Host defense against oropharyngeal and vaginal candidiasis: Site-specific differences

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Summary

Mucosal candidiasis is extremely common in immunocompromised patients. However, the prevalence of site-specific infection (i.e., oropharyngeal, vaginal, and esophageal candidiasis) can be quite variable depending on the immune status of the host. While vulvovaginal candidiasis is common in normal healthy women, oropharyngeal and esophageal candidiasis are more frequently encountered under immunocompromised states. *Candida albicans*, the causative agent in most cases of candidiasis, is a commensal organism of the gastrointestinal and lower female reproductive tracts. Thus, most healthy individuals have demonstrable *Candida*-specific immunity in the peripheral circulation. The pathogenic state is often precipitated by a deficiency or dysfunction in this immunity. Studies from animal models, women with recurrent vulvovaginal candidiasis, and HIV-infected individuals, however, suggest that distinct host defense mechanisms may function against oropharyngeal and vulvovaginal candidiasis. While cell-mediated immunity (CMI) appears important for protection against oropharyngeal candidiasis (OPC), there is little evidence to indicate that T cell-mediated immunity is protective against vulvovaginal candidiasis (VVC). Furthermore, whereas both local and systemically derived immune defenses appear important for protection against OPC, host defenses that protect against VVC appear limited to the local tissue and possibly restricted to innate mechanisms. Thus, current evidence suggests that VVC, unlike OPC, may not represent a strict opportunistic infection.

Key words

Candida albicans, Mucosal candidiasis, Host defense mechanisms, Vaginal candidiasis, Oral candidiasis, Immunosuppression

Diferencias en los mecanismos específicos de defensa del huésped frente a las candidiasis orofaríngea y vaginal

La candidiasis mucosa es extremadamente común en pacientes inmunosuprimidos. Sin embargo, la prevalencia de candidiasis con localizaciones específicas (por ejemplo, orofaríngea, vaginal o esofágica) puede ser variable dependiendo del estado inmunitario del paciente. Mientras que la candidiasis vulvovaginal es común en mujeres sanas, las candidiasis orofaríngea y esofágica son más frecuentes en estados de inmunodeficiencia. *Candida albicans*, agente etiológico de la mayoría de las candidiasis, es un microbionte del tracto digestivo y del aparato reproductor de la mujer. Así, la mayoría de las personas tienen una inmunidad específica contra *Candida* demostrable en la circulación periférica. El estado de patogenicidad es precipitado a menudo por una deficiencia o disfunción de esta inmunidad. Los estudios en modelos animales, en mujeres con candidiasis vulvovaginal recurrente y en personas infectadas por el VIH, sin embargo, sugieren que son distintos los mecanismos que intervienen contra la candidiasis orofaríngea y la vulvovaginal. Mientras que la inmunidad celular parece importante en la protección frente a la candidiasis orofaríngea, hay pocas evidencias que indiquen que la inmunidad celular sea protectora contra la

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candidiasis vulvovaginal. Además, mientras que ambas defensas inmunológicas, local y sistémica, parecen importantes en la protección contra la candidiasis orofaríngea, las defensas del huésped que protegen contra la candidiasis vulvovaginal parecen limitadas a los tejidos locales y posiblemente estén restringidas a mecanismos innatos. Así pues, las evidencias presentes sugieren que la candidiasis vulvovaginal, a diferencia de la orofaríngea, puede no representar una infección oportunista estricta.

Candida albicans, Candidiasis mucosa, Mecanismos inmunitarios, Candidiasis vaginal, Candidiasis oral, Inmunosupresión

