

Type 1 T Helper Cells Specific for *Candida albicans* Antigens in Peripheral Blood and Vaginal Mucosa of Women with Recurrent Vaginal Candidiasis

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The cytokine profile of circulating and vaginal T cells specific for immunodominant mannoprotein antigens of *Candida albicans* was analyzed in patients with recurrent vaginal candidiasis (RVC). Peripheral blood mononuclear cells (PBMC) from patients with RVC proliferated more than those from healthy subjects and expressed higher type 1:type 2 T helper cell cytokine ratios in response to *C. albicans* stimulation. A higher number of *C. albicans*-specific T cells was generated in PBMC from patients with RVC than in PBMC from healthy donors. *C. albicans*-specific T cell clones from patients with RVC produced higher levels of interferon (IFN)- γ and lower levels of interleukin (IL)-4 than clones from control women. More important, a higher proportion of *C. albicans*-specific T cell clones was generated from lesional mucosa of patients with RVC than from normal mucosa, all of which produced IFN- γ but not IL-4. These findings provide direct evidence that RVC is characterized by a highly polarized local and circulating type 1 T helper cell-like response against *C. albicans* antigens.

Candida albicans is the causative agent of vulvovaginal candidiasis in ~90% of women with this disease. Up to 5% of women who experience an initial infection will subsequently develop recurrent vaginal candidiasis (RVC) [1, 2]. *C. albicans* is a normal human commensal that becomes an opportunistic agent of deep-seated disease in severely compromised or otherwise modified hosts [3–5]. However, it is usually present in the gastrointestinal tract and other body sites of all humans, where the microorganism induces host antigen-specific antibodies and reactive T cells that may account for its persistent commensal status [6–8].

Antigen expression on the *C. albicans* cell surface greatly influences the type, magnitude, and effectiveness of the host's immunosurveillance. A few *C. albicans* antigens have been characterized and assessed for their immunogenicity and protective ability, and, among them, mannoproteins (MPs) and mannan polysaccharides have been shown to play a major role in the

host-*Candida* relationship [9]. MPs are cell surface-expressed glycoconjugates that are rich in B and T cell antigens that are relevant to the host's adaptive immunity. Mycelial cell-secreted mannoprotein (M-sMP), an MP-rich material, is released into the external medium during growth and differentiation. MP65, a 65-kDa molecule, is a dominant component of M-sMP and is also present in structural material and recognized by circulating T cells of healthy donors [9]. A proliferative Th1 cell response can be elicited in vitro in cells of mice immunized with whole fungal cells, MP65-rich extract (MP-F2), or the recombinant molecule MP65 [10, 11]. In addition, MP-F2 induces a moderate degree of protection against a highly virulent strain in a model of murine disseminated candidiasis. This protection is increased by interleukin (IL)-12 or anti-IL-10 antibody [12]. Thus, *C. albicans* MPs are a major tool for cell-mediated immune responses against this important human opportunistic pathogen in RVC and other *C. albicans* diseases.

In murine models, T cells play a central role in regulating the immune response to *C. albicans*. It is known that T cells favor cell-mediated and antibody responses; however, the real mechanism of protection is unknown [13–15]. In addition, it may be difficult to extend some results based on rodent models to women. Unlike women, rodents do not constitutively display yeast flora, and there is no evidence of the need for prior sensitization of rodents to *C. albicans* or acidic vaginal pH [13]. On the other hand, it is difficult to clinically evaluate cell-mediated immunity (CMI) in RVC because of the different dynamics of infection, coinfecting or commensal agents, and the effects of the menstrual cycle and of *C. albicans* itself on local immune responses. Nonetheless, analysis of the nature

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and role of the vaginal T cell response may facilitate understanding of the mechanisms involved in the pathogenesis of human RVC.

Patients, Materials, and Methods

Patients. At Demetra Institute (Florence, Italy), 17 consecutive patients with a new diagnosis of RVC, based on their having >3 episodes of vulvovaginal candidiasis per year, were included in the study. The mean age ($\pm$ SD) of the patients was 37 ± 11 years. Fifteen age-matched healthy women were enrolled as control subjects. Blood samples were obtained from all patients during their luteal phase. No study participants had human immunodeficiency virus (HIV) infection, clinical symptoms suggesting other immunodeficiencies, diabetes mellitus, or other chronic infections, and none was being treated with immunosuppressive drugs.

