Differential Susceptibility of Two Species of Macaques to Experimental Vaginal Candidiasis

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Vulvovaginal candidiasis (VVC) caused by Candida albicans is a significant problem in women of childbearing age. Unfortunately, protective host defense mechanisms against VVC are poorly understood. Although rodent models of experimental vaginal candidiasis have been useful, several differences from humans limit the correlation of experimental data. The purpose of the present study was to examine two species of macaques as an alternative model of experimental vaginitis. Screening of pig-tailed and rhesus macaques demonstrated that each had mucosal Candida colonization and prior immune sensitization to C. albicans. Vaginal-associated immunity (cytokines, antibodies, and innate resistance) was also detected in cervicovaginal lavage fluid from both species. Nevertheless, intravaginal inoculation of C. albicans into both species, either untreated or under estrogen-treated conditions, resulted in vaginal infection in rhesus, but not pig-tailed, macaques. Several estrogen-dependent changes in the rhesus immune status coincided with susceptibility to infection. Taken together, these results suggest that pig-tailed and rhesus macaques may be useful in studying pathogenesis and immunity associated with C. albicans vaginitis.

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Vulvovaginal candidiasis (VVC) is one of the most common mucosal fungal infections [1]. In fact, in the United States alone, there are approximately 13 million cases of VVC annually, which account for 10 million visits to gynecologists [2]. Candida albicans, a commensal organism of the gastrointestinal and reproductive tracts, is the causative agent in 85%–90% of all VVC cases [3–5]. Up to 5% of women who experience VVC caused by C. albicans will subsequently develop recurrent VVC (RVVC) [4, 6, 7]. Antifungal therapy, while highly effective for individual attacks of RVVC, does not prevent recurrence [1, 3]. Unlike VVC, which is often precipitated by exogenous factors, including pregnancy, oral contraceptive or antibiotic usage, uncontrolled diabetes mellitus, and hormone replacement therapy [1], RVVC is idiopathic [8].

From early exposure to C. albicans, most healthy individuals have detectable Candida-specific immunity (i.e., positive delayed skin test reactivity and peripheral blood lymphocyte [PBL] responses to C. albicans antigens). Reductions in this immunity are postulated to increase susceptibility to mucosal infection. Indeed, mucosal C. albicans infections occur frequently in patients with reduced cell-mediated immunity (CMI), such as organ transplant recipients [9], patients treated with corticosteroids [10], and patients with AIDS [11, 12]. Experimentally, T helper (Th) 1–type CMI responses characterized by interleukin (IL)–2, interferon (IFN)–γ, and IL-12 production in response to C. albicans have been shown to be associated with resistance to mucosal candidiasis, whereas Th2-type responses (IL-4, IL-5, and IL-10) are associated with susceptibility to infection [13, 14]. Therefore, it has been postulated that deficiencies in CMI also play a significant role in the etiology of RVVC.

To date, studies to examine host defense mechanisms against C. albicans vaginitis have employed both women with RVVC and experimental rodent models [15–23]. Although clinical and animal studies suggest that local, rather than systemic, CMI is more important against C. albicans vaginitis, each has not been without limitations. Clinically, examination of local CMI (i.e., cytokine/antibody production) in cervicovaginal lavage fluid of normal healthy women, as well as those with RVVC, has been difficult to interpret because of several unavoidable problems. These include dynamics of infection (women presenting at varying times during infection), lack of known influences of C. albicans on the cytokines/antibodies present, and influence of the menstrual cycle on the immune parameters.

In rodent models, several distinct properties limit correlation to the human disease. Most importantly, in contrast to humans, rodents do not have detectable yeast flora [19, 24], evidence of prior immune sensitization to C. albicans, or an acidic vaginal pH. Nevertheless, they can be manipulated to conduct highly in-depth studies. Accordingly, vaginal T cells in mice have been found to be phenotypically distinct from their systemic coun-
terparts [25] but do not appear to modulate during a vaginal C. albicans infection [26]. In other studies, murine vaginal epithelialoid cells have been observed to inhibit the growth of C. albicans in vitro [27], suggesting that these unconventional immune cells may represent an innate host defense mechanism against C. albicans in the vaginal mucosa.

