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Protection against Murine Disseminated Candidiasis Mediated by a *Candida albicans*-Specific T-Cell Line

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The role of T lymphocytes in disseminated candidiasis in a mouse model of irradiation-induced immunosuppression was investigated. A continuously cultured *Candida albicans*-specific T-cell line mediated protection of sublethally irradiated mice from disseminated candidiasis as measured by both the fungal load in the kidneys and mortality. These results are the first to demonstrate directly a role for antigen-specific T cells in the protective immune response against murine disseminated candidiasis.

Cases of opportunistic infection caused by *Candida* species, particularly *Candida albicans*, have increased significantly over the past 2 to 3 decades (25). In immunocompromised individuals, *Candida* infections may range in severity from mild, superficial lesions to life-threatening, disseminated infections (19). Superficial (or mucocutaneous) infections are most often associated with defects in cell-mediated immunity, such as in individuals with AIDS (12). Disseminated candidiasis frequently arises as a result of chemotherapy-induced neutropenia in cancer patients, especially those with hematologic malignancies (3). In addition, disseminated candidiasis may be induced by other iatrogenic factors, such as use of intravenous catheters, treatment with broad-spectrum antibiotics, and surgical procedures of the abdomen (25). Disseminated candidiasis in this setting is difficult to diagnose and treat and therefore is a cause of significant morbidity and mortality in critically ill individuals. A detailed understanding of the interaction between *C. albicans* and the host, particularly with regard to the mechanisms of host defense, would likely facilitate the design of effective strategies to combat this important pathogen.

The mechanism(s) which prevents *C. albicans* infections in immunocompetent individuals is not completely understood but is likely to involve both cell-mediated immunity and innate immunity. The finding that individuals with defective cell-mediated immunity most often develop mucocutaneous infections (12, 28) while individuals with phagocyte deficiencies tend to develop disseminated *C. albicans* infections (3, 14, 17) has led many to conclude that T cells mediate immunity to mucocutaneous candidiasis while phagocytes, particularly neutrophils, are responsible for elimination of disseminated infections. While experimental animal studies support the supposition that T cells are important for resistance to mucocutaneous infection (5, 6, 18) and neutrophils are instrumental in resistance to disseminated infection (2, 5, 21), the roles of neutrophils in the former and T cells in the latter type of infection are less clear. In particular, the role of T cells in the immune response to disseminated *C. albicans* infection has been a subject of much disagreement. Evidence both for (1, 16, 23) and against (8, 15, 22) a role for T-cell-mediated immunity in resistance to murine disseminated candidiasis has been presented. Given the natural cooperativity among cells of the immune response, particularly the well-described cytokine-mediated interaction between T cells and phagocytes such as neutrophils (4, 13, 24, 29), it seems reasonable to postulate that T-cell-mediated immunity may contribute to the generation of an effective response against disseminated *C. albicans* infection. Indeed, it has been demonstrated that T-cell-derived cytokines such as gamma interferon and tumor necrosis factor alpha activate in vitro candidacidal activity of murine and human neutrophils (10).

To address directly the question of the role of T cells in resistance to disseminated candidiasis, we developed a *C. albicans*-specific murine T-cell line and tested the effect of the line on the development of disseminated candidiasis in a mouse model of irradiation-induced immunosuppression. *C. albicans*-specific T-cell lines were prepared by immunizing BALB/c mice with a crude cytoplasmic extract of *C. albicans* yeasts (27). Single-cell suspensions were prepared from spleens and mesenteric lymph nodes of mice 14 days after immunization and cultured in vitro in the presence of 100 μg of *C. albicans* extract (CaX) per ml. The resulting *Candida*-

TABLE 1. Proliferation of spleen and lymph node cells from mice immunized with *C. albicans* upon in vitro challenge with CaX[a]

Type (no.) of cells	[^{3}H]thymidine incorporation (cpm)	
	Medium	CaX
None (medium only)	118	158
Immune (2×10^5)	5,590	37,820
Immune (1×10^5)	692	17,937
Nonimmune (2×10^5)	952	1,025

[a] BALB/c mice were immunized subcutaneously at the base of the tail with 200 μg of CaX in complete Freund's adjuvant. Three weeks after immunization, mice were sacrificed and single-cell suspensions were made of spleens and inguinal and mesenteric lymph nodes. Spleen and lymph node cells (2×10^7 of each) were cultured together with CaX at 100 μg/ml in T-cell medium (RPMI 1640 with 8% fetal calf serum, penicillin, streptomycin, glutamine, 2-mercaptoethanol [5×10^{-5} M]). Cells were cultured in round-bottom microtiter dishes at appropriate concentrations in 5% CO_2 at 37°C. Wells were pulsed with [^{3}H]thymidine on day 3 and harvested on day 4 after stimulation. Results are averages of four replicate wells. All standard deviations were less than 8%. Similar results were obtained in at least three different experiments.