Reagents. Complete medium (consisting of RPMI 1640 [Sero-med] supplemented with 2 mM L-glutamine, 1% nonessential amino acids, 1% sodium pyruvate, 2×10^{-5} M 2-mercaptoethanol [all from Gibco] and 100 μ g/mL kanamycin and 10 μ g/mL gentamicin [both from Sigma]) was used throughout the study. PMA and ionomycin were from Sigma, and IL-2 was a gift from Eurocetus. Purified and fluorochrome-conjugated anti-CD3, anti-CD4, anti-CD8, anti-CD14, anti-CD16, and anti-CD20 monoclonal antibodies (MAbs) were from Becton Dickinson. Purified and fluorochrome-conjugated isotype control MAbs were from Southern Biotechnology. Mycelial cell-secreted mannoprotein (M-sMP) and MP65 reagents were prepared as described elsewhere [12], and both preparations were shown by use of the Limulus test to be free of endotoxin.

Proliferation assay to *C. albicans* antigens. Peripheral blood mononuclear cells (PBMC) from patients with RVC and healthy subjects were isolated by Ficoll-Paque density gradient (Pharmacia), and 2×10^5 cells were cultured in triplicate for 6 days in complete medium and 5% autologous serum in 96-well, round-bottomed microwell plates (Nunc), with or without different concentrations of M-sMP or MP65 (from 10^{-3} to 10 μ g/mL) or 200 U/mL streptokinase (SK), as described elsewhere [16]. Sixteen hours before harvesting, 0.5 μ Ci of ^{3} H-TdR (Amersham) was added to each well, and radionuclide uptake was measured by scintillation counting (Tomtec). The mitogenic index was calculated as the ratio between the mean counts per minute obtained in stimulated cultures (less background counts per minute) and those obtained in the presence of medium alone (less background counts per minute). The assay results were considered to be positive when the mitogenic index was ≥ 5 .

In some experiments, PBMC from healthy volunteers were isolated by Ficoll-Paque and then were enriched for T cells and macrophages by a rosetting technique, using neuraminidase-treated sheep red blood cells. The rosetting technique was followed by an immunomagnetic negative-selection procedure, in which 10^7 cells were treated with 2 μ g of anti-CD14, anti-CD16, and anti-CD20 (or anti-CD3, anti-CD16, and anti-CD20) MAbs and the subsequent addition of microbead-conjugated goat anti-mouse IgG (Miltenyi Biotech). Purified T cells contained <1% non-T cells, and purified macrophages contained <1% T, B, and NK cells. Paraformaldehyde-treated or untreated macrophages (1×10^5) were cocultured for 5 days with

purified autologous T cells (1×10^5) in the presence of M-sMP or MP65 at different concentrations (from 10^{-4} to 10 μ g/mL), and radionuclide (0.5 μ Ci of ^{3} H-TdR per well) uptake was measured by scintillation counting (Amersham) after pulsing for 16 h.

Generation of short-term T cell lines and T cell clones from PBMC. M-sMP-specific short-term T cell lines and T cell clones were generated by a technique described in detail elsewhere [16, 17]. In brief, for cloning, T cell blasts from M-sMP-specific T cell lines were seeded under limiting-dilution conditions (0.3 cell/well) in round-bottomed microwell plates (Nunc) containing 10^5 irradiated (Gy 60) allogeneic PBMC as feeder cells, 1% phytohemagglutinin (vol/vol), and 20 U/mL recombinant IL-2 (rIL-2), as reported elsewhere [10]. Growing microcultures were then expanded at weekly intervals with rIL-2 (20 U/mL) and 10^5 irradiated (Gy 60) feeder cells.

T cell clones were generated from surgical vaginal specimens, as described elsewhere [16, 17]. To increase the activated T cell count in the tissue, biopsy specimens were cultured in complete medium and rIL-2 (20 U/mL) for 7 days. Tissue specimens were then disrupted by repeated pipetting, and viable T cell blasts were resuspended in complete medium and counted. T cell blasts were cloned under limiting-dilution conditions (0.3 cells/well) in the presence of irradiated (60 Gy) allogeneic PBMC as feeder cells (10^5 cells/well).

The specificity of short-term T cell lines and clones generated from PBMC and vaginal specimens was assessed as described elsewhere [16]. In brief, 5×10^4 T cell blasts were incubated in triplicate for 48 h in a 0.2-mL volume with 5×10^4 irradiated (60 Gy) autologous PBMC (as antigen-presenting cells [APCs]) in the presence of the appropriate antigen (10 μ g/mL M-sMP or 500 U/mL SK). After a 16-h pulse with 0.5 μ Ci ^{3} H-TdR (Amersham), cultures were harvested, and radionuclide uptake was measured by scintillation counting. T cell lines and clones were considered to be specific when the mitogenic index was ≥ 5 .

