have demonstrated that mouse splenocytes do not affect the growth of the yeast *Candida albicans*.

We designed experiments to determine how effective human NK cells were against *C. albicans*. *C. albicans* is a common opportunistic yeast which causes oral and vaginal infection in normal humans and can cause systemic secondary infections in immunosuppressed individuals. Almost all individuals have immunoglobulin G (IgG) and IgM antibodies to *C. albicans*. The results of our experiments indicate that human NK cells are only marginally cytotoxic to *C. albicans*, with or without antibodies to the yeast; whereas granulocytes kill the yeast effectively. The granulocyte killing of *C. albicans* was expected. However, we did not anticipate that *C. albicans* could inhibit NK cell lysis of K562 tumor cells. We found that this inhibition is not completely dependent upon, but is augmented by, treatment of *C. albicans* with human serum. Results of this study indicate that complex and unanticipated interactions may occur between the NK cells of the immune system and a common yeast which is not normally pathogenic to man.

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MATERIALS AND METHODS

Leukocytic effector cells. Blood was drawn from normal human volunteers and defibrinated. Serum was then collected after centrifugation, and the remaining blood cells were suspended in 0.85% (wt/vol) NaCl. The peripheral blood mononuclear cells (PBMs) and granulocytes were isolated at the interface of Ficoll gradients with densities of 1.077 and 1.119 (Sigma Chemical Co., St. Louis, Mo.), respectively (2, 7), and suspended in RPMI 1640 medium (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 5% heat-inactivated fetal calf serum (Irvine Scientific, Santa Ana, Calif.)–10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid)–2 mM glutamine–100 U of penicillin per ml–0.1 mg of streptomycin per ml. In experiments in which the effects of mononuclear cells and granulocytes on yeast viability were compared, the mononuclear cells containing both lymphocytes and monocytes were added to the wells in 100-μl portions of 2×10^5 cells per well. Granulocytes were plated at 4×10^4 to 9×10^4 cells per well.

Macrophage-depleted peripheral blood lymphocytes (PBLs) and lymphokine-activated lymphocytes were also prepared. Adherent macrophages were depleted after the mononuclear cells were cultured in tissue culture flasks at 37°C for 1 h. Lymphokine activation of cells was accom-

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plished by culturing mononuclear cells at 10^4 to 10^5 cells per well in microtiter plates overight at 37°C, with 25% (vol/vol) crude T-cell growth factor (3)–10% fetal calf serum–1 μg of lipopolysaccharide per ml–65% medium (RPMI 1640 medium, 2 mM glutamine, 10 mM HEPES). This procedure elicits a very high concentration of gamma interferon in the culture supernatant (D. Hudig and L. Kronenberg, unpublished data). Enrichment for activated macrophages was done by allowing the macrophages to adhere to the plastic microtiter plates during activation and subsequently removing the nonadherent lymphocytes from the wells. There was some unavoidable loss of lymphocytes when this approach was used. The activated, nonadherent cells were transferred to additional plates for evaluation of their cytotoxicity to *C. albicans*.

Preparation of *C. albicans*. *C. albicans* 3153A was grown in the medium described by Lee et al. (13) supplemented with 0.2 μM Zn^{2+}–0.4 mM arginine. The culture was grown on a shaker at 25°C in polypropylene flasks for 48 h (to the stationary phase) before use. *C. albicans* 3153A remained in the yeast form under these conditions. Yeast cells were collected by centrifugation at approximately 8,800 × *g* and washed once with sterile 0.85% (wt/vol) NaCl. To treat the yeast with serum, approximately 10^8 yeast cells per ml of serum were incubated at 37°C for 30 min. This serum was either freshly collected and kept on ice or frozen at −70°C to maintain complement activity. These yeast cells were washed and diluted in RPMI 1640 medium with 5% fetal calf serum–10 mM HEPES–antibiotics (as described above) for the cytotoxic assays. Viability of the yeast was determined by the methylene blue stain (8) before they were cultured.

