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Induction of Natural Killer Cell Activity by Inactivated *Candida albicans* in Mice

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Injection of merthiolate-inactivated yeast form cells of *Candida albicans* into the peritoneal cavities of mice induced the appearance of a cytolytic effector population against YAC-1 tumor cell lines. This induction was maximally manifested in 5- to 8-week-old animals 3 to 4 days after injection of 2×10^7 *C. albicans* cells, and the peritoneal lytic population exerted its optimum cytotoxic effect after 4 h of incubation. No significant natural cytotoxic activity was generated by *C. albicans* in the bone marrow or thymus, whereas there was a slight, transient, but significant depression of natural splenic cytotoxicity. Experiments performed to characterize the natural cytotoxic population elicited by the inactivated yeast showed that the effectors were nonadherent, nonphagocytic cells. Moreover, the anti-YAC-1 lytic activity was partially sensitive to anti-Thy1.2 serum and was completely abrogated by treatment of peritoneal nonadherent cells with monoclonal anti-asialo GM1 antibodies. Finally, the peritoneal population of cytotoxic cells induced by *C. albicans* was fully susceptible to Ly5.1 plus anti-immunoglobulin G2a and complement lysis. Although different cell populations could be induced by inactivated *C. albicans*, all of our data support the view that the anti-YAC-1 activity was entirely attributable to natural killer lymphocytes.

Candida albicans is an opportunistic microbial pathogen whose interaction with host immune systems is quite complex. This is aptly exemplified by the controversy existing about the protective role played by specific humoral and cell-mediated immune responses in candidosis (26). On the other hand, recent investigations strongly suggest that *Candida albicans*, like other microorganisms, has a more general impact on host immunity, as shown by its capacity to induce a variety of nonspecific immunomodulatory effects.

Therefore, polysaccharide products of *Candida albicans* have been studied for induction of mitogenic effects in vitro (30, 31), and mannan cell wall components are increasingly suspected to be part of immunosuppression mechanisms in vivo (6, 25, 31). On the other hand, glucan from *Candida* spp. and other yeasts may strongly activate macrophages both in vitro and in vivo (33, 37, 42), thus conferring nonspecific protection against bacterial, fungal, and parasitic infections (16, 33, 43). Whole *Candida* cells (killed or viable) may induce depression of cellular reactivity in vivo (34, 36) but enhance antibody response to humoral and cellular antigens (5). Finally, *Candida* cells and cell wall glucan are endowed with antitumor immunoadjuvant properties (2, 3, 20).

Although the majority of these *Candida* effects have been detected under so different, sometimes peculiar, experimental conditions as to suggest caution in attributing to them any relevance for human disease, there is little doubt that *Candida* cells and products have a diversified range of effects on the immune system and that *Candida* itself may manipulate the immune response.

Hence, analysis of the effects of fungus inoculation into experimental animals may provide useful information both on the response to *Candida* infection and, more generally, on the functions of the immune system challenged with fungal antigens. During our studies on *Candida*-induced immunomodulation, we observed that a single, intraperitoneal injection of chemically inactivated, yeast form cells of *Candida albicans* in CD2F1 mice elicited the appearance of peritoneal natural cytotoxic cells active against YAC-1 tumor target cells. In this paper we describe this induction and show that natural killer (NK) lymphocytes are the most likely effectors of this peritoneal reactivity.

MATERIALS AND METHODS

Mice. Inbred BALB/c Cr(H-2^d), C3H Cr(H-2^k), C57BL/6 Cr(H-2^b), DBA Cr(H-2^d), and SJL/J Cr(H-2) mice, hybrid (BALB/c Cr × DBA/2 Cr) F1 (CD2F1; H-2^d/H-2^d) mice, and outbred CD-1 mice of both sexes were obtained from Charles River Breeding Laboratories, Calco, Italy, and from our own colony.