Cytofluorimetric analysis of cell surface antigens. To evaluate the percentages of T cell subsets in *C. albicans*-specific T cell lines or clones, 2×10^5 cells were collected and stained with anti-CD3, anti-CD4, anti-CD8, anti-CD14, or anti-CD20 fluorochrome-conjugated MAbs (Becton-Dickinson). Cells were analyzed on a FACScalibur cytofluorimeter, using the CellQuest software (Becton Dickinson). The area of positivity was determined by use of an isotype-matched MAb. In all cytofluorimetric analyses, a total of 10^4 events gated for each sample was acquired.

Quantitation of cytokine content in PBMC and T cell clone supernatants. The ability of *C. albicans*-specific T cell clones to produce cytokines was evaluated after stimulation of viable T cell blasts (10^6 /mL) for 36 h with PMA (20 ng/mL) plus anti-CD3 MAb (10 ng/mL) or with *C. albicans* antigen (1 μ g/mL) in the presence of irradiated autologous PBMC as APCs. In some experiments, 5-day culture supernatants from PBMC stimulated with *C. albicans* antigens were also assayed for cytokine content. For the quantitation of IL-2 in supernatants, an immunoassay (Quantikine; R&D Systems) was used according to the manufacturer's instructions [16]. IL-4, IL-5, and interferon (IFN)- γ were quantified, as described elsewhere [16], by use of a capture ELISA that was developed in-house. Cytokine levels 5 SD higher than those of the control supernatant (derived from irradiated feeder cells alone) were considered to be positive.

Statistical analysis. The *t* and χ^2 tests were used for statistical analyses. $P < .05$ was considered to be significant.

Results

High proliferation of circulating T cells from women with RCV in response to *C. albicans* antigens. PBMC from 13 women with RVC and 12 age-matched healthy control women were stimulated for 5 days in the presence of M-sMP or SK, a control antigen. The proliferative response to M-sMP (0.1–10 $\mu\text{g/mL}$) was significantly higher in PBMC from patients with RVC than in PBMC from healthy subjects; however, no difference was noted between the 2 groups in their response to SK (figure 1). In addition, PBMC from 4 patients with RVC showed a higher proliferative response to purified MP65 than PBMC from 6 healthy control women (data not shown).

To exclude the prospect that the T cell response to M-sMP and MP65 could result from contamination of molecules by superantigenic activity, purified T cells from 3 healthy subjects were cocultured in the presence of fresh or paraformaldehyde-pretreated M-sMP- or MP65-pulsed autologous APCs. Since T cells could proliferate only in response to fresh antigen-pulsed APCs, this suggests the processing requirement for both M-sMP and MP65 and excludes the possibility that the response resulted from contaminating superantigens (data not shown). Thus, the higher proliferative response to *C. albicans* antigens in PBMC from patients with RVC can reflect either an increased proportion of *C. albicans*-specific memory T cells in these patients or the production by these cells of different concentrations of cytokines promoting T cell growth.

Circulating *C. albicans*-specific T cells in patients with RVC exhibit a prevalent Th1-like profile of cytokine production. To evaluate the cytokine profile of *C. albicans*-specific T cells, 5-day culture supernatants were obtained, using PBMC from patients with RVC and from healthy donors that had been stimulated with different doses of M-sMP, and the supernatants were assayed for their IL-4 and IFN- γ content. As shown in figure 2, the mean values of IFN- γ production by M-sMP-stimulated PBMC from patients with RVC were similar to those of control supernatants at all M-sMP doses used. In contrast, the mean levels of IL-4 production by PBMC from patients with RVC were significantly lower ($P < .05$) than those produced by PBMC from control donors when low antigen doses (10^{-3} and 10^{-2} $\mu\text{g/mL}$) were used. When the ratios between the mean levels of IFN- γ and IL-4 were considered, the ratios were significantly higher in the PBMC supernatants from patients with RVC than in those from healthy donors ($P < .05$ for 10, 10^{-2} , and 10^{-3} $\mu\text{g/mL}$ M-sMP) (data not shown), suggesting a Th1 predominance among circulating *C. albicans*-specific T cells in this disease.

To support this possibility, short-term M-sMP-specific T cell lines and clones were generated from tissue samples from 3 patients with RVC and 3 healthy women by using a high dose

(10 $\mu\text{g/mL}$) of M-sMP, which was previously shown to elicit strong T cell proliferation both in patients with RVC and in healthy control women (see figure 1). In total, 247 T cell clones were obtained from the T cell lines derived from healthy women, and 341 were derived from T cell lines of patients with RVC. Most T cell clones exhibited the $\text{CD4}^+\text{CD8}^-$ phenotype; the $\text{CD4}^-\text{CD8}^+$ T cell clone phenotype was detected in $<2\%$ of patients with RVC and in 3.7% of control women. The high clonal efficiency (range, 25%–43%) and the comparable mean ratios between CD4^+ and CD8^+ T cells in both short-term T cell lines and T cell clones (data not shown) suggest that no substantial selection in favor of one T cell subset occurred during the cloning procedure.