Limiting dilution assays for cytotoxicity to yeast. Three dilutions of yeast cells pretreated with serum were dispensed into 96-well, flat-bottom microtiter plates (Linbro Scientific, Hamden, Conn.) in 100-μl portions to yield approximately 0.25, 0.5, and 1 yeast cell per well. After the addition of fresh mononuclear cells, granulocytes, or activated NK cells to the yeast cells, the microtiter plates were incubated for 48 h in sealed gas boxes (CBS Scientific, Del Mar, Calif.) at 37°C in 5% CO_2. Control plates contained the yeast dilutions without human effector cells. There were five microtiter plates for each condition. After 48 h of incubation, outgrowth of yeast colonies was clearly visible to the unaided eye, and wells were scored as positive or negative. Although with limiting dilutions a few wells are innoculated with more than one yeast, because of our sample size we considered each well to represent one colony. The wells that appeared to be negative for yeast growth were also examined microscopically to verify the absence of minimal outgrowth.

NK assays. K562 tumor cells were obtained from Lozzio and Lozzio (14) and used as targets in 4-h ^{51}Cr release assays (4) in round-bottom microtiter plates. K562 target cells were radiolabeled with 0.4 mCi of $Na_2{}^{51}CrO_4$ (50 to 500 Ci/g of Cr; ICN Pharmaceuticals, Inc., Irvine, Calif.) in RPMI 1640 medium for 3 h at 37°C, washed, and plated at 2 × 10^4 cells per well. The effector cells were human PBMs. Quadruplicate wells were prepared at each effector to target cell ratio of 40:1, 20:1, 10:1, 5:1, and 2.5:1.

To measure inhibition of NK lysis, the yeast cells were added to the NK assay at 100:1, 10:1, and 1:1 ratios of yeast to K562 cells. To obtain positive controls for cold target inhibition of K562 cytolysis, unlabeled K562 cells were added at 10:1, 3:1, and 1:1 ratios of unlabeled to labeled K562 cells. All NK assays were performed in RPMI 1640 medium supplemented with heat-inactivated 5% fetal calf serum–10 mM HEPES–2 mM glutamine. Positive controls for NK activity contained only effector and K562 target cells. Unless otherwise indicated in the text, all results were reproduced in three separate experiments.

The percentage specific chromium release was calculated as follows: [(experimental ^{51}Cr release − background ^{51}Cr release)/(maximum ^{51}Cr release − background ^{51}Cr release)] × 100. Standard errors of quadruplicate wells were never above 2.0% ^{51}Cr release. The number of lytic units per 10^7 PBMs was calculated after one lytic unit was designated as the number of mononuclear cells required to lyse 30% of the K562 cells.

Statistical analysis. The two-sample *t* test was used for analysis of the limiting dilution assays, and the paired *t* test was used for the inhibition assays and flow cytometry data (6).

Conjugates. For examination of NK cell and yeast conjugate formation by light microscopy, 1:1 and 10:1 mixtures of yeast to mononuclear cells were incubated at 37°C for 15 min, spun at 170 × *g* for 3 min, and then placed on ice for 1 h. After gentle suspension with a Pasteur pipette, the conjugates were analyzed by direct counts on a hemacytometer.

Joseph Phillips examined the yeast-lymphocyte conjugates by three-color flow cytometry at the Becton Dickinson Immunocytometry Center, Mountain View, Calif. Monocyte-depleted PBLs were stained red with phycoerythrin-conjugated Leu-4 (anti CD3, the T-cell receptor accessory molecule) and green with fluorescein isothiocyanate-conjugated Leu-11a (anti CD16, an Fc gamma receptor) monoclonal antibodies. After serum pretreatment, the yeast cells were adjusted to 5 × 10^8 cells per ml in 0.1 M $NaHCO_3$ (pH 7.8 to 8.0), and 1 mg of biotinyl-*N*-hydroxysuccinimide in 2 mg of dimethyl sulfoxide per ml was added dropwise to the yeast while the suspension was vortexed gently. The mixture was incubated at room temperature for 1 h and then washed in phosphate-buffered saline 5 to 10 times. The biotinylated yeast were then stained blue with allophycocyanin-conjugated streptavidin. The stained yeast cells and PBLs were combined at a yeast to PBL ratio of 50:1, pelleted at slow speed, and incubated at 4°C for 1 h. After gentle resuspension of the pellet, the conjugate mixture was run on a FACS 440 flow cytometer equipped with a 90-μm tip to reduce the shear forces on the conjugates. The percentage of Leu-11a or Leu-4 positive cells that bound the yeast was determined by gating on either the Leu-11a$^+$ or Leu-4$^+$ cells and analyzing the percentage of these cells that costained with the yeast.