In a rat model of experimental vaginitis, studies show that Candida-specific IgA antibodies may play a role in protection against vaginitis [22, 23]. However, there is little support for this clinically, as women with RVVC have been shown to have normal or elevated levels of Candida-specific antibodies in vaginal secretions [28]. Furthermore, individuals with B cell deficiencies are not susceptible to mucosal candidiasis [29, 30].

In contrast to rodents, healthy nonhuman primates are known to be colonized with yeast on skin and in the alimentary and reproductive tracts [31, 32]. In fact, numerous studies have shown that nonhuman primates experience Candida species infections from similar predisposing factors as humans, including immunodeficiency [33–35]. Furthermore, rhesus macaques have been shown to have vaginal-associated Langerhans’ cells, macrophages, and CD4+ and CD8+ T cells similar to those of humans [36, 37].

The purpose of this study was to investigate two species of macaques, Macaca nemestrina (pig-tailed) and M. mulatta (rhesus), as an alternate model system to study pathogenesis and host defense against C. albicans vaginitis.

Materials and Methods

Primates. Female M. nemestrina (pig-tailed; 5–15 years old) and M. mulatta (rhesus; 4–13 years old) macaques, 6–8 kg, housed at the Tulane Regional Primate Research Center, Covington, LA (a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care), were used in this study. The animals were singly housed in accordance with The Guide for Care and Use of Laboratory Animals [38] and were fed standard primate diet twice daily and provided water ad libidum.

Specimen collection: anesthesia. Pig-tailed and rhesus macaques were given ketamine HCl (Fort Dodge, Overland Park, KS) intramuscularly at a concentration of 10 mg/kg prior to any specimen collections.

Blood. Blood (10–20 mL) was collected from the femoral vein in heparinized Vacutainer tubes (Becton Dickinson Microbiology Systems, Cockeysville, MD).

Swabs. Oral, rectal, and vaginal transtissal swabs were performed by use of sterile culturette swabs (Precision Dynamics, San Fernando, CA). Sterile specula were used to facilitate vaginal sampling.

Vaginal lavage. Sterile specula were inserted into the vaginal lumen, followed by the administration of 3–4 mL of sterile PBS. The lavage was performed by use of a sterile transfer pipette with constant aspiration for 20–30 s and the fluid collected in a sterile test tube. Processing of lavage fluid included removing a portion for quantitative assessment of microorganism burden followed by processing for soluble and cellular fractions. For the latter, the lavage fluid was centrifuged, and the supernatants were collected, filtered, aliquotted, and stored at −70°C until use in cytokine and immunoglobulin analyses. The lavage fluid cell pellet was resuspended in 1 mL of PBS and stored at −70°C until use in tissue culture assays.

Vaginal pH. Vaginal swabs were performed by use of sterile culturette (Precision Dynamics) as described above. Each swab was then rolled onto pH hydrion paper (Micro Essential Laboratories, Brooklyn, NY) of range 3.0–5.5, and the pH value was recorded after 10–15 s.

Mucosal microflora screening. Oral, rectal, and vaginal swabs were plated on Sabouraud-dextrose agar (SAB; Becton Dickinson) and CHROMagar (CHROMagar Microbiology, Paris) plates. Colonies were enumerated and characterized after 48–72 h incubation at 30°C (SAB) or after 24–48 h incubation at 37°C (CHROMagar). Yeast colonies were confirmed by microscopy on wet-mount slides with heat-killed blastospores65 (for identification of C. albicans) were performed by inoculating a single colony-forming unit (cfu) into 0.5 mL of fetal bovine serum (FBS; Life Technologies Industries, Gaithersburg, MD). After 2 h incubation at 37°C, 7.5% CO2, aliquots were examined by microscopy for germ tube formation. Germ tubes were defined as tubes at least twice the length of the mother cell, with no septae separating the two [39].