The specificity of a panel of T cell clones was assessed by measuring the proliferative response of 10^5 T cell blasts to 10 $\mu\text{g/mL}$ M-sMP in the presence of 10^5 autologous irradiated PBMC. The proportion of CD4^+ T cell clones derived from patients with RVC (32/167; 19.2%) that were specific for M-sMP or MP65 antigens was significantly greater ($P < .05$) than that of M-sMP- or MP65-specific T cell clones generated from healthy donors (21/197; 10.7%). All MP65-positive T cell clones were also reactive to M-sMP antigen. The proportion of M-sMP-specific T cell clones reactive to purified MP65 antigen derived from the patients with RVC (9% of clones tested) was similar to that of clones derived from the healthy donors (7.1% of clones tested) (table 1). In all subjects, the proportions of M-sMP-positive/MP65-negative clones were higher than the

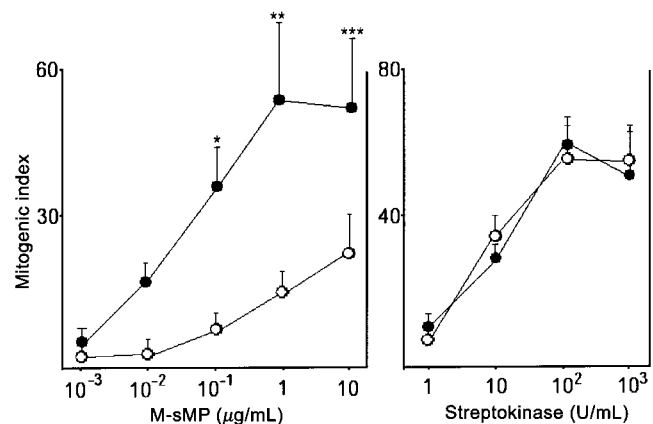


Figure 1. Proliferative response of peripheral blood mononuclear cells from patients with recurrent vaginal candidiasis (RVC) to mycelial cell-secreted mannoprotein (M-sMP) antigen. In total, 2×10^4 cells from 13 patients with RVC (●) and from 12 healthy donors (○) were cocultured for 5 days in complete medium plus 5% autologous serum with increasing doses of M-sMP antigen, as described in Patients, Materials, and Methods. Parallel cultures were done with different doses of streptokinase as control antigen. After a 16-h pulse with ^3H -TdR, cultures were harvested, and radioactivity was counted by liquid scintillation spectroscopy. For each dose, results are presented as mean ($\pm$ SE) mitogenic indexes, as described in Patients, Materials, and Methods. * $P < .005$; ** $P < .001$; *** $P < .0005$.

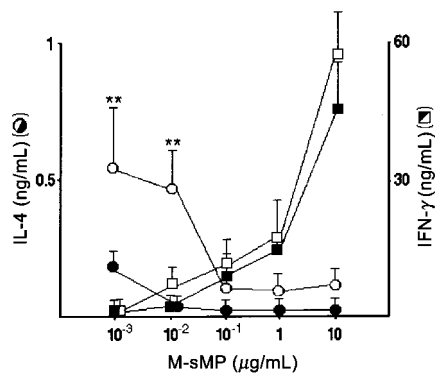


Figure 2. Cytokine production from peripheral blood mononuclear cells (PBMC) of patients with recurrent vaginal candidiasis (RVC) after stimulation with *Candida albicans* antigens. Interleukin (IL)-4 (● and ○) and interferon (IFN)- γ (■ and □) levels were measured in 5-day culture supernatants of PBMC from patients with RVC (●, ■) and from healthy donors (○, □) (the same patients and donors described in figure 1). Results are presented as mean ($\pm$ SE) cytokine levels. * $P < .01$; ** $P < .05$. M-sMP, mycelial cell-secreted mannoprotein.

proportions of MP65-reactive clones; thus, it is reasonable to hypothesize that other *C. albicans* antigens present in M-sMP are also involved in the anti-*C. albicans* T cell response in patients with RVC.