Mucosal candidiasis is a significant problem in immunocompetent as well as immunocompromised individuals, especially those infected with HIV [1-4]. The most common forms of mucosal candidiasis are oropharyngeal, esophageal, and vaginal [5]. In fact, esophageal candidiasis is considered an AIDS defining illness [2]. The majority of cases of episodes of mucosal candidiasis are caused by *Candida albicans*, a dimorphic fungal commensal organism of the gastrointestinal and lower female reproductive tracts. As a commensal, *C. albicans* asymptomatically colonizes epithelial surfaces presumably in the blastoconidia form that it takes in nature. As a result of this exposure, most healthy individuals have developed detectable *Candida*-specific immunity (i.e., cutaneous skin test and peripheral blood lymphocyte responses to *Candida* antigen, as well as *Candida*-specific antibodies in sera and mucosal secretions) (reviewed in [5]). On the other hand, during symptomatic attacks of mucosal candidiasis, *C. albicans* is observed as elongated hyphae or pseudohyphae and superficially invades the mucosa. Depending on the site, signs and symptoms of infection can include itching, burning, pain, and a white curd-like substance at the site of the infection/lesion [5].

Table 1. Epidemiology and risk factors for *Candida albicans* at the oral and vaginal mucosa.

	Oral Mucosa	Vagina
Percent of healthy individuals normally colonized by *C. albicans*	5-50% (mean 25%)	5-20% (mean 15%)
Candidiasis in healthy women	Rare	50-75%
C. albicans as causative agent	> 95%	75-90%
Predisposing factors for infection		
- Antibiotics	+	+++
- Hormone contraceptive therapy	-	++
- Steroids	++	?
- Chronic mucocutaneous candidiasis	++++	+/-
- Chemotherapy		
- Lymphoma/hematologic malignancy	++	+/-
- Transplantation (allogeneic)	+++	+/-
- AIDS	++++	+?
Recurrent infection in healthy women	< 1% (HIV-negative)	5-10% (idiopathic)

There are several epidemiologic and microbiologic differences for how *C. albicans* presents at the oral and vaginal mucosa, both in the commensal and pathogenic states (Table 1). Although both the oral mucosa and vagina are normally colonized with *C. albicans*, the oral mucosa is colonized at higher rates (up to 50%, mean 25% vs. 5 to 25%, mean 15%) [6,7]. While oropharyngeal candidiasis (OPC) (oral thrush) is rare in healthy women of any age, 50 to 75% of women will experience at least one episode of symptomatic vulvovaginal candidiasis (VVC) during their lifetime [7,8].

Clinical experience shows that factors influencing the incidence of infection are quite distinct. Hormonal influences and antibiotic usage appear to predispose to VVC more than OPC. On the other hand, corticosteroids more often predispose to OPC than VVC. Furthermore, OPC is much more common than VVC in patients with chronic mucocutaneous candidiasis, chemotherapy patients with lymphoma/hematologic malignancies, allogeneic transplantation, and AIDS. Finally, recurrent OPC is rare in healthy women, whereas approximately 5% of healthy, HIV-negative women will experience recurrent VVC (RVVC) with no known exogenous predisposing factors [7,9].

Although both innate and adaptive immune mechanisms, including humoral responses, have been shown to play significant roles in host defense against *C. albicans* infection [5,10-14], clinical observations show that cell-mediated immunity (CMI) by T cells and cytokines are critical against mucosal *C. albicans* infections [1-5]. Vaginal candidiasis, however, has not been well represented in these observations as until recently, few case-controlled studies had been conducted exclusively in immunocompromised women. What is now emerging from clinical studies and experimental animal models is a realization that host defense mechanisms against *C. albicans* at the oral and vaginal mucosa may be distinct. This review will summarize the current understanding of host defense mechanisms important at the oral and vaginal mucosa against *C. albicans*.

HOST DEFENSE AGAINST VAGINAL CANDIDIASIS

Until recently, little was known regarding host defense mechanisms important at the vaginal mucosa against any vaginal pathogen, including *C. albicans*. Furthermore, while a limited number of clinical immune-based studies have been conducted on women with RVVC, none have been conducted on women with VVC, immunocompetent or otherwise. Based on animal data suggesting that Thl (IL-2, IFN-γ, IL-12)- and Th2 (IL-4, IL-5, IL-10)-type CMI by CD4+ T cells is associated with resistance and susceptibility, respectively, to mucosal *C. albicans* infection [10,11], studies were conducted in an estrogen-dependent murine model of experimental vaginal candidiasis to evaluate the role of systemic CMI against vaginitis. The first series of studies showed that an experimental vaginal *C. albicans* infection induced a systemic Thl-type response (evidenced by *Candida*-specific