Menstrual cycle screening: serum estradiol concentrations. Serum estradiol concentrations were determined by use of a Coat-A-Count 125I radioimmunoassay kit (Diagnostic Products, Los Angeles) according to the manufacturer’s instructions.

Sex skin determination. “Sex skin” is a phrase used to describe the skin that swells around the female genitals, including the buttocks and the rear thighs, in response to estrogen [40]. Sex skins were scored from grades 0 to 5 (most swelling) and recorded.

Immunological screening: PBL proliferation. PBLs were isolated by density gradient centrifugation by use of Ficoll-Paque (Pharmacia Biotechnology, Piscataway, NJ). PBLs were resuspended in serum-free AIM V media (Life Technologies) supplemented with 100 mM glutamine (Life Technologies) and were co-cultured (× 106 cells/mL) in triplicate wells with 20 μg/mL of the mitogen phytohemagglutinin (Sigma, St. Louis), or C. albicans antigens, including 5 × 104 C. albicans heat-killed blastospores (HKb)/mL or 125 μg/mL C. albicans soluble cytoplasmic substances (SCS) at 37°C, 7.5% CO2, for 3 (mitogen) or 6 (antigen) days, respectively. On the final day of culture, 1 μCi 3H-thymidine (ICN, Costa Mesa, CA) was added to each well in a volume of 25 μL, and 6 h later the wells were harvested and the incorporated thymidine counted in a liquid scintillation counter (Beckman Instruments, Irvine, CA). Results were expressed as proliferation indices (PIs) reflecting the mean counts per minute (cpm) of cells cultured with mitogen- or antigen-stimulated cultures compared with the mean cpm for cells cultured in medium alone. Mean PIs from several experimental determinations (groups of 4–5 macaques) were used for statistical analyses.

Cytokine analysis in vaginal lavage fluid. Vaginal lavage fluid was assayed for IL-12, IFN-γ, IL-4, and IL-10 by ELISA by use of commercially available capture and biotinylated detection antibodies (Becton Dickinson). Preliminary studies showed that antibodies against human cytokines cross-reacted with the primate specimens. Standard curves were generated by use of recombinant
human cytokines. The assays were performed in high-protein-binding EIA/A2 96-well tissue culture plates (Costar, Cambridge, MA), as described elsewhere [41]. The absorbance values of each cytokine was determined by use of a Ceres 900 automated microplate reader (Bio-Tek, Winooski, VT) and Kinetic software (Bio-Tek). The total protein of each sample was also determined by the Lowry method (Sigma). Total protein concentrations in vaginal lavage fluid from pig-tailed and rhesus macaques were 0.03–3.6 mg/mL and 0.006–3.0 mg/mL, respectively. Cytokine concentrations were expressed as pg cytokine/mg protein.

Total antibody analysis in vaginal lavage fluid. IgG and IgA antibodies in lavage fluids were quantified by ELISA, as described elsewhere [41], by use of human IgA and human IgG (Sigma) as standards and the concentrations of each expressed as ng antibody/µg protein. Human IgG and IgA were found to be as efficient as primate IgG and IgA at reacting with anti-human IgG and IgA antibodies (data not shown). Candida-specific antibody analysis in vaginal lavage fluid. Candida-specific IgG and IgA antibodies were quantified by ELISA, also as described elsewhere [41], by use of C. albicans culture filtrate antigen [19] to capture Candida-specific antibodies. Because there is no commercially available Candida-specific IgG or IgA, a pooled sample of lavender fluids from patients with RVVC that gave absorbance values at least 2 SD higher than lavender fluid from normal healthy women was used as the standard for all determinations. Standard concentrations were identified by diluting lavender fluid in the ELISA and arbitrarily defining 1000 U/mL as the highest concentration for which a dose response could be achieved. Results were expressed as units of Candida-specific antibody/mg protein.

