CONCISE COMMUNICATION

Growth Inhibition of Candida albicans by Human Vaginal Epithelial Cells

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Vulvovaginal candidiasis (VVC) is a common mucosal infection caused by Candida species in women of childbearing age. Although acute VVC affects a large number of women and is often precipitated by hormonal fluctuations involving high estrogen levels, recurrent VVC (RVVC) affects another 5%-10% of women with no known predisposing factors. We have recently reported that vaginal epithelial cells from nonhuman primates and mice inhibit the growth of Candida albicans in vitro, which may represent an innate host defense mechanism against C. albicans at the vaginal mucosa. In the present study, we show that vaginal epithelial cells collected from healthy women with no history of VVC also exhibit anti-Candida activity, with no differences in activity at various stages of the menstrual cycle. Women diagnosed with RVVC, on the other hand, have reduced epithelial cell anti-Candida activity. These results are further evidence that vaginal epithelial cells provide an innate host resistance mechanism against Candida and that reduced activity may contribute to RVVC.

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Vulvovaginal candidiasis (VVC) is a common opportunistic fungal infection caused by Candida species that affects ~75% of otherwise healthy women of childbearing age in the United States [1]. Several factors predispose women to acute VVC, most of which involve elevated hormone levels (e.g., pregnancy, the luteal phase of the menstrual cycle, and use of oral contraceptives or hormone replacement therapy) or antibiotic use. Another 5%-10% of women are seemingly healthy and suffer from recurrent hormone replacement therapy) or antibiotic use. Another 5%-10% of women are seemingly healthy and suffer from recurrent VVC (RVVC) affects another 5%-10% of women without any known predisposing factors. We have recently reported that vaginal epithelial cells from nonhuman primates and mice inhibit the growth of Candida albicans in vitro, which may represent an innate host defense mechanism against C. albicans at the vaginal mucosa. In the present study, we show that vaginal epithelial cells collected from healthy women with no history of VVC also exhibit anti-Candida activity, with no differences in activity at various stages of the menstrual cycle. Women diagnosed with RVVC, on the other hand, have reduced epithelial cell anti-Candida activity. These results are further evidence that vaginal epithelial cells provide an innate host resistance mechanism against Candida and that reduced activity may contribute to RVVC.

Although cell-mediated immunity (CMI) by Th1-type T cells is considered to be the predominant host defense mechanism against mucosal Candida albicans infections, the role of CMI with regard to Candida at the vaginal mucosa has recently been challenged. First, several clinical studies have demonstrated that most women experience RVVC despite having normal levels of Candida-specific Th1-type CMI in the peripheral circulation (reviewed in [2]). Second, experimental studies that have used an estrogen-dependent murine model of vaginal candidiasis have paralleled the clinical findings [2]. Furthermore, our most recent data suggest that some form of immunoregulation may inhibit a more profound CMI response of systemic or local origin against vaginal candidiasis [3, 4]. Humoral immunity against vaginal candidiasis is equally controversial. Experimental studies in mice have provided some evidence for protection, using Candida-specific antibodies [5], but, clinically, women with RVVC have normal or elevated levels of Candida-specific antibodies [6]. With regard to innate resistance, although polymorphonuclear leukocytes (PMNL) have significant killing activity against Candida in vitro (reviewed in [7]), PMNL do not affect Candida at the murine vaginal mucosa [8]. Although macrophages that also have significant killing activity against Candida in vitro [7] have not been investigated functionally at the vaginal mucosa, NK cells have been shown to have little to no anti-Candida activity in vivo or in vitro against Candida [9]. In contrast, we have recently shown that primary vaginal epithelial cells from mice and nonhuman primates, as well as human oral epithelial cells and epithelial cell lines, have the ability to inhibit the growth of Candida in vitro [10, 11]. Given that epithelial cells are the host cells with the most contact with C. albicans at mucosal tissues, we have postulated that epithelial cells may represent an important local anti-Candida host defense mechanism. Epithelial cell-mediated anti-Candida activity, as it is currently understood, involves cell contact between a carbohydrate moiety on the epithelial cells and Candida, with no role for soluble factors, phagocytosis, or oxidative killing [12].
Significant data on vaginal epithelial cell–mediated anti-
Candida activity are available; however, a survey of such activity in
adult women has not been conducted. The purpose of the present
study was to evaluate the anti-Candida activity of epithelial cells
collected from the human vaginal mucosa in a large population
of women at different stages of the menstrual cycle, as well as
from women with a history of RVVC.