RESULTS

Human mononuclear cells with NK cell activity do not kill *C. albicans*. We selected an approach that was biased toward the detection of an initial loss in viability of the yeast, as opposed to a reduction in the growth rate of the yeast. *C. albicans* was preincubated with autologous human serum, washed, and diluted so that no more than one yeast cell was plated per well. Greater than 40,000-fold excesses of human mononuclear cells or granulocytes were added. After culturing for 48 h, the wells were examined for outgrowth of the individual yeast cells. ^{51}Cr release assays were done simultaneously to ensure that NK cell activity was present in the mononuclear cell population. Only marginal differences in the viability of the yeast were found between controls and cultures with mononuclear cells (Table 1). In contrast, 70 to 90% of the yeast cultured with granulocytes lost viability (Table 1). Granulocyte killing of *C. albicans* is a well-documented phenomenon (5, 19) and served as a positive control for the assay conditions.

TABLE 1. Viability of *C. albicans* is affected by granulocytes but not by NK lymphocytes[a]

No. of yeast/well	No. of wells with yeast colonies/plate ± SEM (% of control) for[b]:		
	Control	Mononuclear cells[c]	Granulocytes[c]
0.25	22.8 ± 1.7 (100)	19.2 ± 1.0 (84.2)	2.6 ± 0.8 (11.4)[d]
0.5	40.2 ± 1.4 (100)	35.4 ± 1.2 (88.1)[e]	5.6 ± 0.9 (13.9)[d]
1.0	61.2 ± 1.2 (100)	56.8 ± 2.4 (92.8)	9.4 ± 2.1 (15.4)[d]

[a] Outgrowth of single yeast cells cultured in the presence of mononuclear cells or granulocytes. NK cell activity was 238.1 lytic units per 10^7 PBMs.
[b] There were 96 wells per plate and an average of five plates per set. The numbers represent values from one of two similar experiments.
[c] There were 2×10^5 macrophage-depleted mononuclear cells per well and 9×10^4 granulocytes per well.
[d] $P < 0.0005$.
[e] $0.01 < P < 0.025$.

To determine whether NK cells could kill the yeast after activation of NK cytotoxicity, the mononuclear cells were incubated in the presence of lymphokines to stimulate NK cells (25) and lipopolysaccharide to stimulate monocytes (27). This combination elicits high concentrations of interleukin 2 and also gamma interferon, which is a key lymphokine for macrophage activation. NK assays were performed on the mononuclear cells before or after incubation with the lymphokines and lipopolysaccharide. Normal two- to threefold activation of NK to K562 cells occurred under these conditions. Despite these pretreatments, neither the activated nonadherent NK lymphocytes nor the total mononuclear cells (with adherent macrophages) resulted in substantial killing of yeast (data not shown).

***C. albicans* can block NK cell lysis of K562 cells.** To investigate whether there were any interactions between *C. albicans* and human NK cells, the effect of the yeast on NK activity was determined by adding yeast to K562 ^{51}Cr release assays. Inhibition of NK lysis of the K562 cells became evident at a 10:1 ratio of serum-treated yeast to ^{51}Cr-labeled K562 cells. The inhibition increased as the concentrations of these yeast cells increased (Fig. 1). Untreated yeast cells also showed noteworthy NK cell inhibition at a 100:1 yeast to K562 target cell ratio (Fig. 2), but not at ratios of 1:1 or 10:1 (data not shown). In contrast to the inhibition by live yeast, heat-killed, untreated yeast did not inhibit NK cells at a 10:1 or 100:1 yeast to K562 ratio. Therefore, the inhibition observed at these ratios is not due to a mass effect from an excess number of yeast in the system. The spontaneous release of ^{51}Cr in the control NK cell assays without yeast was always equivalent to that observed in the NK assay with yeast, indicating that the yeast do not absorb the ^{51}Cr released from K562 cells. Exclusion of trypan blue, which was determined by microscopic examination of the mononuclear cells after incubation with the yeast, showed that the lymphocytes remained viable. As a positive control to illustrate that NK cell activity could be readily inhibited, unlabeled K562 cells competed with ^{51}Cr-labeled K562 cells for lysis in all of the experiments (data not shown).