Growth inhibition assay. The C. albicans growth inhibition assay was performed as described elsewhere for murine cells [27]. Briefly, vaginal epithelial cells were pelleted through density gradient centrifugation and collected. The epithelial cell pellet (consisting of 85%–90% epithelial cells as determined by enumerating 5 separate fields of 100 cells and expressed as the mean percentage of cells having an epithelial morphology) was resuspended in RPMI-1640 medium supplemented with penicillin (100 U/mL), streptomycin (100 µg/mL), L-glutamine (2 mM), 2-mercaptoethanol (5 × 10−5 M), sodium pyruvate (2 mM), HEPES buffer (20 mM), and 10% FBS (complete media [CM]; all from Life Technologies). Stationary-phase C. albicans strain 3153A (National Collection of Pathogenic Fungi, London, UK) kindly provided by D. Soll, University of Iowa, Iowa City) blastoconidia were grown in 750 µL CM. The cultures were incubated for 9 h at 37°C, 7.5% CO2 in the presence of 1 µCi/mL 3H-glucose (ICN). Controls included small wells containing C. albicans at various effector-to-target ratios (E:T) (in a volume of 100 µL CM) and 3H-glucose (ICN). Controls included C. albicans and effector cells cultured in medium alone. Thereafter, the blastoconidia were collected, washed twice, and counted with a hemocytometer, and added to individual wells of a microtiter plate (Costar) at 1 × 105 cells/mL in a volume of 100 µL complete media. The epithelial cells were then added to triplicate wells containing C. albicans at various effector-to-target (E:T) ratios in a volume of 100 µL CM. The cultures were incubated for 9 h at 37°C, 7.5% CO2 in the presence of 1 µCi/mL 3H-glucose (ICN). Controls included C. albicans and effector cells cultured in medium alone. Thereafter, 50 µL of bleach was added to all wells for 5 min, and the cell extracts were harvested onto glass fiber filters. The incorporated glucose was quantified by liquid scintillation. Uptake of glucose by C. albicans and effector cells during the 9-h assay was generally 8000–12,000 and 100–500 cpm, respectively. Percentage of growth inhibition was calculated as follows:

\[
\% \text{ growth inhibition} = 1 - \frac{\text{mean cpm experimental} - \text{mean cpm effector cell}}{\text{mean cpm } C. \text{ albicans}} \times 100.
\]

Vaginal infection (untreated). Anesthetized pig-tailed or rhesus macaques were inoculated intravaginally with stationary-phase C. albicans 3153A (clinical isolate maintained in the laboratory for several years) blastoconidia (5 × 106–1 × 107 total) in a volume of 0.5 mL PBS. In some experiments, a more recent vaginal clinical isolate, DB597.94, kindly provided by J. D. Sobel (Wayne State University School of Medicine, Detroit), was used. Macaques were immobilized and monitored for leakage over a 30-min period. Of the oral yeast isolates identified in both macaque species, 50 µg estradiol valerate (Sigma) dissolved in sesame seed oil subcutaneously in a volume of 0.5 mL. Estrogen was administered twice daily, beginning 7 days prior to vaginal inoculation and continuing through day 14 postinoculation. Serum estradiol measurements and sex skin scores were used to confirm the effects of estrogen. After confirmation, animals were inoculated intravaginally with C. albicans as described above.

Statistical analysis. The unpaired Student’s t test was used to analyze the data. Significant differences were defined at a confidence level where P < .05.