delayed-type hypersensitivity (DTH) and Thl-type cytokines, IL-2 and IFN-γ, produced by draining lymph node cells in response to *Candida* antigens) that was indistinguishable from that induced by systemic immunization with *Candida* antigen and adjuvant [15,16]. However, subsequent studies showed that preinduced *Candida*-specific systemic Thl-type responses (systemic immunization) could not protect mice against experimental vaginitis [17]. These results were the first evidence that systemic CMI expressed in the peripheral circulation may not represent an important or critical protective host defense mechanism against *C. albicans* at the vaginal mucosa. Partial protection against vaginitis was, however, achieved in animals given a second inoculation of *C. albicans* following the spontaneous resolution of a primary infection in the absence of estrogen [18]. Interestingly, although anamnestic DTH occurred during the secondary infection, suppression of this systemic Thl-type reactivity, either by *Candida*-specific suppressor T cells or depletion of all systemic CD4+ cells, had no affect on the protection against vaginitis [18,19], providing additional evidence that systemic CMI had a limited role in protection against a vaginal *C. albicans* infection. In support of these observations, we also showed that mice either resistant or susceptible to systemic *C. albicans* infection [20] were equally susceptible to *C. albicans* vaginitis and could equally be partially protected against a second vaginal infection [21]. We concluded from these studies that some form of locally acquired mucosal immunity, T cell and/or antibody-mediated, was responsible for protecting mice against a vaginal *C. albicans* infection, and that the vaginal mucosa had some level of immunological independence or immune compartmentalization.

These data correlated well with the limited number of immune-based clinical studies conducted in women with RVVC, although such studies have not been without controversy. Since idiopathic RVVC occurs in the absence of known exogenous predisposing factors for vaginitis (i.e., oral contraceptives and antibiotic usage, diabetes mellitus, pregnancy), it had been postulated for some time that RVVC results from some form of immune dysfunction or deficiency. Early studies showing normal levels of *Candida*-specific antibodies in sera and vaginal secretions in RVVC patients [22,23] prompted studies to examine CMI. Several such studies conducted in RVVC patients either described a dysfunction in systemic CMI (usually *Candida*-specific) [24-28], or found no obvious abnormalities [27,29,30]. In a more comprehensive study from our laboratory, we showed that although some RVVC patients may experience a loss in *Candida*-specific cutaneous skin test reactivity similar to several previous studies [27,29], the majority of RVVC patients, both during symptomatic infections and infection-free periods of remission, had normal levels of *Candida*-specific Thl-type CMl in the peripheral circulation as detected by cytokine production *in vitro* [31]. We concluded from these results that the immune dysfunction/deficiency, if present in RVVC patients, was at the local rather than systemic level and that the loss of *Candida*-specific skin test reactivity was the result rather than a cause of infection [31]. These results also correlated with two relevant clinical observations; women with RVVC are generally not susceptible to oral, esophageal, or other forms of cutaneous candidiasis [7], and women with CMC, who have reduced *Candida*-specific systemic CMI, are rarely susceptible to RVVC [5]. Taken together, we have postulated, that as in animal studies, the proposed immune deficiency in RVVC patients is primarily localized to the vaginal mucosa and does not involve systemic CMI.