The cytokine profile of M-sMP- and MP65-specific T cell clones was analyzed by measuring the concentrations of IL-4 and IFN- γ in culture supernatants after a 24-h stimulation with PMA plus anti-CD3 MAb or antigens (10 μ g/mL) in the presence of irradiated autologous PBMC. As shown in figure 3A, the mean ($\pm$ SE) levels of IFN- γ production by M-sMP- and MP65-specific T cell clones derived from patients with RVC were significantly higher than those for healthy subjects in response to both polyclonal and antigenic stimulation. The mean levels of IL-4 produced by M-sMP- and MP65-specific T cell clones were significantly lower than those produced by control counterparts after antigenic stimulation, but no difference was seen following polyclonal stimulation. However, when categorized into Th1, Th2, or Th0 cells (according to their ability to produce IFN- γ alone, IL-4 alone, or both, respectively), the proportions of Th2 clones derived from patients with RVC were significantly lower than those of control clones, even after polyclonal stimulation (figure 3B).

Some randomly selected *C. albicans*-specific T cell clones from patients with RVC and control women also were assessed for the production of the cytokines IL-5 and IL-2, which are preferentially associated with the Th2 and Th1 profiles, respectively. Although the levels of IL-5 production by 37 M-sMP- and MP65-specific T cell clones from patients with RVC and 21 from control women were comparable, the mean levels of IL-2 produced by the same T cell clones derived from patients with RVC were significantly higher than those of control clones (3.0 ± 0.2 ng/mL vs. 2.1 ± 0.2 ng/mL; $P < .01$). This finding fur-

ther supports the Th1 predominance of *C. albicans* antigen-specific T cells in patients with RVC and can also account, at least in part, for the strong proliferative response of their T cells to *C. albicans* antigens.

C. albicans-specific T cells derived from the vaginal mucosa of a patient with RVC produced high levels of IFN- γ but little or no IL-4. The cytokine profile of *C. albicans* antigen-specific T cells infiltrating the vaginal mucosa of 2 patients with uterine prolaxus, one of them displaying RCV, was also assessed. To do this, infiltrating T cells were derived by coculturing rIL-2 for 7 days with biopsy specimens obtained from vaginal mucosa and then cloning the T cells under limiting-dilution conditions. *C. albicans* antigen-specific T cell lines and clones derived from PBMC from the 2 women were used as controls. On the whole, 229 CD4⁺ T cell clones were derived from vaginal mucosa (157 from the patient with RVC and 72 from the control subject), and 134 CD4⁺ T cell clones were derived from the corresponding PBMC (60 from the RVC patient and 74 from the control subject). The numbers of T cell clones specific for *C. albicans* antigens derived from vaginal mucosa or PBMC are summarized in table 2. The proportion of *C. albicans* antigen-specific clones derived from vaginal mucosa of the patient with RCV was significantly greater ($P < .01$) than that derived from the control subject. In contrast, no significantly different T cell clones were derived from the PBMC of the 2 women. After stimulation with antigen or PMA plus anti-CD3, the mucosal CD4⁺ T cell clones from the patient with RVC had significantly higher levels ($P < .01$) of IFN- γ and significantly lower levels ($P < .05$) of IL-4 than the corresponding clones from mucosa of the control woman (figure 4A). Accordingly, when vaginal mucosa-derived *C. albicans* anti-

Table 1. Specificity of T cell clones generated from mycelial cell-secreted mannoprotein (M-sMP)-specific T cell lines derived from peripheral blood mononuclear cells (PBMC) of patients with recurrent vaginal candidiasis (RVC).

Source, subject	Antigenic specificity of T cell clones ^a	
	M-sMP ⁺ , MP65 ⁻	M-sMP ⁺ , MP65 ⁺
Healthy subjects		
1	7/60 (12)	5/60 (8)
2	6/50 (12)	3/50 (6)
3	8/87 (9)	6/87 (7)
Total	21/197 (10.7) ^b	14/197 (7.1) ^c
Subjects with RVC		
1	19/64 (30)	5/64 (8)
2	6/36 (17)	6/36 (17)
3	7/67 (10)	4/67 (6)
Total	32/167 (19.2) ^b	15/167 (9) ^c

^a Data are no. specific/no. tested (% specific). In total, 10⁴ T cell blasts were cocultured with 10⁴ irradiated (60 Gy) autologous PBMC and 10 μ g/mL M-sMP for 3 days, as described in Patients, Materials, and Methods. After a 16-h pulse with ³H-TdR, radioactivity was counted. T cell clones were considered to be positive (+) when the mitogenic index was ≥ 5 and negative (−) when the mitogenic index was < 5 . MP65, mannoprotein 65.

^b $P < .025$ for total for healthy subjects vs. total for subjects with RVC.

^c P was nonsignificant for total for healthy subjects vs. total for subjects with RVC.