Results

Yeast flora and Candida-specific lymphocyte responsiveness in macaques. To determine whether macaques could serve as an animal model in studying pathogenesis and host responses to experimental C. albicans vaginal infections, we first examined whether pig-tailed and rhesus macaques were colonized by yeast at mucosal sites. Swabs were collected from the oral, rectal, and vaginal mucosa of both species and plated onto selective media for colonization and identification. Sixty percent of pig-tailed and rhesus macaques were culture-positive for yeast species at the rectal mucosa. Candida species identified by CHROMagar included C. albicans, C. tropicalis, and C. krusei. Of the oral yeast isolates identified in both macaque species, 33% were positively identified as C. albicans. Fifty percent and 25% of the rectal isolates from pig-tailed and rhesus macaques, respectively, were identified as C. albicans. In contrast, although the vaginal mucosa had detectable yeast (20% and 60% in pig-
Figure 1. Vaginal-associated cytokines in untreated macaques. Vaginal lavages were collected for untreated pig-tailed (n = 5) and rhesus (n = 5) macaques, and the soluble fraction was assayed for the presence of interleukin (IL)-12, interferon (IFN)-γ, IL-4, and IL-10. The figure shows cumulative results from several experiments for each species (mean ± SE). *, significant differences between groups (P < .05).

Figure 2. Vaginal-associated total and Candida-specific antibodies in untreated macaques. Vaginal lavages were collected for untreated pig-tailed (n = 5) and rhesus (n = 5) macaques, and the soluble fraction was assayed for the presence of total (top) and Candida-specific (bottom) IgG and IgA by ELISA. The figure shows cumulative results for each species (mean ± SE).

Vaginal-associated immunity in untreated macaques. To determine whether untreated pig-tailed and rhesus macaques had vaginal-associated cell-mediated and humoral immunity, vaginal lavage fluid was collected from both species and analyzed for the presence of Th1- (IL-12 and IFN-γ) and Th2- (IL-4 and IL-10) type cytokines and total, as well as Candida-specific, IgG and IgA. The results in figure 1 show that vaginal lavage fluid from both species had detectable cytokines. Th2-type cytokines predominated in both species, with rhesus macaques having significantly higher concentrations of each (IL-12, P < .027; IFN-γ, P < .026; IL-4, P < .013; IL-10, P < .016). Figure 2, top and bottom show that lavage fluid from pig-tailed and rhesus macaques also had detectable amounts of total IgG and IgA (figure 2, top; range, 2–323 ng/μg protein), as well as Candida-specific IgG and IgA (figure 2, bottom; range, 557–19,810 U/mg protein).

To determine whether the epithelial cell–mediated anti-Candida activity observed in cells from the murine vaginal mucosa and human vaginal lavages [27] was present in the vaginal mucosa of primates, we enriched for epithelioid cells from vaginal lavages of both macaque species and examined them for C. albicans growth inhibition. Results in figure 3 show that epithelioid-enriched cells collected from vaginal lavage fluid of both pig-tailed and rhesus macaques inhibited the growth of C. albicans at E : T ratios as low as 2.5 : 1.

Experimental C. albicans vaginal infection in macaques. Initial experiments to determine whether macaques could be infected vaginally with C. albicans sought to establish an infection in untreated animals. For this, pig-tailed and rhesus macaques were inoculated intravaginally with a long-term laboratory-cultivated C. albicans strain, and vaginal fungal burden was quantified in vaginal lavage fluid over a 21-day period (preliminary studies comparing vaginal fungal burden in lavages and swabs showed that longitudinal lavage sampling had minimal effects on relative levels of vaginal fungal burden). The results illustrated in figure 4, top show that a low-grade (10^5–10^7 cfu/mL) infection was established in rhesus, but not pig-tailed, macaques, with an infection rate in the rhesus animals of approximately 50% over the 21-day period. Results did not differ when a more recent clinical isolate was used or when a 2-fold higher inoculum was employed (data not shown). Animals with positive vaginal fungal cultures after 21 days postinoculation...
Vaginal epitelioid cell–mediated anti-\textit{Candida} activity in untreated macaques. Epitelioid-enriched populations were isolated by density gradient centrifugation of the resulting lavage cell pellet and examined for the in vitro growth inhibition of \textit{C. albicans}. The figure shows cumulative results for each species (mean ± SE).