The lack of effects of systemic CMI at the vaginal mucosa appear to extend as well to the HIV-infected patient although again the studies are not without controversy. Over the past 5 years there have been several cohorts established to evaluate epidemiological data in women infected with HIV. Prior to this, women's health issues had not received much attention. These studies represented the first formal opportunity to collect data on the natural history of genital tract infections in a large number of immunocompromised women. In some early uncontrolled studies, it was reported that vaginitis was more common in HIV+ women and the incidence of infection increased as the CD4 cell counts decreased [32-34]. In contrast, other studies reported that *Candida* vaginitis was not more common in HIV+ or AIDS patients compared to HIV- women [35-38], and the frequency did not correlate with decreased CD4 cell counts [39,40]. There are several possible explanations for these controversial findings. A serious problem with some of these studies is the lack of appropriate control groups. For example, comparing parameters between HIV+ and HIV- individuals requires careful matching of behavioral risk factors. However, the design of too many studies failed to also include a matched control group that can be used to identify true baseline levels [32-34]. In fact, when the appropriate groups were inclusive, it was determined that while vaginal colonization by *C. albicans* and VVC was more common in HIV+ individuals, similar increases were observed in HIV- individuals with high risk exposure to HIV, when compared to an HIV- low risk group [36,38]. Thus, it appears that high risk behavior rather than HIV was responsible for the observed results. Additionally, the lack of knowledge of host defense mechanisms important for protection against vaginal candidiasis has hampered the ability to adequately interpret these clinical data. Nevertheless, taking into account the lack of correlation between reduced CD4+ cells and VVC in HIV+ women and a similar lack of effects of systemic CMI in HIV- women with RVVC, it would not appear that systemic CMI governs host resistance/susceptibility to candidiasis at the vaginal mucosa.

Accordingly, our most recent studies have focused on local CMI at the vaginal mucosa. The vaginal mucosa without organized lymphoid areas such as the gastrointestinal tract-associated Peyer's patches or tonsils, nevertheless, from animal data, has all the necessary components for competent adaptive immune responses. This includes immunoglobulin expression, T cells, and MHC class II+ cells (i.e., Langerhan's cells, macrophages) to serve as antigen presenting cells [41,42]. Of critical importance, several investigators have reported that vaginal lymphocytes in mice are phenotypically distinct from their systemic counterparts [43-45]. Flow cytometric data indicate that although CD4+, α/β T cell receptor (TCR)+ cells dominate the T cell repertoire at the murine vaginal mucosa in a manner similar to lymph node cells, there is a 5 to 50-fold higher percentage of γ/δ TCR+ cells (15 to 50%) and very few, if any, CD8+ cells (normally 20% in blood) [44,45]. In contrast to γ/δ T cells at other mucosal sites, the γ/δ T cells at the vaginal mucosa of mice express a homogenous site-specific Vγ4/Vδ1 TCR [44,46] potentially indicative of a unique function(s). With respect to CD4+ cells, results from several independent assays suggest that vaginal CD4+ T cells express the CD4 protein in a different conformation, or at least atypically, compared to lymph node cells. This is based upon differential recognition by epitope-distinct anti-CD4 antibodies under non-denaturing conditions, whereas normal recognition occurs under denaturing conditions [45,47]. Interestingly, the

putative differences in the vaginal CD4 protein may extend to the level of transcription and result from a unique mRNA [47]. The predominance of CD4+ α/β TCR+ cells at the vaginal mucosa ensures that those the cells that are most critical for host defense against candidal infections at other mucosal sites, are in close proximity to the site of infection. At present, it remains unclear what an atypical expression of the CD4 protein has on the function of the CD4+ cells, but a significant effect would be expected since the CD4 protein is integral to T cell activation through its interaction with MHC class II antigens [48]. Nevertheless, these observations further support the concept of immunological independence or compartmentalization of T cells at the vaginal mucosa. Additional support for this concept comes from animal experiments showing difficulty in trafficking of immune mediators into the vagina. The intravenous administration of complement-fixing antibodies specific for Thy-1 depleted T cells in the periphery, but not in the vagina. In contrast, intravaginal administration of the same antibodies depleted T cells in both the vagina and the periphery [49]. Unfortunately, there have been few studies examining vaginal CMI in humans. One study showed the presence of CD8+ cytotoxic T lymphocytes in the vaginal mucosa which are not readily found in the naïve mice [50]. Primates, on the other hand, have been studied in some detail. To date, reports in primates have described α/β (CD4+ and CD8+) and γ/δ T cells along with Langerhans cells [51]. In these studies, as in humans, vaginal CD8+ T cells had some level of cytotoxic activity [52].