***Candida albicans* and peritoneal stimulants.** The strain of *Candida albicans* used throughout this study (strain BP) was isolated from a clinical specimen and was identified by using established taxonomic criteria (3, 18). The organism was grown at 28°C with slight agitation in a low-glucose Winge medium composed of 0.2% (wt/vol) glucose and 0.3% (wt/vol) yeast extract (BBL Microbiology Systems, Cockeysville, Md.) until the stationary phase of growth was reached (~24 h). The culture yielded approximately 2.8×10^8 cells per ml. Under these conditions, the organism grew as an essentially pure yeast form population (24). After growth, cells were harvested by low-speed centrifugation, washed twice in sterile distilled water, and inactivated by treatment with sodium merthiolate (0.01%, wt/vol) for 24 h. After inactivation, the organism was thoroughly washed with sterile distilled water and lyophilized; 1 mg of dry material corresponded to approximately 5×10^7 yeast cells. The lyophilized material was suspended in a 0.85% NaCl sterile solution immediately before use; this material is referred to below as CA. Pyran copolymer (type NSC 46015) was obtained from the Hercules Research Center, Wilming-

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ton, Del. Pyran was dissolved in a sterile 0.9% NaCl solution, and the pH was adjusted to 7.0 by using 1 N NaOH. Thioglycolate broth (Brewer thioglycolate medium) was obtained from Difco Laboratories, Detroit, Mich. Mice were injected with 1 ml of a 10% solution of this medium. After each treatment, the cellularity of the elicited cell populations was evaluated by cell counting with a Burker hemacytometer.

Tumors. Cell lines YAC-1 (a tissue culture cell line of YAC, a Moloney-induced lymphoma of A/Sn origin [4]), P-815 (an ascitic lymphoma of DBA/2 origin [8]), MBL-2 [a Moloney-induced lymphoma of B6 (H-2^b) mice], and EL-4 (a benzopyrene-induced lymphoma of C57BL/6 origin [7]) were maintained in RPMI 1640 medium (Eurobio Laboratories, Paris, France) supplemented with 10% fetal calf serum (GIBCO Laboratories, Grand Island, N.Y.), 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer (Eurobio), and 0.1% gentamicin sulfate (this medium is referred to below as complete RPMI 1640 medium). Tumor cells were transferred into fresh, complete RPMI 1640 medium 24 h before use in the ^{51}Cr release assay.

Cell fractionation procedures. (i) Plastic adherence. A total of 4×10^7 effector cells suspended in 10 ml of complete RPMI 1640 medium were incubated for 3 h at 37°C in 93-mm petri dishes (Nunc, Roskilde, Denmark) under a 5% CO_2 atmosphere. After incubation, the dishes were extensively washed with RPMI 1640 medium to remove the nonadherent cells. The adherent cells were recovered by scraping with a rubber policeman, washed, and resuspended (viability, 80 to 90%) in complete RPMI 1640 medium.

(ii) Nylon column. Effector cells were passed over a nylon fiber column as previously described (14). Briefly, the sterile nylon columns were rinsed with 20 ml of RPMI 1640 medium supplemented with 5% fetal calf serum. The columns were drained of excess medium; they were then replaced in the sterile syringe covers and put into a CO_2 incubator at 37°C for at least 1 h before loading of cells. Then 10^8 cells in 2 ml were added to the column and washed into the nylon wool with 0.5 to 1 ml of warm (37°C) medium. The columns were replaced in the sterile syringe covers and left for 45 min at 37°C. The columns were then washed slowly with warm (37°C) medium, the first 25 ml of each effluent was collected in a 50-ml conical tube, and cells were pelleted by centrifugation at 290 $\times$ g for 10 min at 4°C. The level of cell recovery was about 30%.

(iii) Carbonyl-iron powder and magnet. Phagocytic cells were removed from the effector cell population as previously described (17). Briefly, 25 ml of a spleen cell suspension (10^7 cells per ml) was incubated with 25 mg of carbonyl-iron powder (GAF Corp., New York, N.Y.) in a 50-ml conical tube (BD Labware, Oxnard, Calif.) for 60 min at 37°C. To remove the cells that ingested iron particles, the tube was placed on top of a magnet, and the supernatant was removed. This last step was repeated six to eight times. The cells were then washed and used as effectors in the microcytotoxicity assays.