Effects of estrogen-treated and infection on immune reactivity. Because estrogen treatment enhanced susceptibility to infection and has been shown to inhibit immune reactivity [46, 47], we examined whether estrogen treatment altered immune reactivity in pig-tailed and rhesus macaques and what influence, if any, infection had on such reactivity in rhesus macaques. Parameters monitored included PBL responses to \textit{C. albicans} antigens, vaginal cytokine and immunoglobulin concentrations, and epithelioid cell-mediated anti-\textit{Candida} activity. Results in table 1 show that pig-tailed macaque PBL responses to \textit{C. albicans} HKB significantly decreased with 50, but not 30, µg estradiol doses, compared with untreated macaques \(P < .04\). No effects of estradiol were observed in response to \textit{C. albicans} SCS. In rhesus macaques, while no effects were observed for responses to HKB, responses to SCS were reduced in animals beginning stages of anemia, which has been attributed to high levels of estrogen [44, 45].
treated with 30 μg estrogen (P < .027). PBL responses in infected rhesus macaques remained positive and relatively unchanged (data not shown).

Th1/Th2 vaginal cytokine concentrations under estrogenized conditions, compared with untreated macaques, are illustrated in figure 5, top. Although a Th2-type cytokine profile remained evident in estrogen-treated pig-tailed and rhesus macaques, 3 of the 4 cytokines in rhesus macaques were significantly reduced under estrogenized conditions relative to those of untreated animals (dashed lines; IFN-γ, P < .035; IL-4, P < .012; IL-10, P < .016). In contrast, cytokine concentrations in estrogen-treated pig-tailed macaques were not different from their untreated controls. During infection in the rhesus macaques (figure 5, bottom), a further decline in cytokine concentrations was observed throughout the 21-day period, compared with uninfected estrogen-treated macaques.

In contrast to cytokines, vaginal antibodies (total and Candida-specific IgG and IgA) in pig-tailed and rhesus macaques during an estrogenized state and during infection in estrogen-treated rhesus macaques were not different, compared with their untreated counterparts (data not shown).

The in vitro growth inhibition (GI) of C. albicans by vaginal lavage fluid–derived epithelioid cells from both untreated and estrogen-treated pig-tailed and rhesus macaques is illustrated in figure 6. Growth inhibition by vaginal epithelioid cells from estrogen-treated rhesus macaques was significantly reduced, compared with that from untreated animals at all E : T ratios examined (10 : 1, P < .031; 5 : 1, P < .018; 2.5 : 1, P < .027; 1.2 : 1, P < .047). In contrast, epithelioid cells collected from untreated or estrogen-treated pig-tailed macaques had similar levels of C. albicans GI.

Discussion

Results from this study show that macaques appear to be useful in studying pathogenesis and immunity associated with experimental C. albicans vaginitis. First, pig-tailed and rhesus macaques, unlike rodents, are clearly colonized with yeast at mucosal tissues similar to humans. Second, both macaque species had detectable systemic immune responses to C. albicans antigens (PBL proliferation to Candida antigens), suggesting prior Candida-specific exposure and CM1 sensitization similar to those of humans. Third, at least one of the species could be experimentally infected in the vagina with C. albicans. Thus, many of the limitations associated with such studies in rodents appear to be circumvented with the use of macaques.

To date, vaginal immunity in humans has been evaluated by measuring cytokine and antibody concentrations in vaginal secretions [28, 41]. Because of the ability to quantify soluble primate immunomodulators in vaginal lavage fluids by use of human reagents, we were able to compare such immunomodulators between the two primate species. The basal Th2-type profile (IL-4, IL-10 > IL-12, IFN-γ) in pig-tailed and rhesus macaques contrasts a recent observation in normal healthy women that revealed a predominant Th1-type profile (IL-2, IL-12, IFN-γ > IL-4, IL-5, IL-10) in vaginal lavages [41]. In humans, the Th1-type cytokines were speculated to be part of a putative homeostatic immune mechanism in response to normal bacterial and/or fungal flora. Perhaps the Th2-type cytokines

Table 1. Effects of estrogen treatment on peripheral blood lymphocyte proliferation.