In the mouse model, studies have now been initiated to evaluate vaginal T cells during a *C. albicans* vaginal infection. Previously, the only studies examining the role of local T cells during vaginitis were conducted by Balish and co-workers. In these studies, immunodeficient mice were used to study the natural history of *Candida* vaginal infections [53]. The nude strain of mice (nu/nu) deficient in T cells showed no increase in susceptibility to vaginitis. However, since the animals were not maintained in a state of pseudoestrus and vaginal T cells were not evaluated, it is difficult to interpret these findings. More recently, a study showed that mice depleted of γ/δ T cells had an increased susceptibility to vaginitis [54]. This together with the increased percentage of γ/δ T cells in the vagina suggest a potential role for these cells as a first-line defense mechanism. In our laboratory, findings by flow cytometry, immunohistochemistry, and RT-PCR, all show little evidence for changes in the percentage or composition of specific T cell populations during either primary or secondary experimental vaginal *Candida* infections [55]. These results provide two important pieces of information. First, there is no evidence for systemic T cell infiltration during infection, providing additional support for the lack of systemic T cells during a vaginal *Candida* infection. Second, the results indicate that if the local T cells are active against the infection, they are doing so without detectable changes from naïve conditions. Studies currently underway to evaluate the local expression of cytokines, chemokines, and adhesion molecules during infection will be important to fully recognize the significance of our findings. In fact, such studies may shed some light on possible immunoregulatory mechanisms restricting the action of the systemic and/or local T cells.

In the clinical setting, taking into account the role of CMI in resistance or susceptibility to *Candida* infection at other mucosal sites [10,56,57], in parallel to animal studies, we evaluated Thl- and Th2-type cytokines in vaginal lavage fluid from women with RVVC [58]. Interestingly, we unexpectedly found a constitutive dominant presence of Thl-type, but not Th2-type, cytokines in lavage fluid of normal healthy control women that might reflect a natural immune homeostasis, possibly in response to normal bacterial or yeast flora, including *C. albicans*. With respect to RVVC patients in the study, although some differences in cytokine concentrations were detected between vaginal fluids collected from control women and RVVC patients with or without a symptomatic infection, we did not detect any characteristic pattern of cytokine changes in symptomatic or asymptomatic RVVC patients that one would associate with susceptibility to infection (i.e., lack of Thl-type cytokines or increased Th2-type cytokines). In an alternative experimental design to assess *Candida*-specific vaginal immune responses, we have preliminary evidence that the intravaginal administration of commercial *Candida* skin test antigen to normal healthy women during the follicular stage of the menstrual cycle (minimal influence of reproductive hormones) results in an elevation of Thl-type cytokines in vaginal secretions [58]. This novel approach provides a possible mechanism of establishing whether or not CMI is involved in immune homeostasis within the vagina and contributes to local protection against *C. albicans* vaginal infections in those women normally resistant to vaginitis. Such a design might also be useful to evaluate local immune reactivity in women susceptible to vaginitis (i.e., women with RVVC) or in HIV+ women.

In addition to a potential role for resident T cells and cytokines, immunoglobulins represent another putative protective mechanism functioning against *C. albicans* vaginal infections. Although clinical observations do not support this [59], some studies in a rat model of experimental vaginitis indicate that *Candida*-specific IgA may in fact play a significant role in protection against experimental vaginal candidiasis [60-63]. Such contrasting results to clinical observations may be explained by the type and concentration of *Candida*-specific antibodies present. Casadevall recently proposed that protective, non-protective, and indifferent (neither protective nor non-protective) antibodies are present in a pool of antibodies, and that those in the highest concentration dominate the level of protection, if any [14]. If so, protective *Candida*-specific antibodies do not appear clinically dominant, whereas protection against vaginitis in the rat model may be reflective of high concentrations of "protective" antibodies.