The degree of inhibition of NK cell activity by *C. albicans* varied among experiments, depending primarily upon the serum used to pretreat the yeast. Serum-treated yeast inhibited NK activity to a greater extent than did the untreated yeast for five of seven serum donors (Table 2). Pretreatment of *C. albicans* with the sera from the two other donors did not increase the ability of the yeast to inhibit NK activity above that observed with the untreated yeast. Furthermore, heat inactivation of inhibitory sera at 56°C for 30 min, which would reduce complement C3 deposition, did not diminish their inhibitory effects (data not shown). Experiments were done to determine whether the sources of the NK lymphocytes or the sera used to pretreat the yeast were responsible for these variations in inhibition of NK activity. PBMs of one donor were compared by examining the inhibitory effects of yeast treated with either autologous serum or sera from four other donors (Table 3). Both the autologous and

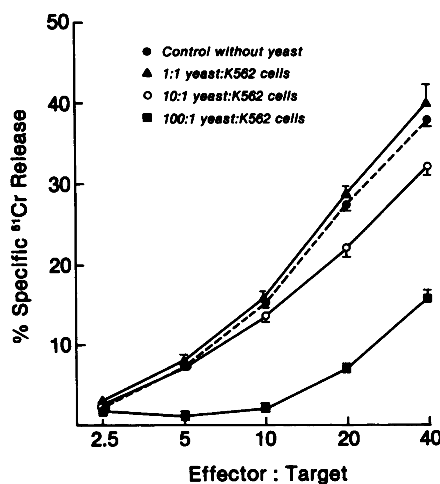

FIG. 1. Inhibition of human NK cell cytotoxicity by *C. albicans*. NK activity against K562 tumor cells was measured in the presence of increasing concentrations of *C. albicans* pretreated with fresh human serum. Serum and PBMs were from donor 6.

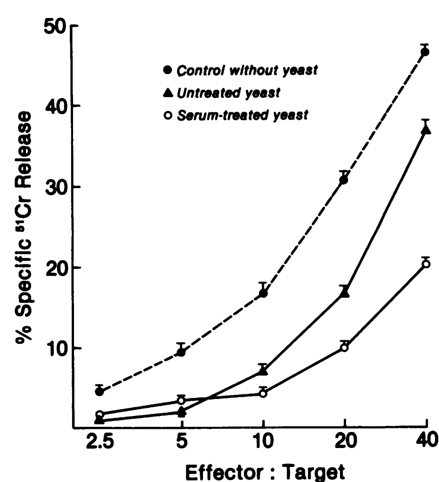

FIG. 2. *C. albicans* pretreated with human serum inhibits NK cells more than does untreated yeast. The yeast cells were added to the NK cell assay at a 100:1 yeast to K562 cell ratio. The serum and PBMs were from donor 1.

TABLE 2. Cytotoxic activity of human NK cells is inhibited by both untreated yeast and yeast pretreated with fresh autologous serum[a]

Serum donor	NK cell activity (lytic units/10^7 PBMs) in:		
	Control	Untreated yeast[b]	Serum-treated yeast[b]
1	25.6	15.9	5.2
2	121.9	56.8	20.0
3	30.3	10.9	6.9
4	30.3	10.9	1.3
5	227.3	38.5	53.2[c]
6	20.8	2.5	4.0[c]
7	138.9	66.7	33.3

[a] Either untreated yeast or yeast pretreated with serum were included in the ^{51}Cr release assays to determine the NK cell activity of PBMs from seven different donors. The ratio of yeast cells to K562 cells was 100:1. The serum and PBMs were autologously paired.