<table>
<thead>
<tr>
<th>Species (μg estrogen/bid)</th>
<th>HKB</th>
<th>SCS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig-tailed (none)</td>
<td>24.8 ± 8.1</td>
<td>5.2 ± 2.2</td>
</tr>
<tr>
<td>Pig-tailed (30)</td>
<td>35.2 ± 11.8</td>
<td>11.6 ± 3.9</td>
</tr>
<tr>
<td>Pig-tailed (50)</td>
<td>7.2 ± 6.0</td>
<td>1.6 ± 0.7</td>
</tr>
<tr>
<td>Rhesus (none)</td>
<td>15.5 ± 4.6</td>
<td>3.4 ± 0.7</td>
</tr>
<tr>
<td>Rhesus (30)</td>
<td>17.7 ± 3.4</td>
<td>2.1 ± 0.6</td>
</tr>
</tbody>
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* Mean cpm of cells cultured with antigen-stimulated cultures, compared with the mean cpm for cells cultured in medium alone ± SE. HKB, C. albicans heat-killed blastospores; SCS, C. albicans soluble cytoplasmic substances.

- Figure 5. Effects of estrogen treatment and infection on vaginal cytokine concentrations. Vaginal lavages were performed on pig-tailed (n = 5) and rhesus (n = 5) macaques in an estrogenized state 7–10 days after start of estrogen treatment (top) or in rhesus macaques during an infection (bottom), and the soluble fraction was assayed for the presence of interleukin (IL)-12, interferon-γ, IL-4, and IL-10 by ELISA. Dashed lines, mean concentration in untreated animals. The figure shows cumulative results for each species (mean ± SE).
in the vaginal mucosa of healthy primates also represent a response to bacterial and/or fungal flora. With respect to antibodies, detection of Candida-specific IgG and IgA in vaginal lavages provides additional evidence of prior immune sensitization to C. albicans. Interestingly, macaque vaginal concentrations of total IgG were higher than those of IgA, findings consistent with that recently reported in humans [48]. Finally, vaginal concentrations of Candida-specific IgA were higher than IgG, similar to those of humans [41].

Recently, we reported that vaginal epithelioid cells from mice and humans, as well as purified epithelial cell lines, have the ability to inhibit the growth of C. albicans in vitro [27]. Here, we show that epithelioid cells from both macaque species have a similar ability to inhibit the growth of C. albicans in vitro at equally low E : T ratios. Thus, in primates, like humans and rodents, epithelial cells may represent an innate host defense mechanism against C. albicans at the vaginal mucosa.

When the two species were inoculated intravaginally with C. albicans, we unexpectedly found that rhesus, but not pig-tailed, macaques showed evidence of vaginal infection. Of the two species, our bias was to establish and study a vaginal infection in pig-tailed macaques based on their human-like nonseasonal infection either untreated or after estrogen treatment by use of either laboratory or clinical C. albicans isolates. In contrast, the infection in rhesus macaques was attained under either untreated or estrogen-treated conditions, although clearly the estrogenized condition produced the higher organism titers and better rates of infection. Evidence of infection rather than colonization in rhesus macaques was based on vaginal fungal burden and the presence of hyphae in lavage fluid. Similar to rodents, however, signs and symptoms of vaginitis (i.e. edema, evidence of itching) were not detected, although hair and sex skin properties could have masked such evidence. Whereas some rhesus macaques spontaneously resolved the infection over the 21-day observation period irrespective of the presence or absence of the estrogenized condition, those that had not were treated with an over-the-counter antifungal drug and responded accordingly. This intriguing differential susceptibility to vaginitis in macaques is the first described for vaginal candidiasis. In fact, very different results were observed in the mouse model, where strains of mice that were differentially susceptible to experimental systemic candidiasis were equally susceptible to experimental vaginal candidiasis [49].