(iv) Treatment of anti-Thy1.2 antiserum plus complement. Monoclonal antibodies to Thy1.2 were purchased from New England Nuclear Corp., Boston, Mass. (lot LK 114). A total of 3×10^7 cells were preincubated with anti-Thy1.2 antiserum diluted 1:100 in complete RPMI 1640 medium for 30 min at room temperature, washed once in complete RPMI 1640 medium, suspended in a 1:4 dilution of low-tox-m rabbit complement (Cedarlane Laboratories, Hornby, Ontario, Canada), and incubated for 45 min at 37°C. The surviving cells were then washed twice and counted. In all experiments a complement control was included, in which the first incubation was in medium alone and the second incubation was with the complement. The level of cell recovery was between 50 and 60%.

(v) Treatment with anti-asialo GM1 antiserum plus complement. Antiserum previously shown to react selectively with mouse NK cells (15) was obtained from Wako Chemicals GmbH, Düsseldorf, West Germany. Peritoneal exudate cells (PEC) (see below) were treated with a 1:200 dilution of antiserum in RPMI 1640 medium for 30 min at room temperature, washed twice, suspended in a 1:4 dilution of low-tox-m rabbit complement, and incubated for 1 h at 37°C. The surviving cells were then washed twice, counted, and tested for residual activity in the microcytotoxicity assays. Monoclonal anti-Ly5.1 and anti-immunoglobulin G2a (IgG2a) rat antimouse antibodies were obtained from New England Nuclear Corp. (lots FPD.398 and FPC.001, respectively). A total of 3×10^7 cells were preincubated with Ly5.1 antiserum (diluted 10^{-3}) for 20 min at room temperature and then washed and suspended in complete RPMI 1640 medium with anti-IgG2a antibody (diluted 10^{-2}) for 20 min at room temperature. After washing and suspension in fresh medium, rabbit complement (diluted 1:4) was added, and cells were incubated for 60 min at 37°C. The surviving cells were washed twice and counted. For controls we omitted Ly5.1 serum or Ly5.1 serum and anti-IgG2a antibodies or simply incubated the preparations in complete RPMI 1640 medium alone.

In vitro cytotoxicity assay. (i) Preparation of effector cells. PEC were harvested by massaging the peritoneal cavity after injection of 5 ml of cold RPMI medium containing 5 U of heparin per ml and aspirating the exudate with a syringe and 26-gauge needle.

Spleens and thymuses were excised, minced, and pressed through a 60-mesh wire screen into cold complete RPMI 1640 medium. Bone marrow cells were flushed out of femurs by using complete RPMI 1640 medium and a 26-gauge needle. Debris was removed by brief centrifugation. All cells were centrifuged and washed three times in cold complete RPMI 1640 medium. Cells were suspended in complete RPMI 1640 medium, and viable cells were counted with a hemacytometer by the dye exclusion method. All cells were kept on ice until use.

(ii) ^{51}Cr release assay. The ^{51}Cr release assay has been described previously (20). Different concentrations of effector cells were incubated with 10^4 ^{51}Cr-labeled tumor target cells (5×10^6 cells labeled with 100 μCi of $Na_2{}^{51}CrO_4$ for 45 min) for different times at 37°C in U-shaped 96-well plastic microtiter plates (Greiner Labortechnik, Nürtingen, West Germany). Unless otherwise specified, the release time was 4 h. After incubation, the plates were centrifuged at 800 $\times$ g for 10 min, and the radioactivity in 0.1 ml of the supernatant was measured with a γ-scintillation counter. All groups were tested in quadruplicate. The base-line ^{51}Cr release value was determined by using an autologous control with equal numbers of unlabeled target cells in the place of effector cells. The percentage of specific lysis was obtained as follows: % cytotoxicity = (test cpm − autologous cpm)/(total cpm incorporated/2) $\times$ 100, where test cpm was the mean cpm released in the presence of effector cells.