Subjects and Methods

Subjects. Eighty-two healthy, nonpregnant women without a
history of VVC and 10 women with RVVC in remission were
recruited for this study. Among the 82 women who did not have
RVVC, epithelial cells obtained from 29 women during the follicular
phase of the menstrual cycle, from 14 during the ovulatory
phase, and from 39 during the luteal phase were tested. Epithelial
cells obtained from 11 of these women were tested at all 3 stages of
the menstrual cycle over the course of a 2-year period. Venous
blood was collected from all women, and serum samples were used to
confirm the follicular and luteal stages of the menstrual cycle via
progesterone concentrations measured at Wayne State University
Clinical Endocrinology Laboratory (Detroit) by use of competitive
enzyme immunoassays. The ovulatory stage was determined by a
urine-based luteinizing hormone (LH) detection kit (OvuQuick;
Quidel Pharmaceuticals) that identifies the 24-h period of
ovulation. Day of cycle from the start of menses and local cellular
maturation indices from vaginal swabs examined by Papanicolaou
stain were also used to confirm the stage of the cycle. Of the women
who participated in the study, 37% were white and 63% were
black. The median age of all women was 40 years (range, 20–51
years). All women were confirmed to be free of vaginitis by vaginal
swab culture on Sabouraud dextrose agar (Becton-Dickinson), had
negative results of testing with potassium hydroxide stain of a
vaginal smear, and lacked common symptoms of vaginitis. All
yeast colonies from asymptomatic colonized women were tested
to confirm the absence of pathogenic yeast. The enriched population
was resuspended in phytone-peptone (PP) medium (Becton-Dickinson)
for germ-tube formation and/or speciated by use of the API 20
AUX identification system (bioMérieux).

A diagnosis of RVVC was made on the basis of a history of ≥3
episodes of VVC during the course of 1 year. Women with RVVC
were asymptomatic at the time of testing. Eighty percent of these
women were receiving fluconazole maintenance therapy at the
time of testing. Serum progesterone levels and vaginal cellular
maturation indices confirmed the follicular and luteal stages of
the menstrual cycle at the time of testing (table 1). Ovulation
was confirmed by LH surge. Vaginal swab cultures indicated
14%–21% detectable asymptomatic colonization at various
stages of the menstrual cycle; the majority of colonized women
were colonized with C. albicans (≥75%) (table 1). None of
the women with RVVC had detectable yeast colonization.

Results

To further investigate the anti-Candida activity of vaginal epider-
phelial cells, we tested the growth-inhibition potential of primary
vaginal epithelial cells collected from a large number of women
at different stages of the menstrual cycle, as well as from a
small subset of patients with RVVC who were asymptomatic at
the time of testing. Serum progesterone levels and vaginal cellular
maturation indices confirmed the follicular and luteal stages of
the menstrual cycle at the time of testing (table 1). Ovulation
was confirmed by LH surge. Vaginal swab cultures indicated
14%–21% detectable asymptomatic colonization at various
stages of the menstrual cycle; the majority of colonized women
were colonized with C. albicans (≥75%) (table 1). None of
the women with RVVC had detectable yeast colonization.

The results illustrated in figure 1 show that epithelial cells
collected from women without a history of VVC at each phase of the
menstrual cycle, as well as those from patients with RVVC, were
capable of inhibiting the growth of C. albicans in vitro. At high
E:T ratios, vaginal epithelial cells from each stage of the men-
strual cycle inhibited the growth of C. albicans at moderate
levels (19%–29%). Cells from the ovulatory phase had the high-
est activity (27%–40%). In each case, a dose response was
observed as E:T ratios decreased. Generally, no significant
differences were seen in the activity of cells at each of the 3

Human vaginal epithelial cell line. A human vaginal epithelial
cell line (VK2; R. Fichorova, Harvard Medical School, Boston)
was used [13]. The VK2 cell line, immortalized with human papillo-
mavirus 16E6E7, was maintained in keratinocyte serum-free med-
ium (Gibco) supplemented with 50 μg/mL bovine pituitary extract,
0.1 ng/mL epidermal growth factor, 100 U/mL penicillin, and 100
mg/mL streptomycin and passaged every 3–4 days.