The continued resistance of pig-tailed macaques to the experimental vaginal infection under high doses of estrogen was remarkable. First, C. albicans vaginal infections in rodents are easily achieved under a pseudoestrous state, even when maintained at near physiologic levels [50]. Second, humans most often experience VVC when estrogen levels are elevated (i.e., oral contraceptive usage, hormone replacement therapy, pregnancy, and the luteal phase of the menstrual cycle) [1, 8]. This differential susceptibility of pig-tailed and rhesus macaques to experimental C. albicans vaginitis poses an interesting question as to whether a subset of women exists who are resistant to vaginal candidiasis. Indeed, an estimated 25% of women are not expected to experience an episode of C. albicans vaginitis during their lifetime [1].

Although vaginal pH was not found to be a factor in the differential susceptibility of the two species to vaginitis, there are several differences between the two species that may contribute to the resistance of pig-tailed macaques to the vaginal infection. One possibility was the difference in the magnitude of PBL proliferation to Candida antigens as evidence of systemic CMI. Indeed, pig-tailed macaques had significantly higher PBL responses to C. albicans antigens than did rhesus macaques. However, it would appear unlikely that this difference alone could account for the differences in susceptibility to infection, especially in light of the fact that there is little evidence that systemic CMI influences protection against C. albicans vaginal infections [1, 51, 52].

A second possibility was the difference in the levels of Th2-type cytokines between the two species. The low levels of vaginal-associated Th2-type cytokines in pig-tailed versus rhesus macaques is consistent with that associated with resistance to Candida infections [13, 53]. However, the susceptibility of rhesus macaques to infection under estrogenized conditions when Th2-type cytokines were reduced does not support this theory. In fact, despite similar levels of vaginal Th2-type cytokines in both species under estrogenized conditions, the differential susceptibility to infection remained.

Another possible factor is vaginal-associated Candida-specific antibodies. In the rat model of vaginal candidiasis, Candida-specific IgA antibodies have been shown to be protective.
against infection [22], and recently an IgM antibody that protects against systemic candidiasis was shown to protect against vaginitis [54]. Clinical studies, however, have shown no identifiable role for Candida-specific IgG and IgA, as these antibodies are similar in women with and without vaginal candidiasis [55]. In our study, similar to humans, no difference in Candida-specific IgG and IgA was detected in untreated and estrogen-treated pig-tailed and rhesus macaques, suggesting a minimal role, if any, for these antibodies in susceptibility to, or protection against, infection.

Epithelioid cell–mediated anti-Candida activity represents yet another possible factor. In contrast to pig-tailed macaques, which maintained high levels of anti-Candida activity irrespective of the preinoculum condition, vaginal epithelioid cell activity from estrogen-treated rhesus macaques was significantly reduced, compared with their untreated counterparts. Thus, the absence of vaginal epithelioid cell–mediated anti-Candida activity correlated with increased susceptibility to infection. This cause and effect relationship provides the strongest support to mechanism against the tissue or other immune mechanisms. Indeed, as part of the normal vaginal flora in either species.

In summary, although the murine and rat models of experimental Candida vaginitis have been used for years to study pathogenesis, efficacy of antifungal drugs, and host defenses against infection, our results suggest that a primate model of experimental vaginal candidiasis may be more suitable for these investigations. The differential susceptibility of pig-tailed and rhesus macaques provides the added unique opportunity to study the mechanisms responsible for resistance against, and susceptibility to, C. albicans vaginal infection. The results will be expected to have a significant impact on immunotherapy or other strategies to prevent or treat vulvovaginal candidiasis.

References