Statistical analysis. Differences in specific radiolabel released in the in vitro microcytotoxicity assays were determined by using Student's t test. Each experiment was repeated three to five times; the data reported are the means of quadruplicate samples. Standard errors (usually $<1.5\%$) have been omitted.

TABLE 1. Cytotoxic activity of murine PEC after stimulation with CA[a]

Stimulant[b]	Cellularity (no. of cells per mouse, $\times 10^6$)[c]	% of specific cytolysis against:[d]			
		YAC-1 cells	P-815 cells	MBL-2 cells	EL-4 cells
None (saline)	3.2	1.5	1.3	4.2	2.7
Thioglycolate	10	5.8	2.5	4.8	3.1
CA	4.9	25.3[e]	3.7	5.8	3.8
Pyran	5.1	32.5[e]	6.2	6.9	4.4

[a] PEC were obtained from 8-week-old CD2F1 mice 3 days after intraperitoneal injection of stimulant.
[b] Intraperitoneal injection of 2×10^7 CA cells, 50 mg of pyran copolymer per kg, 2 ml of 10% thioglycolate broth, or 2 ml of saline.
[c] Number of cells recovered from peritoneal cavity after stimulation.
[d] Lysis of target cells was measured by a 4-h chromium release assay at a ratio of effector cells to target cells of 50:1.
[e] Statistically significant difference of treated mice compared with untreated mice ($P < 0.01$).

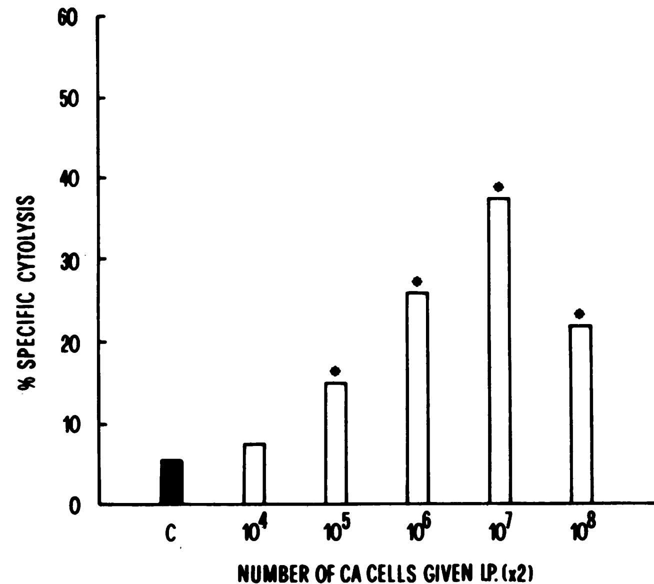

FIG. 1. Cytolytic activities of PEC from CA-treated mice measured as a function of the number of inactivated CA cells. CD2F1 mice that were 8 weeks old were given different doses of CA cells intraperitoneally (I.P), and their PEC were harvested 4 days later. These cells were assayed with ^{51}Cr-labeled YAC-1 target cells for 4 h at a ratio of effector cells to target cells of 100:1. The numbers of cells were as follows: 1.8×10^6 (control); 1.7×10^6 (2×10^4 CA cells); 2.1×10^6 (2×10^5 CA cells); 4.0×10^6 (2×10^6 CA cells); 5.0×10^6 (1×10^7 CA cells); and 3.4×10^6 (2×10^8 CA cells). The asterisks indicate significant differences ($P < 0.01$) for CA-treated mice compared with untreated controls. For other experimental details, see the text.