Growth inhibition assay. An established modified [3H]glucose
uptake assay was conducted [11]. In brief, C. albicans isolate strain
3153A, grown to stationary phase, was added to individual wells of
a microtiter plate at 1 × 10⁵ cells/mL in a volume of 100 μL of
PP-FBS medium. Epithelioid-enriched cells were then added in trip-
llicate to a microtiter plate in a volume of 100 μL of PP-FBS at vari-
ous ratios of effector to target cells (E:T ratio) based on viable cell
numbers, beginning at 40:1 and serially diluted to 1.25:1. The cul-
ture was incubated at 37°C in 5% CO₂ for 9 h in the presence of
1 μCi [3H]glucose (ICN Pharmaceuticals). After incubation, 100
μL of sodium perchloride (bleach) was added to each well for 5
min, the cell extracts were harvested, and the incorporated [3H]glu-
cose was measured by liquid scintillation (Beckman Instruments).

Uptake of glucose by C. albicans and effector cells cultured alone
(positive and negative controls, respectively) during the 9-h incubation
was generally 15,000–30,000 and 200–1000 cpm, respec-
tively. Percentage of growth inhibition was calculated as follows:
1 − ([mean cpm of epithelial cells and C. albicans − mean cpm of
epithelial cells]/mean cpm of C. albicans) × 100.

Statistical analysis. The unpaired Student’s t test was used to
analyze data. P < .05, calculated using a 2-tailed test, was con-
sidered to be significant.
stages of the menstrual cycle at any E:T ratio. The exception was a significant decrease in activity by cells collected during the follicular phase, compared with those collected during the luteal phase and evaluated at a 10:1 E:T ratio (P = .0345). In contrast, vaginal epithelial cell anti-Candida activity for women with a history of RVVC was significantly reduced, compared with cells collected during the follicular and ovulatory phases of the menstrual cycle evaluated at E:T ratios of 10:1 (P < .0329) and 5:1 (P < .0085) and compared with cells collected during all stages of the menstrual cycle evaluated at E:T ratios of 2.5:1 (P < .0210) and 1.25:1 (P < .0428). The vaginal epithelial cell line exhibited a wider range of Candida inhibition at the same E:T ratios (>60% at 40:1 to <10% at 5:1 and less).

Discussion

Although the role of Candida-specific T cells and antibodies at the vaginal mucosa in adaptive immunity has been challenged, as have the limited roles of PMNL and NK cells against C. albicans in the vagina [8, 11, 14], vaginal epithelial cells from mice and nonhuman primates have recently been shown to inhibit the growth of C. albicans in vitro [10, 11]. This potentially important innate host defense mechanism has

<table>
<thead>
<tr>
<th>Phase of menstrual cycle (no. of subjects)</th>
<th>Colonization, % of subjects</th>
<th>Colonization with Candida albicans, % of subjects</th>
<th>Serum progesterone level, ng/mL ± SEM</th>
<th>Superficial epithelial cells, %</th>
<th>Estimated day of cycle, mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follicular (31)</td>
<td>14</td>
<td>75</td>
<td>0.65 ± 1.4</td>
<td>38</td>
<td>6.6</td>
</tr>
<tr>
<td>Ovulatory (14)</td>
<td>21</td>
<td>100</td>
<td>2.9 ± 0.47</td>
<td>54</td>
<td>14</td>
</tr>
<tr>
<td>Luteal (39)</td>
<td>15</td>
<td>83</td>
<td>8.6 ± 1.22</td>
<td>33</td>
<td>19.8</td>
</tr>
</tbody>
</table>

*Normal ranges for menstrual cycle: follicular, 1–3 ng/mL; ovulatory, 2–3 ng/mL; and luteal, >8 ng/mL.