[b] All inhibition by treated or untreated yeast was statistically significant, with $P < 0.05$.

[c] No additional inhibition was observed after treatment of the yeast with serum.

heterologous sera were inhibitory to various extents, which indicates that the variation in inhibition of NK activity could be determined by the source of serum. Results of another experiment with PBLs from a different donor, but with the same pool of sera, were similar (data not shown).

Additional experiments did not help to explain why some sera promoted inhibition of NK cells more than did other sera. In a separate series of flow cytometry experiments, we could find no correlation between the ability of the serum-treated yeast to block NK activity and the amounts of complement C3, C-reactive protein, IgG, or IgM that were bound to the serum-treated yeast (data not shown).

***C. albicans* treated with serum can bind to lymphocytes.** We initially thought that the yeast might be inhibiting NK cell activity by binding to the NK cells via selected NK cell receptors for serum proteins, such as Fc receptors for IgG (11, 20, 28), receptors for the complement component C3 (21), or receptors for C-reactive protein (1). To determine whether the yeast cells actually bound to the lymphocytes, conjugate assays were done with untreated and serum-treated yeast and observed by direct microscopic examination or by flow cytometry. Direct microscopic observation indicated that there was rare binding between the individual lymphocytes and one to two untreated yeast cells. More lymphocytes bound only one or two yeasts when the yeast

TABLE 3. Inhibition of NK cells by serum-treated yeast is dependent upon the source of serum

Serum donor[a]	% of control NK cell activity in serum-treated yeast[b]
None	
1	25.0
2	10.3
3 (autologous)	22.9
4	4.1
5[c]	37.5

[a] PBMs were from donor 3.

[b] All activities were calculated from lytic units per 10^7 PBMs. The percentage of control NK cell activity in controls and with untreated yeast was 100 and 35.9%, respectively. All inhibition by treated or untreated yeast was statistically significant, with $P < 0.05$.

[c] Serum from donor 5 also did not enhance yeast-mediated inhibition of autologous NK cell activity (see Table 2).

TABLE 4. Serum-treated yeast bind to CD16$^+$ (Fc gamma receptor) lymphocytes better than do CD3$^+$ T cells

Serum donor	% of each lymphocyte subset with bound yeast for[a]:	
	CD16$^+$ cells	CD3$^+$ cells
Untreated	1.0	0
1	8.0	0
2	30.0	6.0
3	5.0	3.0
4	8.0	3.0
5	20.0	3.0

[a] Determined by flow cytometry. Similar results were obtained with PBLs from a different donor. Leu-11a monoclonal antibody binds to the Fc receptor (CD16), which is characteristic of NK cells; Leu-4 monoclonal antibody binds to the T-cell receptor accessory molecule (CD3), which is not characteristic of NK cells. The difference between percentages of CD16$^+$ and CD3$^+$ subsets which bound serum-treated yeast was significant at $P < 0.05$. A total of 20% of the PBLs were CD16$^+$ cells and 70% were CD3$^+$ cells.

cells were pretreated with serum (data not shown). This serum-mediated binding was associated with the CD16$^+$ lymphocyte phenotype that is characteristic of NK cells (11, 12), as indicated by flow cytometric analysis (Table 4). The difference in binding of yeast to the CD16$^+$ NK cells and to the CD3$^+$ T lymphocytes was significant, $P < 0.05$, by paired *t* test analysis. The degree of binding to CD16$^+$ cells, however, did not correlate with the degree of inhibition of NK activity. Sera from donors 1, 2, 3, and 4 increased inhibition of NK activity, whereas serum from donor 5 did not (Table 3). Sera from donors 2 and 5 promoted binding of the yeast to lymphocytes better than did sera from the other three donors (Table 4). No binding of the yeast to the K562 cells was observed by direct microscopic examination.

DISCUSSION

We found that human NK cells do not kill *C. albicans*, which now confirms that human, as well as mouse splenic NK cells (26), are unable to affect the growth of this common yeast. The remarkable finding of our experiments is that *C. albicans*, with or without serum components, can block human NK activity against K562 cells. Marconi et al. (15) have reported that murine NK-mediated cytotoxicity is enhanced by *C. albicans* in vivo. This enhancement occurred several days after intraperitoneal administration of inactivated yeast cells and is not an analogous situation to our in vitro NK cell assays.