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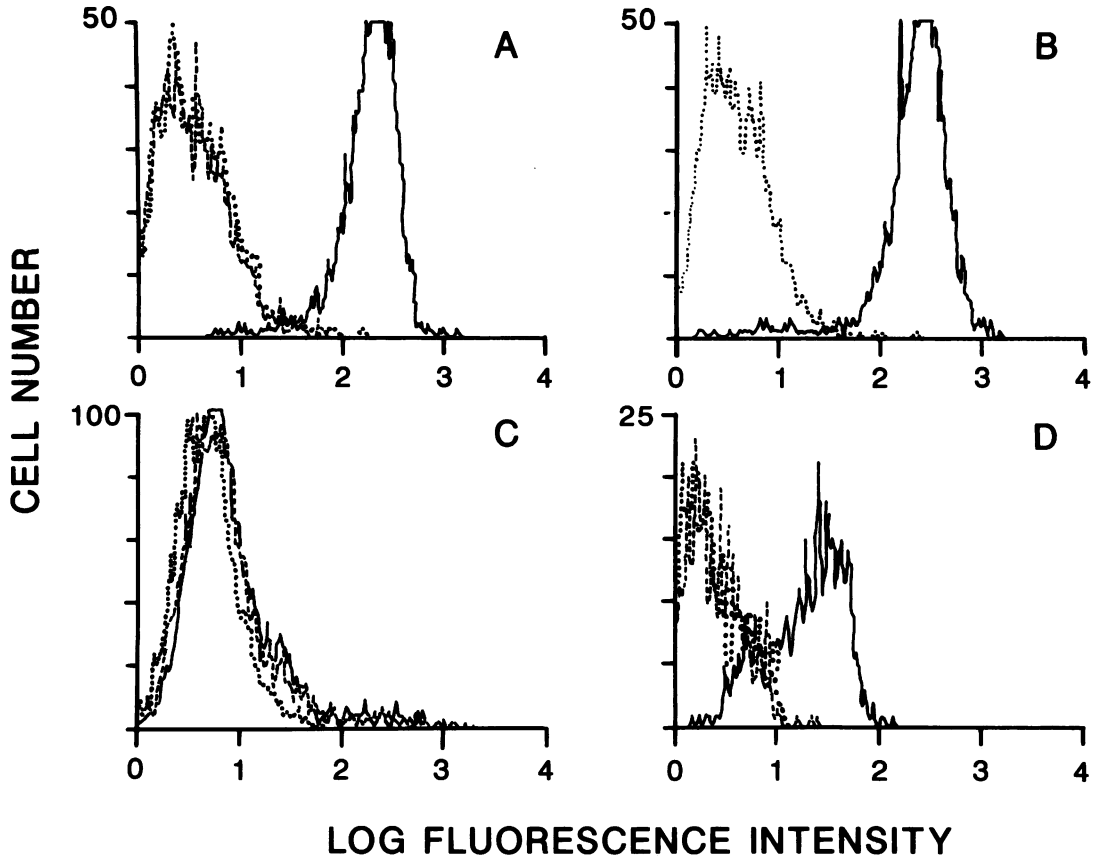