RESULTS

Induction of natural cytotoxic activity by intraperitoneal inoculation of *Candida albicans*. To study the effect of *Candida albicans* on natural reactivity in vivo, CA was injected intraperitoneally into 8-week-old CD2F1 mice, and the PEC, harvested 3 days after inoculation, were tested in vitro for their capacity to lyse a number of tumor cell lines in a ^{51}Cr release assay. Control mice received saline or thioglycolate as aspecific irritants or pyran copolymer, a strong inducer of natural cytotoxic effectors (32). As shown in Table 1, treatment with CA induced highly cytotoxic PEC against YAC-1, an NK cell-sensitive cell line, but not against EL-4, P-815, or MBL-2 cells, all of which are known to be resistant to murine NK effectors (8, 12). The effectiveness and target spectrum of CA-induced PEC paralleled the effectiveness and target spectrum shown by pyran, whereas low or negligible reactivity was detected in thioglycolate-stimulated PEC, despite the elevated number of cells induced by this agent in peritoneal cavities (Table 1). The cytolytic activity of CA-induced PEC was optimally expressed in the first 4 h of effector-target contact when the target was the sensitive YAC-1 line, whereas the insensitive EL-4 line resisted 10 h of contact with the effectors. Merthiolate-inactivated cells of *Saccharomyces cerevisiae* were unable to elicit peritoneal cytotoxic effectors against YAC-1 target cells, whereas some activity was obtained by injecting a crude glucomannan-protein fraction of *Candida albicans* cell walls (data not shown).

TABLE 2. Effect of route of CA injection on production of natural cytotoxic effectors in peritoneal cavities and spleen[a]

Treatment	Day of assay	Cellularity (no. of cells per mouse, $\times 10^6$)		% of specific cytolysis[b]	
		PEC	Spleen	PEC	Spleen
Saline, i.p.	4	1.25	45.3	6.0	24.7
CA, i.p.	4	4.1	56.0	25.6[c]	14.4[c]
CA, s.c.	4	2.0	38.6	9.1	24.3
CA, i.v.	4	1.25	56.0	6.6	22.1
Saline, i.p.	10	4.2	50.5	5.4	21.4
CA, i.p.	10	4.0	48.3	15.6[c]	21.4
CA, s.c.	10	3.8	63.7	7.2	22.5
CA, i.v.	10	3.9	35.6	8.4	21.8

[a] CA was injected into 8-week-old CD2F1 mice intraperitoneally (i.p.), subcutaneously (s.c.), or intravenously (i.v.) at zero time. Pooled lymphoid cells were prepared from PEC or spleens of normal or CA-immunized mice. In the case of CA-immunized mice, cells were harvested either 4 or 10 days after CA injection.
[b] The lymphoid cells were assayed as effector cells in a 4-h chromium release assay against ^{51}Cr-labeled YAC-1 target cells at a ratio of effector cells to target cells of 50:1.
[c] Highly significant difference ($P < 0.01$) between CA-treated mice and untreated controls.

The injection of CA in a dose sufficient to raise peritoneal anti-YAC-1 activity did not generate significant cytotoxic activity in the thymus or bone marrow, whatever the target or the route of CA administration, nor did it increase the spontaneous natural cytotoxicity of spleen cells against YAC-1 tumor cells. On the contrary, mice injected intraperitoneally with CA manifested a slight, transient depression of natural splenic cytotoxicity (Table 2).

Other aspects of CA induction of PEC cytotoxicity. The experiments described below were designed to assess the influence of the dose of CA and the influence of the age and strain of mice, as well as the kinetics of cytotoxic PEC induction by CA. Figure 1 shows that there was a clear dose-response effect in the induction of cytotoxic PEC by CA, with maximum induction measured when the animals were given 2×10^7 CA cells intraperitoneally. However, low but significant activity was observed with a dose as low as 10^5 cells, corresponding roughly to 1 μg (dry weight) of material. After injection of an optimal dose of CA, the cytolytic activity of PEC peaked on day 3, although activity persisted at significant levels until day 7. A decline to the