We cannot readily explain this unexpected inhibition of human NK activity by *C. albicans*. *C. albicans* did not bind to K562 cells. Also, *C. albicans* did not entrap the lymphocytes because strain 3153A did not form mycelia during the 4-h NK cell assay. K562 target cells were not rendered sterically inaccessible to the NK cells by the yeast because only the live *C. albicans* could affect inhibition. The dead yeast could not cover up cells to block access. Furthermore, since the live yeast did not affect the lymphocyte viability or absorb the ^{51}Cr that was released from K562 cells, trivial explanations are unlikely to account for the results of this study. NK cells preferentially bound *C. albicans* after pretreatment of the yeast with serum. The binding pattern of yeast treated with serum from donor 5, however, is inconsistent with the relative ability of the sera to promote yeast inhibition of NK cells. We considered that these discrepancies might be due to the fact that there were more ligands on some of the treated yeast than on others, creating potential

differences in the net avidity which would not appear in a simple binding assay. Although NK cells have receptors for IgG, complement C3 in several forms, and C-reactive protein, the different amounts of these proteins that bound to *C. albicans* could not explain the variation among sera in augmenting the inhibition of NK cells by *C. albicans*. Although ready explanations are currently unavailable, the data with the untreated yeast suggest that some interactions always occur. Additionally, these interactions which we have observed may initiate the release of lymphokines, which then induce additional cytolytic activity from granulocytes, as observed by J. Y. Djeu (The Fourth International Workshop on Natural Killer Cells, abstr., 1986).

Our results are in direct agreement with data for mouse splenic NK cells and *C. albicans* reported previously for a murine system (26). Our cytotoxic assays, which began with single yeast cells, provided an overwhelming ratio of effector cells to yeast cells and should have ensured the killing of any susceptible *C. albicans* by NK cells. These results differ from reports that mouse splenic NK cells can inhibit the growth of two yeasts, *Cryptococcus neoformans* (16) and *P. brasiliensis* (10). *P. brasiliensis*, like *Candida albicans*, is a dimorphic fungus which has no capsule. Although *Cryptococcus neoformans* is an encapsulated organism, it appears that NK cells may recognize some structure on the *Cryptococcus neoformans* cell wall below the capsule, since removal of the capsular material does not interfere with NK cell-mediated growth inhibition of this yeast (17). Thus, encapsulation cannot account for sensitivity or resistance to NK cells.

The inhibition of NK cell activity by *Candida albicans* may not require direct binding of the yeast to the NK cell. The yeast may have absorbed NK cell cytolytic factors that are secreted into the medium (29). The binding of lymphocytes to untreated yeasts was rarely detectable by light microscopy and undetectable by flow cytometry, indicating that any binding which may have occurred would have to be of low avidity. Ross et al. (22) have indicated that complement receptor type three binds glucan, which is a component of the cell wall of *C. albicans*. Therefore, the complement receptor type 3 of NK cells might bind the glucan of *C. albicans*. Yeast cells preferentially bind to lymphocytes which have the NK cell phenotype (i.e., $CD16^+$) only after pretreatment of the yeast with serum. Thus, both yeast cell wall and serum components may interact with NK cells and may be involved in the inhibition of NK cells. This inhibition might also be caused by steric hindrance of the interaction between NK cells and K562 cells or by the blocking of NK cell receptors which may stabilize NK cell-K562 cell interactions.

The most unusual results of this study are that NK activity could be inhibited by *C. albicans*. Although the reasons for inhibition of NK cells by *C. albicans* remain unclear, it may indicate a significant role in vivo for the inhibition of human NK activity by *C. albicans*. This role may be especially apparent in an invasive yeast infection, in which the yeast cells have entered both tissues and the vasculature. Results of this study also indicate that information is lacking concerning normal lymphocyte-*C. albicans* interactions which might be suppressed in immunocompromised patients with *Candida* thrush.

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