* Determined by vaginal smear stained with Papanicolaou. Normal ranges for menstrual cycle: follicular, 20%; ovulatory, 70%; and luteal, 60%. “Superficial” is defined as the end-stage differentiation of epithelial cells after exposure to estrogen.

Figure 1. Vaginal epithelial cell–mediated growth inhibition of Candida albicans. Vaginal epithelial cells were collected from healthy women at different stages of the menstrual cycle and from women who had recurrent vulvovaginal candidiasis (RVVC) but were asymptomatic at the time of collection. A vaginal epithelial cell line (VK2) was also used. In all cases, the cells were evaluated for anti-Candida activity by a [3H]glucose uptake assay. Results are expressed as percentage of inhibition by epithelial cells of total glucose uptake by C. albicans at various ratios of effector to target cells (E:T). The mean ± SEM for individual tests and for 3 repeated tests of the vaginal cell line are shown. φ, P < .05, compared with data from all stages of the menstrual cycle; ψ, P < .05, compared with data from follicular and ovulatory stages; τ, P < .05, data from follicular stage compared with data from luteal stage.
also been reported for human oral epithelial cells and epithelial cell lines but not for lymphoid cell lines [15]. The present study shows that human vaginal epithelial cells collected at various stages of the menstrual cycle from women without RVVC and a vaginal epithelial cell line also inhibit the growth of *C. albicans* in a dose-dependent manner. In fact, compared with anti-*Candida* activity by epithelial cells from the vaginal mucosa of mice and nonhuman primates [10, 11], primary and immortalized human vaginal epithelial cells have similar levels of activity at all E:T ratios. In contrast, human, macaque, and mouse vaginal epithelial cell–mediated anti-*Candida* activity is considerably less than that the activity reported for human oral epithelial cells and may, in fact, be considered to be nonexistent in some women [15]. If epithelial cells do indeed represent an innate host defense mechanism, the lesser activity of vaginal epithelial cells, in comparison with that of oral epithelial cells, may account for the increased frequency of VVC, vaginal epithelial cells, in comparison with that of oral epithelial cell lines but not for lymphoid cell lines [15].

The mechanism of the epithelial cell–mediated anti-*Candida* activity currently is not fully understood. Murine vaginal and human oral epithelial cells function by cell contact; soluble factors have no role, and phagocytosis and surface phospholipids and proteins are not involved. On the other hand, the activity is abrogated by periodic acid, which suggests that surface carbohydrates have a role [12]. It is expected that human vaginal epithelial cells function in a similar manner.

Vaginal epithelial cells from women with RVVC who were in remission at the time of testing displayed anti-*Candida* activity similar to that of cells from women with no history of RVVC at high E:T ratios. However, at lower E:T ratios, epithelial cells from women with RVVC had significantly less activity than did cells from women without RVVC, regardless of the stage of the menstrual cycle at which cells were collected. These data may indicate a dysfunction in epithelial cells in women with RVVC, at least when *Candida* and epithelial cells approach equal numbers. We recognize that the activity is fairly weak and that the differences observed reflect the lower inhibition ranges. Nevertheless, if epithelial cells represent an innate vaginal host defense mechanism, weaker activity in women with RVVC may promote quicker overgrowth of *Candida* and contribute to susceptibility to infection. Testing of larger numbers of women with RVVC is needed to confirm this hypothesis. Testing of women with RVVC while they are asymptomatic would also be of interest. However, infectious levels of *Candida* associated with epithelial cells interfere with the epithelial cell controls necessary for the growth inhibition assay.

In conclusion, this is the first evidence that human vaginal epithelial cells can inhibit the growth of *C. albicans*. The anti-*Candida* activity of these cells, although detectable, is fairly weak, compared with that of oral epithelial cells. We hypothesize that, in the absence of a significant adaptive response against *C. albicans*, vaginal epithelial cells provide an innate host resistance mechanism to control commensalism but can be easily overwhelmed, resulting in frequent episodes of VVC. Furthermore, reduced levels of epithelial cell anti-*Candida* activity in women with RVVC may contribute to recurrent episodes.

References