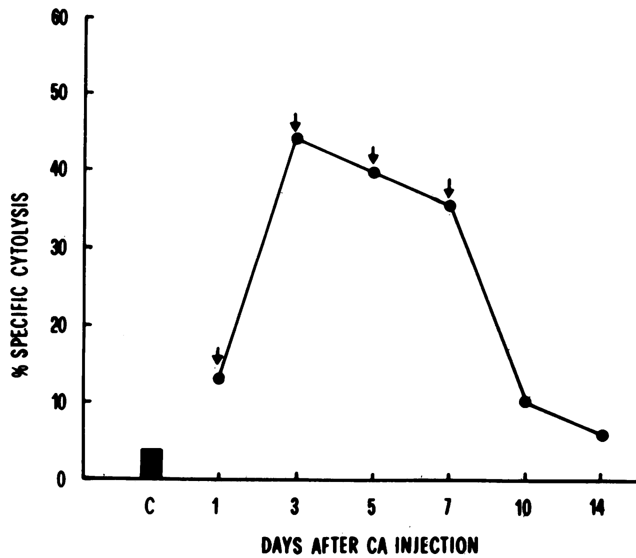

FIG. 2. Cytolytic activity of PEC as a function of time after CA administration. CD2F1 mice were injected intraperitoneally at zero time with 2×10^7 CA cells. The PEC were harvested from 1 to 14 days later and assayed with ^{51}Cr-labeled YAC-1 target cells at a ratio of effector cells to target cells of 100:1. The solid bar indicates PEC from untreated control mice (C). Symbol: ●, PEC from CA-treated mice. The arrows indicate significant differences ($P < 0.01$) for CA-treated mice compared with untreated controls.

basal level of activity shown by the untreated control was observed 12 to 14 days after inoculation (Fig. 2). Induction of cytolytic PEC by CA was detected in almost all strains of mice examined, although CD2F1 and C57BL/6 mice were maximally reactive. The only exception was strain SJL/J, which did not respond to CA. For each mouse strain, the best responses to CA induction were obtained with 5- to 8-week-old animals (data not shown).

Characterization of CA-induced reactive PEC. We addressed the question of the nature of the reactive peritoneal cells elicited by intraperitoneal injection of CA. Since both CA and its insoluble glucan cell wall components have been shown to be powerful macrophage activators and to be able to enhance macrophage tumoricidal properties (37, 42), we first tested whether CA-induced PEC activity could be attributed to adherent phagocytic cells. To do this, we examined the adherence and phagocytic properties of CA-induced PEC harvested from 8-week-old CD2F1 mice 3 days after a single intraperitoneal injection of 2×10^7 CA cells. PEC were passed in tissue culture dishes and through nylon wool columns and separated into adherent and nonadherent populations. Table 3 shows that no loss of cytolytic activity was detected after removal of adherent cells; instead, there was a clear enrichment of activity in the nonadherent population. We also observed that treatment of CA-induced PEC with iron and a magnet did not reduce the cytolytic activity of these cells (Table 3).

In subsequent experiments, phenotypic characterization of PEC was attempted by using specific antisera, as shown in Tables 4 and 5. PEC were passed through a nylon wool column and then treated with anti-Thy1.2 serum plus complement. This treatment reduced (to about one-third at a ratio of effector cells to target cells of 50:1) but did not abolish the lytic activity of PEC. If nylon wool-passed PEC were treated with anti-asialo GM1 antibodies and complement, the cytolysis of YAC-1 target cells was completely abrogated. A similar abrogation occurred when CA-induced

TABLE 4. Sensitivity of nonadherent CA-induced peritoneal effector cells to anti-Thy1.2 or anti-asialo GM1 serum in the presence of complement[a]

Nonadherent effector cell population[b]	% Recovery	% of specific cytolysis at an effector-to-target cell ratio of:[c] 50:1	25:1	12.5:1
Untreated cells	100	57.3	43.6	38.9
Complement-treated cells	91	52.1	33.2	31.7
Cells treated with anti-Thy1.2 + complement[d]	36	32.6[e]	24.7	16.1
Cells treated with anti-asialo GM1 + complement[d]	46	5.8[e]	4.2	3.1

[a] CD2F1 mice that were 8 weeks old were injected with 2×10^7 CA cells intraperitoneally, and peritoneal exudates were harvested 4 days later.
[b] Nonadherent cells were obtained after nylon wool passage of PEC.
[c] Measured in a 4-h chromium release assay against ^{51}Cr-labeled YAC-1 target cells.
[d] The protocol used for antiserum treatment is described in the text.
[e] $P < 0.01$ (treated cells compared with untreated cells).