Innate immunity may also play a significant role in protection against vaginitis, although few studies have addressed it. Indeed, a variable leukocyte infiltrate predominated by polymorphonuclear leukocytes (PMNL) is often observed associated with hyphae and/or epithelial cells in vaginal lavage fluid of infected mice. However, this infiltrate in animals rarely correlates to lower vaginal fungal burdens during an experimental infection (Fidel, unpublished observations). Additionally, this leukocyte infiltrate is generally not observed in clinical cases of *Candida* vaginitis. Nevertheless, PMNL and macrophages (two types of leukocytes with considerable effector function against *C. albicans* (reviewed in [5])) are potential candidates for anti-*Candida* innate resistance and are present at or near the vaginal mucosa. Experimentally, however, Balish and co-workers showed that animals with the beige mutation (bg/bg) immunodeficient in phagocytic cells were not more susceptible to a natural *C. albicans* infection under non-estrogenized conditions [53]. More recently Black *et al*. showed that depletion of PMNL under pseudoestrus conditions had no effect on vaginal fungal burden, although microabscesses within the tissue were significantly reduced [64]. The authors speculated

that PMNL may in fact play a significant role against *C. albicans* in the vagina, but the PMNL cannot consistently be deployed in high enough numbers during pseudoestrus to be effective. We recently found that depletion of PMNL had no effect on vaginal fungal burden under estrus or non-estrus conditions [55] suggesting that PMNL do not in fact appear to play a significant role against *C. albicans* in the vagina. These results are consistent with the clinical observation that VVC is rare in neutropenic women (J. Sobel, personal communication). Natural killer (NK) cells represent another potential innate resistance mechanism against *C. albicans* at the vaginal mucosa. However, most studies have shown little or no role for NK cells against *C. albicans* [65,66]. Furthermore, NK cells do not appear to be resident cells of the vaginal mucosa [67].

In light of the uncertainties regarding the role of conventional innate resistance against *C. albicans* at the vaginal mucosa, some attention has shifted to somewhat unconventional immune cells (i.e., epithelial and endothelial cells) in the vaginal mucosa as potential anti-*Candida* effector cells. Epithelial cells have been shown to produce a variety of cytokines and chemokines [68], and endothelial cells have been shown to be phagocytic for *Candida* [69]. Epithelial cells are particularly intriguing as innate immune effector cells as they represent the cell type most often associated with *Candida* in the vagina. Recognizing this, we recently tested and demonstrated that vaginal epithelioid cells extracted from naive mice, or collected from human and primate vaginal lavages, inhibit the growth of *C. albicans in vitro* [70]. Thus, although more studies need to be performed, epithelial cells may represent an important innate resistance mechanism against *C. albicans* at the vaginal mucosa.

Based on information collected from animal models, women with RVVC, and HIV+ women, there is little evidence to suggest that *C. albicans* is a strict vaginal opportunistic pathogen employing the conventional definition of the organism's enhanced ability to cause infection primarily during periods of systemic immunosuppression. First, *Candida*-specific Thl-type CMI in the peripheral circulation provides little to no protection against *C. albicans* vaginitis. Second, although *Candida*-specific antibodies may be protective in some cases, there is no evidence clinically that susceptibility to vaginitis is associated with a deficiency in local or systemic *Candida*-specific antibodies. It remains unclear though if *C. albicans* is an opportunistic vaginal pathogen relative to local immunity. Protective defense cells might include resident vaginal T cells despite little evidence to date supporting a protective role for such cells against experimental vaginitis. Other candidates might include conventional innate resistance cells (i.e., PMNL, macrophages, and NK cells) although none to date have shown a convincing role against *C. albicans* at the vaginal mucosa. On the other hand, vaginal epithelial cells have been demonstrated to inhibit *C. albicans in vitro*. It is interesting to consider that epithelial cell-mediated anti-*Candida* activity may represent a means by which *C. albicans* is held in a commensalistic relationship. But this innate defense is probably quite weak and can be overwhelmed easily in the presence of large numbers of organisms or organisms in a virulent growth phase. Critical studies to evaluate the anti-*Candida* activity by vaginal epithelial cells, PMNL, macrophages, or T cells in women with RVVC or HIV+ women may shed some light as to whether vaginal *C. albicans* infections are opportunistic based on local immune deficiencies.

HOST DEFENSE AGAINST OROPHARYNGEAL CANDIDIASIS

In contrast to a considerable number of experimental studies conducted to identify protective host defenses against vaginal candidiasis, clinical or animal studies on oropharyngeal candidiasis (OPC) have been few. Nevertheless, the data are not only more clear, but significantly different from what has been described for VVC.