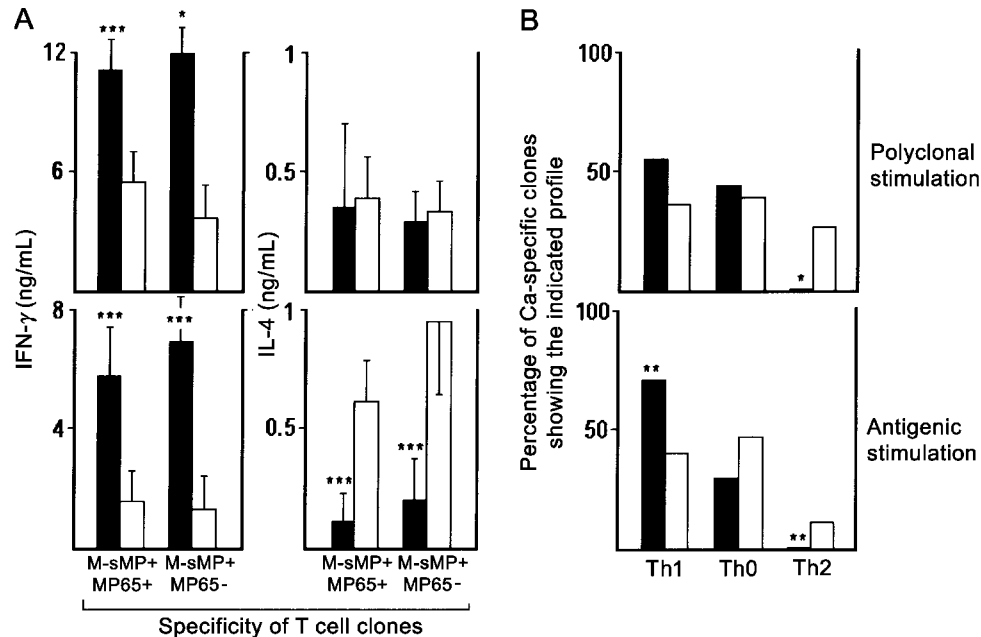


Figure 3. Cytokine production of CD4⁺ T cell clones specific for *Candida albicans* antigens (Ca) derived from patients with recurrent vaginal candidiasis (RVC). T cell clones were generated from *C. albicans*-specific T cell lines generated from 3 patients with RVC (■) and 3 healthy donors (□) and were evaluated for phenotype and specificity, as described in Patients, Materials, and Methods. Interleukin (IL)-4 and interferon (IFN)- γ levels were evaluated in the 36-h supernatants obtained after stimulation, in the presence of autologous irradiated peripheral blood mononuclear cells, with PMA plus 100 μ g/mL anti-CD3 monoclonal antibody or with 10 μ g/mL mycelial cell-secreted mannoprotein (M-sMP) antigen and mannoprotein (MP) 65, as described in Patients, Materials, and Methods. *A*, Mean ($\pm$ SE) levels of cytokine production induced by MP65-positive (+) and MP65-negative (-) *C. albicans*-specific T cell clones. *B*, Percentage of *C. albicans*-specific T cell clones categorized as Th1 (production of IFN- γ), Th2 (production of IL-4), or Th0 (production of both IFN- γ and IL-4). * $P < .01$; ** $P < .025$; *** $P < .05$.

gen-specific T cell clones were categorized as Th1, Th2, or Th0 cells, a clear-cut polarization toward the Th1 phenotype was observed in clones from the patient with RCV, compared with those from the control woman ($P < .01$; figure 4B).

Discussion

CMI has been considered to play an essential role in anti-*Candida* defense of the mucosa. On that basis, it was hypothesized that patients with RVC have a defect in anticandidal CMI. One study reported that the T cell proliferative response toward undefined *C. albicans* extracts was significantly reduced in patients with RVC and was associated with a decrease in IFN- γ [17]. Another reported that the T cell proliferative response was completely abrogated in patients with chronic vaginal candidiasis [18], whereas yet another observed that T cell proliferation was higher in RVC subjects and that patients with chronic vaginal candidiasis had a minimal proliferative response [19].

Another important observation of the current study was that most *C. albicans* antigen-specific T cells in PBMC from patients with RVC (compared with those from control women) showed a prevalent Th1 profile, which was indicated by the high level of IFN- γ induced by either polyclonal or antigenic stimulation

and by the reduced production of IL-4 after stimulation with antigen alone. This finding is consistent with those obtained in an earlier study of murine vaginal candidiasis [13].