TABLE 3. Adherence and phagocytic properties of CA-induced peritoneal exudate effector cells[a]

Effector cell population[b]	Peritoneal resident cells: Cellularity (no. of cells per mouse, $\times 10^6$)	% Recovery[c]	% of specific cytolysis at an effector-to-target cell ratio of:[d] 50:1	12.5:1	CA-induced PEC: Cellularity (no. of cells per mouse, $\times 10^6$)	% Recovery	% of specific cytolysis at an effector-to-target cell ratio of: 50:1	12.5:1
Unseparated cells	3.2	100	3.9	1.1	5.7	100	28.5	14.5
Plastic surface-nonadherent cells		5	5.4	2.6		12	32.4	16.8
Nylon wool-nonadherent cells		2	6.2	3.4		21	59.0[e]	31.6[e]
Cells treated with carbonyl-iron + magnet		4	5.1	3.3		45	27.6	12.6

[a] CD2F1 mice that were 8 weeks old were not treated or were treated with 2×10^7 CA cells intraperitoneally, and peritoneal exudates were harvested 4 days later.
[b] The separation procedures used are described in the text.
[c] Percentage of the original cell population recovered after in vitro treatment.
[d] Lytic activity was measured in a 4-h chromium release assay against ^{51}Cr-labeled YAC-1 target cells.
[e] $P < 0.01$ (nonadherent cells compared with unseparated cells).

TABLE 5. Sensitivity of CA-induced peritoneal effector cells to anti-Ly5.1 serum and anti-IgG2a in the presence of complement[a]

Effector cell population[b]	% Recovery	% of specific cytolysis at an effector-to-target cell ratio of:[c]		
		100:1	50:1	25:1
Untreated cells	100	35.1	25.3	15.4
Complement-treated cells	97.1	35.7	24.9	15.1
Anti-IgG2a-treated cells	98.2	33.1	22.3	12.0
Cells treated with anti-IgG2a + complement	97.0	26.9	18.1	9.9
Anti-Ly5.1-treated cells		34.2	23.9	13.4
Cells treated with anti-Ly5.1 + anti-IgG2a + complement	42.8	4.2[d]	2.6[d]	1.5[d]

[a] CD2F1 mice that were 8 weeks old were injected with 2×10^7 CA cells intraperitoneally, and peritoneal exudates were harvested 4 days later.

[b] The protocol used for antiserum treatment is described in the text.

[c] Measured in a 4-h chromium release assay against ^{51}Cr-labeled YAC-1 target cells.

[d] Significant difference ($P < 0.01$) between treated and untreated effectors.

PEC were treated with anti-Ly5.1 plus anti-mouse IgG2a antiserum plus complement (Table 5). Thus, the naturally reactive PEC elicited by CA in the peritoneal cavities of mice were nonphagocytic, nonadherent cells and had a surface phenotype compatible with NK lymphocyte populations (see below).

DISCUSSION

Recently, it has become increasingly evident that the opportunistic pathogen *Candida albicans* is capable of inducing a variety of nonspecific immunomodulatory effects. It is important to gain information on these effects to understand how this microorganism interacts with the immune system and, more particularly, whether this immunomodulation has any supportive or causative role in the pathogenesis of candidosis. To do this, we showed that CA, given in a proper dose, at a proper time, and by a proper route of inoculation, is able to induce powerful natural cytotoxic activity in the PEC populations of several mouse strains. This activity was not merely attributable to aspecific irritation or to an artifact of modified peritoneal cellularity, as shown by suitable controls. On the contrary, the CA-induced PEC activity closely paralleled in intensity, time of induction, and spectrum of sensitive targets the activity shown by pyran, a classical stimulator of natural cytotoxicity (12, 32).

There are reasons to believe that at least one dominant fraction of the cytolytic populations induced by *Candida albicans* in the peritoneal cavities of mice consists of NK cells, which have been shown to be primarily active in controlling tumor growth and viral infections but are also increasingly suspected to play a role against other infectious agents, including fungi (13).