FIG. 1. Phenotypic analysis of continuously cultured anti-*Candida* cells by flow cytometry. Cells (3×10^5) were placed in polystyrene tubes, centrifuged, and resuspended in 0.05 ml of a 1/50 dilution of the appropriate monoclonal antibody (145-2c11 [Boehringer Mannheim] for CD3, PK136 [American Type Culture Collection] for natural killer cells, GK1.5 [Becton Dickinson] for CD4, LyT2 [Becton Dickinson] for CD8, H57-597 [Pharmingen] for the α/β TCR, GL3 [Pharmingen] for the γ/δ TCR, goat anti-mouse immunoglobulin [Southern Biotechnology Associates, Inc.]). Cells were placed on ice for 45 min, washed three times, and resuspended in 1% paraformaldehyde–0.02% NaN_3 in phosphate-buffered saline. Cells were analyzed on a FACScan apparatus (Becton Dickinson). Panel A: – – – –, autofluorescence; · · · · · · ·, LyT2-fluorescein isothiocyanate (CD8); ----------, 145-2c11-fluorescein isothiocyanate (CD3). Panel B: · · · · · · · ·, autofluorescence; ----------, GK1.5-phycoerythrin (CD4). Panel C: · · · · · · · ·, autofluorescence; – – – – –, PK136 (natural killer cell); ----------, goat anti-mouse immunoglobulin-fluorescein isothiocyanate (B cell). Panel D: · · · · · · ·, autofluorescence; – – – – –, GL3-fluorescein isothiocyanate (γ/δ TCR); ----------, H57-597-fluorescein isothiocyanate (α/β TCR). The antigens recognized by the various antibodies are in parentheses next to the designations for the antibodies.

specific proliferative response was measured by [^{3}H]thymidine uptake (Table 1). Cells from immunized mice demonstrated a pronounced proliferative response to the CaX (6.7-fold increase over cells incubated with medium alone), while cells from nonimmune mice failed to mount a detectable proliferative response.

To produce a *Candida*-specific line, cell cultures derived from immune mice were maintained in vitro by restimulation every 10 days with CaX in the presence of syngeneic spleen cells (2,000 R) as antigen-presenting cells. Sixty days after initial explant and in vitro culture (and 20 days after the previous stimulation), the cells were again tested for specificity to *C. albicans*. These continuously cultured cells generated a strong proliferative response against *C. albicans* (Table 2) (compare cells incubated with CaX with cells incubated in medium alone in group 2). However, these cells failed to mount a response to a control antigen, ovalbumin. Further, the *Candida*-specific response was *H-2* restricted, since strong proliferation was observed in the presence of syngeneic (group 2, incubated with CaX) but not allogeneic (group 4, incubated with CaX) antigen-presenting cells (Table 2).

The finding that the in vitro proliferative response of cultured cells from *Candida*-immune mice to *Candida* antigens was *H-2* restricted suggested that the responding cells were T cells. Phenotypic analysis by flow cytometry indicated that these continuously cultured cells were positive for CD3, CD4, and the α/β T-cell receptor (TCR) and negative for CD8, surface immunoglobulin, the γ/δ TCR, and Pk126, a natural killer cell marker (Fig. 1a to d). This phenotypic profile, along with the *H-2*-restricted proliferation of cells shown in Table 2, indicates that we generated a *C. albicans*-specific, major histocompatibility complex-restricted, $CD4^+$ T-cell line in continuous culture.

To determine whether this T-cell line had the capacity to confer protection against disseminated candidiasis, BALB/c mice were sublethally irradiated (550 R) 2 days prior to challenge with *C. albicans* (day −2) to abrogate primary immune responsiveness (9). Unirradiated animals were included as a control. Some animals were inoculated intrave-

TABLE 2. Proliferation of continuously cultured lymphoid cells from *C. albicans*-primed mice is *Candida* specific and *H-2*^{d} restricted[a]

Group	Cells	[^{3}H]thymidine incorporation (cpm)			
		Medium	CaX, 200 μg/ml	CaX, 25 μg/ml	Ovalbumin, 200 μg/ml
1	BALB/c anti-*Candida*	279	171	198	192
2	BALB/c anti-*Candida* + irradiated BALB/c spleen	786	7,045	11,291	1,375
3	Irradiated BALB/c spleen	182	171	117	129
4	BALB/c anti-*Candida* + irradiated C57BL/6 spleen	2,653	3,160	2,405	ND[b]

[a] Immune cells described in the footnote to Table 1 were restimulated every 10 days. For restimulation, 10^5 responder cells were harvested from culture, washed twice, and cultured together with 6×10^6 irradiated (2,000 R) syngeneic or allogeneic spleen cells as antigen-presenting cells and 100 μg of CaX per ml in 2 ml of T-cell medium. Responder cells had been in culture for 60 days and were last restimulated with CaX 20 days prior to this assay. The proliferation assay was performed with 10^4 responder cells and 2×10^5 stimulator cells in round-bottom microtiter plates. Wells were pulsed with [^{3}H]thymidine on day 3 and harvested on day 4. Results are averages of four replicate wells. All standard deviations were less than 9%.