Despite the few data, owing to the difficulty in obtaining vaginal specimens and in analyzing their infiltrating cells under sterile conditions, the results of this study provide, to our knowledge, the first direct demonstration of the existence of human vaginal T cells specific for *C. albicans* antigens. In accord with this, *C. albicans* antigen-specific vaginal T cells were found in immunized rats and in nonimmunized rats into which vaginal Th1 cells from immunized rats were adoptively transferred (authors' unpublished data). *C. albicans* antigen-specific vaginal T cells from a patient with RVC produced levels of IFN- γ significantly higher than those of the control clones, and they were virtually unable to produce IL-4 after polyclonal or antigenic stimulation. This strongly suggests that the human T cell response to *C. albicans* antigens is strongly Th1 polarized, even at the vaginal level, a finding that is consistent with results published elsewhere [15] showing that intravaginal challenge with *C. albicans* antigens in patients with RVC leads to increased production of Th1 cytokines in the vaginal fluids. Furthermore, in rat vaginal candidiasis, IL-12 was found in the vaginal fluids after the first infection, and there was an increase in IL-2 and IFN- γ levels after subsequent infections [20].

Table 2. Specificity for *Candida albicans* antigens of T cell clones derived from vaginal mucosa and peripheral blood mononuclear cells (PBMC) of patients with recurrent vaginal candidiasis (RVC).

Source	Antigenic specificity of T cell clones ^a		
	M-sMP ⁺ , MP65 ⁻	M-sMP ⁺ , MP65 ⁺	Total
Healthy subjects			
PBMC	3/74 (4)	1/74 (1)	4/74 (5.4) ^b
Vaginal mucosa	3/72 (4) ^b	4/72 (6) ^c	7/74 (9.5) ^d
Subjects with RVC			
PBMC	6/60 (10)	5/60 (8)	11/60 (18.3) ^b
Vaginal mucosa	20/157 (13) ^b	17/157 (11) ^c	37/157 (23.6) ^d

^a Data are no. specific/no. tested (% specific). In total, 10⁴ T cell blasts were cocultured with 10⁴ irradiated (60 Gy) autologous PBMC and 10 µg/mL M-sMP for 3 days, as described in Patients, Materials, and Methods. After a 16-h pulse with ³H-TdR, radioactivity was counted. T cell clones were considered to be positive (+) when the mitogenic index was ≥5 and negative (−) when the mitogenic index was <5. MP65, mannoprotein 65; M-sMP, mycelial cell-secreted mannoprotein.

^b *P* < .05 for healthy subjects vs. subjects with RVC.

^c *P* was nonsignificant for healthy subjects vs. subjects with RVC.

^d *P* < .025 for healthy subjects vs. subjects with RVC.

Remaining to be answered are the questions of what induces amplification of Th1-specific responses in patients with RVC and whether this is a primary or secondary event in the pathogenesis of the disease. It was demonstrated recently that the mycelial (but not yeast) form of *C. albicans* induces a strong APC stimulation with production of proinflammatory cytokines (namely, IL-12 and IFN-α), which are essential to skew the specific T cell response toward the Th1 profile [21]. Whether the disease is related to overexpression of such cytokines by vaginal macrophages or APCs of draining lymph nodes or to an impairment of the production of regulatory cytokines (IL-10 and transforming growth factor [TGF]-β1) by the same cells has not been established. An increased expression of TGF-β1 in vaginal mucosa and in draining lymph nodes that was associated with a lack of demonstrable vaginal CMI was reported in a study of murine vaginal candidiasis [22]. By contrast, the experiments reported here show the presence of greater proportions and similar or increased reactivity of MP-specific vaginal T cells in comparison with their circulating counterparts, thus suggesting an excessive cell-mediated vaginal immune response. This is of relevance, considering the well-known predominance of mycelial forms of *C. albicans* in clinical and experimental vaginitis.

There is consensus that T helper cells in rodents play a central role in regulating the immune response to *C. albicans* antigens. Protection usually correlates with the occurrence of Th1 responses, whereas Th2 responses are associated with disease exacerbation and pathology [7, 10]. Thus, it has been suggested that the local anti-*C. albicans* antigen T cell response in experimental vaginal candidiasis (EVC) could be responsible for recurrent and chronic infection, whereas the increased numbers of systemic anti-*C. albicans* antigen Th1 cells may control dissemination of the pathogen [23]. Results obtained from an es-

trogen-dependent model of EVC suggest that local CMI is more important than systemic CMI for protection against vaginitis [23]. On the other hand, other experimental studies argue against the possibility that CMI is the predominant host defense mechanism against vaginal *C. albicans* infections [24]. Neither predisposing factors nor local immunologic abnormalities have been found to account for the high prevalence of vaginitis in most women with idiopathic RVC [23]. Moreover, in carefully performed case-control studies, the incidence of symptomatic vaginal candidiasis in HIV-positive women was not different from the frequency of disease in HIV-negative subjects at risk for HIV infection [25]. By contrast, a genetic predisposition to protection from EVC after peripheral immunization has been observed throughout mouse strains. Protection against EVC could be transferred to nonimmunized resistant mice by