In fact, CA-induced PEC lysed only NK-sensitive target cells and showed several characteristics, such as time of induction, kinetics of lysis, age, and strain distribution of reactivity, which are distinctive of NK cells. It should be stressed that other natural effectors could be induced by fungus inoculation, as shown by the presence in the peritoneal cavities of cells distinct from lymphocytes and morphologically attributable to promonocytes and macrophages, which under particular circumstances could be good candidacidal effectors (19). However, the following results collectively support the conclusion that the anti-YAC-1 activity elicited by CA described above was entirely due to NK cells: (i) the effectors were nonadherent, nonphagocytic, and partially sensitive to Thyl.2 antiserum, and (ii) the activity was abrogated by treatment with anti-asialo GM1 antibodies plus complement and by treatment with Ly5.1 plus anti-IgG2a plus complement (9, 10, 13, 15, 23, 38, 39, 44).

Our results add another piece of experimental evidence that host immune systems may be manipulated by *Candida* cells. We have previously shown that cells of *Candida albicans* strongly synergize with antiblastic chemotherapy in eradicating aggressive, fully histocompatible experimental leukemias (2, 3). For this effect to occur, collaboration of the immune system was necessary (22), and immunological processing of both tumor and yeast cells had to take place in the peritoneal cavity (20, 21). The data reported above apparently favor some involvement of NK cells in the antitumor immunoadjuvant mechanisms of *Candida* spp., as it is well established that NK lymphocytes are potentially able to control tumor growth and metastases both in experimental animals and in humans (9, 12, 35). However, the antitumor immunoadjuvant properties of *Candida albicans* require an intact T-cell compartment (22), whereas T cells are not required for NK cell activation (9, 12). The capacity of *Candida albicans* to elicit NK peritoneal cells parallels what has been shown with other antigenically unrelated microbial immunostimulants, such as *Mycobacterium bovis* BCG, *Corynebacterium parvum*, streptococcal preparation OK432, and other preparations (27, 28, 40, 41, 44, 45). Interferon induction is common to many of these immunoadjuvants, but we were never able to detect any significant level of interferon in sera or peritoneal fluids of CA-injected mice (22) (data not shown). However, nondetectable amounts of interferon could still play a crucial role in eliciting augmented NK cell activity. Studies are in progress to elucidate this aspect of the mechanism of immunomodulatory effects of *Candida albicans*. Nevertheless, it may be possible that the augmentation of NK cell activity does not rely upon interferon induction, as, for instance, in the case of OK432 preparations (41, 45).

We also found that the splenic NK cell basal activity of young mice can be transiently but significantly depressed after intraperitoneal injection of killed *Candida* cells. Although no attempt has yet been made to characterize the nature of this immunodepression, a trivial dilution phenomenon can be excluded since the cellularity of the spleens was not substantially augmented in CA-treated mice compared with untreated control mice. One possibility is that *Candida albicans* induces selective depletion of NK cells or that there was an expansion of suppressor cells. In our experiments, removal of adherent cells from the spleens of CA-treated animals by passage through a nylon wool column did not eliminate the suppressive activity (data not shown), thus ruling out the possibility that adherent cells are directly involved in the CA-mediated decline in natural splenic reactivity.

Altogether, the results of this study document another aspect of the immunomodulatory activity of the opportunistic organism *Candida albicans*, i.e., its capacity to modulate NK cell activity. No data are presently available to establish whether the appearance of NK effectors in the peritoneal cavities of CA-treated mice results from local activation of resident precursors or from recruitment of effector lymphocytes from other sites, as suggested by other workers (39). We do not even know at the moment what might be the relevance, if any, of NK cell induction on control of tumor

growth by *Candida* vaccine (3, 20). Finally, recent data suggest that elicitation of NK effectors may also have a protective role against fungal pathogens (13). Our demonstration that *Candida albicans* modulates NK cell activity in peritoneal cavities and, possibly, in spleens of mice suggests further studies on the possible role of this modulation in control of *Candida* pathogenicity.

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