[b] ND, not done.

nously on day −1 with BALB/c anti-*Candida* T cells. All animals were challenged intravenously on day 0 with 5×10^4 live *C. albicans* yeasts as described previously (17). Development of disseminated infection was assessed at days 12 and 19 postchallenge by enumerating the *C. albicans* CFU in the kidneys (17), a primary site of replication of intravenously inoculated *C. albicans* (20). In addition, deaths in each group were noted. Table 3 indicates that the *Candida*-specific T-cell line significantly reduced the fungal load in the kidneys of irradiated mice at days 12 and 19 postchallenge, compared with irradiated mice which received no T cells. At day 12 postchallenge, the average number of CFU recovered from the kidneys of irradiated mice which received *Candida*-specific T cells ($n = 10$) was approximately 1 log unit less than the number recovered from mice which were irradiated but did not receive T cells ($n = 10$) ($P < 0.05$). The average number of CFU recovered from control, unirradiated, immunocompetent mice ($n = 10$) was not significantly different from the number of CFU recovered from irradiated mice which had received *Candida*-specific T cells ($n = 10$) ($P = 0.430$). At day 19, there was a significant difference in mortality between irradiated mice which had received *Candida*-specific T cells (20%) and mice which had not (80%) ($P < 0.05$). In addition, the average number of CFU recovered from surviving animals at day 19 was significantly less for irradiated mice which had received *Candida*-specific T cells ($n = 9$) than for mice which had not ($n = 4$) ($P = 0.05$). Taken together, these results demonstrate that this *Candida*-specific T-cell line conferred protection against overgrowth of *C. albicans* in the kidneys and subsequent *C. albicans*-induced death in irradiation-immunosuppressed mice.

TABLE 3. Enumeration of *C. albicans* CFU in kidneys[a]

Mouse no.	*C. albicans* CFU/kidney				
	Day 12			Day 19	
	I + T	I, no T	No I, no T	I + T	I, no T
1	35	490	112	2,460	7,300[b]
2	237	837	90	75	560
3	440	600	125	968	27,600[b]
4	935	10,000	172	103	5,905
5	120	40,300	2,410	1,795	Died
6	277	657	32	728	Died
7	270	12,900	35	25	Died
8	1,875	440	350	677	Died
9	302	5,785	437	9,200[b]	Died
10	2,270	26,700	290	Died	Died
Avg	676	9,871	405	1,779	10,341

[a] Mice were sublethally irradiated (I) with 550 R on day −2, injected intravenously with T cells (5×10^6) on day −1 (T), and injected with 5×10^4 *C. albicans* cells intravenously on day 0. The T cells were restimulated with *C. albicans* 10 days prior to the assay. Control mice were either unirradiated or received no T cells. Mice were sacrificed on day 12 or 19, and kidneys were aseptically removed and homogenized in 10 ml of phosphate-buffered saline. A 0.1-ml volume of a 1/10, 1/100, or 1/1,000 dilution of the homogenate was added to 13 ml of Sabouraud dextrose agar at 56°C in 60-mm-diameter culture plates. The plates were cultured for 36 h at 37°C, and *Candida* colonies were enumerated. Significance of differences between groups as stated in the text were calculated by using Student's *t* test. Similar results were obtained in two different experiments.

[b] Animal was moribund. Animals dead or moribund were included when determining mortality as described in the text. Significance of differences in mortality between the two groups at day 19 was determined by chi-square analysis.

A recent study by Romani et al. (23) supports the involvement of T cells, particularly of the Th1 type (26), in the immune response to murine disseminated candidiasis. In this study, administration of an anti-interleukin 4 monoclonal antibody, which inhibited Th2-type responses, resulted in the generation of a *Candida*-specific delayed-type hypersensitivity (Th1-type) response which was associated with a decreased fungal load in the kidneys and increased survival in infected animals. We are currently assessing the cytokine pattern of our *Candida*-specific T-cell line and developing clones from this line. Preliminary data, however, suggest that the line produces gamma interferon, a Th1-type cytokine.

In summary, our results are the first to provide direct evidence that *C. albicans*-specific T cells are instrumental in the control of disseminated *Candida* infection in mice and provide an impetus for further examination of the specific effector mechanisms by which T cells mediate resistance in this model. In particular, the generation of a Th1-type immune response may significantly enhance neutrophil-mediated (and possibly macrophage-mediated) killing of yeasts and hyphal organisms in deep tissues by generation of phagocyte-activating cytokines such as gamma interferon.

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