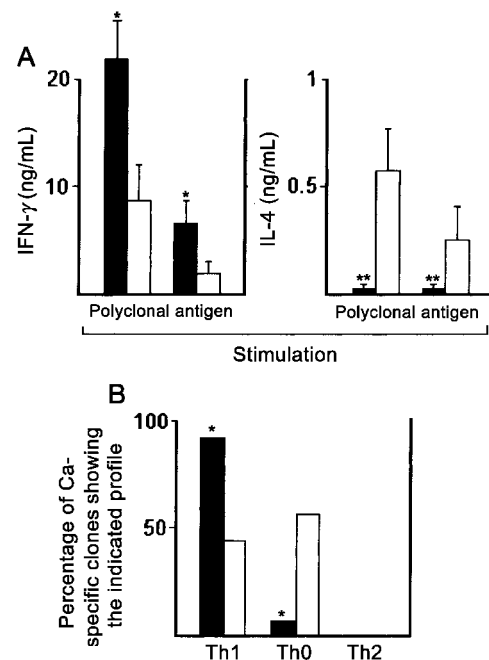


Figure 4. Cytokine production from CD4⁺ T cell clones specific for *Candida albicans* antigen (Ca) derived from a vaginal biopsy sample from a patient with recurrent vaginal candidiasis (RVC). Surgical specimens from 1 patient with RVC (■) and 1 control subject (□) were cultured for 7 days in medium plus recombinant interleukin (IL)-2, and T cell blasts were subsequently cloned under limiting-dilution conditions, as described in Patients, Materials, and Methods. A, Mean (±SE) levels of interferon (IFN)-γ and IL-4 production by *C. albicans*-specific T cell clones derived from vaginal lesions after polyclonal stimulation (PMA plus anti-CD3 monoclonal antibody) or stimulation with 10 µg/mL mycelial cell-secreted mannoprotein (M-sMP) antigen, in the presence of autologous irradiated peripheral blood mononuclear cells (as antigen-presenting cells). B, Percentages of *C. albicans*-specific T cell clones, derived from vaginal lesions, after polyclonal stimulus, categorized as Th1 (production of IFN-γ), Th2 (production of IL-4), or Th0 (production of both IFN-γ and IL-4). **P* < .01; ***P* < .05.

CD3⁺CD4⁺ T cells from immunized mice [26], and, more important, it was associated with some loci of intracellular adhesion molecule (ICAM)-1, the ICAM-1-related sequence 1, and Fcε-RII), thus suggesting that an impaired binding of T cells to mucosal (vaginal) endothelial cells [27] may be the main cause of susceptibility to RVC. A differential susceptibility to EVC also has been observed in 2 species of nonhuman primates, thus confirming the role of genetic predisposing factors in facilitating *C. albicans* infection [27]. On the basis of all these findings, it is generally accepted that the mechanisms for resistance and susceptibility to *C. albicans* infection in RVC may be multifactorial. Modifications of genetic background, vaginal pH, T cell binding to vaginal endothelial cells, epithelial mucosal integrity, and local specific humoral and cell-mediated immune responses likely play important parts in explaining RVC, but they probably do not fully explain this condition.

The direct demonstration that the anti-*C. albicans* antigen T cell response in vaginal mucosa in patients with RVC is Th1 oriented could yield new insights into the induction and maintenance of RVC. It may, indeed, be hypothesized that *C. albicans* antigens in patients with RVC locally recruit and amplify a vigorous *C. albicans* antigen-specific Th1 response, which is usually associated with a strong cytolytic activity by CD4 and CD8 T cells, macrophages, and NK cells. The excess of lytic activity by vaginal anti-*C. albicans* antigen Th1 cells, not balanced by a local production of Th2-type cytokines, might induce a vicious cycle by modifying the epithelial barrier and facilitating new rounds of infection. Indeed, it has been shown that vaginal epithelioid cells from mice, nonhuman primates, and humans can inhibit the growth of *C. albicans* in vitro [28], thus serving as a crucial local host defense mechanism against *C. albicans* antigens in the absence of an inflammatory, neutrophilic infiltrate. Last, the continuous local stimulation could allow *C. albicans* antigens to pass through vaginal mucosa and amplify memory Th1-like cells specific for *C. albicans* antigens in regional lymph nodes and peripheral blood.

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