Clinically, individuals with chronic mucocutaneous candidiasis who have reduced *Candida*-specific CMI inevitably acquire OPC [5,12]. Furthermore, OPC is often diagnosed during immunocompromised conditions, and specifically when CD4+ T cells are reduced [1,4,40,71]. These data strongly suggest a protective role of systemic CMI against OPC. In a pilot study that assessed *Candida*-specific peripheral blood lymphocyte (PBL) proliferation and cytokine responses in HIV+ individuals with or without OPC, it was reported that the incidence of OPC correlated not only with decreased CD4 cell numbers, but also with reduced Th1-type reactivity (without a shift to Th2-type reactivity) [36]. Interestingly however, although PBL from most HIV+ individuals with OPC in this study proliferated less efficiently compared to those without OPC, responses were still considered positive. Furthermore, it did not appear that HIV infection caused an early Th cell-associated susceptibility to OPC as no difference in Thl/Th2 cytokine production between HIV+ individuals (without OPC) and HIV- individuals with high-risk exposure to HIV was observed. Nevertheless, there is an HIV-related susceptibility to OPC since OPC will occur in HIV+ individuals with normal CD4 cell numbers and no obvious systemic immunosuppression [6]. Furthermore, clinical experience shows that OPC is much more common in AIDS patients than in those immunosuppressed therapeutically following an organ transplant or as treatment for lymphoma (J. Sobel, personal communication). A possible explanation is that local host defenses that play a role in susceptibility to OPC are affected by HIV. Indeed, there is a clear dichotomy of cytokines in saliva of HIV- and HIV+ individuals with or without OPC. Specifically, while a Th0-type (mixed) salivary cytokine profile is present in HIV- individuals, a dominant Th2-type cytokine profile has been observed in HIV+ individuals and is more pronounced in those with OPC [72]. Interestingly, the Th2-type cytokine profile appeared to result from a decrease in Thl-type cytokines (putative protective) rather than an elevation in Th2-type cytokines. Alternatively, HIV may directly influence *Candida* virulence. Indeed HIV viral load has been detected in the oral mucosa of HIV-infected individuals (Luftig and Fidel, unpublished observations).

Although animal models have and continue to be used to study OPC and host defense mechanisms against infection, the literature contains few reports and little information has been forthcoming. Experimental gastrointestinal (stomach) *Candida* infections are relatively easy to induce, however, it has been difficult to induce persistent oral *Candida* infections in animals. The most reproducible model is a hyposalivary rat model where the removal of salivary glands increases susceptibility to infection [73], presumably by removing salivary antimicrobial compounds. There also exists a cyclosporin immunosuppression rat model, and an immunocompetent mouse model [74]. Infections in each model are relatively short-lived (7 days). To date, the mouse model has been the only one exploited with respect to specific CMI host defenses. Data from naive mice show the presence of α/β CD4+ or CD8+ T cells as well as γ/δ T cells [74,75].

During OPC, all these T cell subpopulations were shown to infiltrate into the oral mucosa with the presence of γ/δ T cells correlating with clearance of infection [75]. In a murine AIDS model (MAIDS), 30% of the animals showed recurrent OPC with CD8+ cells recruited into the tissue [76]. There is no indication to date, whether any of these orally associated T cells are phenotypically or otherwise distinct from their systemic counterparts similar to that described at the vaginal mucosa.

Clinically, much of the data regarding the presence of oral-associated T cells comes from HIV+ individuals with gingivitis or periodontitis. In these studies, CD4+ and CD8+ T cells were shown to be present in infected, but not normal, gingiva [77,78] with CD8+ cells dominating infected tissues of HIV+ individuals. Although it is not known what percentage of these cells are local versus systemically derived, these results show that immune cells can accumulate at a site of oral infection. Besides the studies in animals, there is no information whether immune cells, T cells or otherwise, accumulate in *Candida*-infected oral lesions in humans.

The role of humoral immunity against OPC has been studied exclusively in the clinical setting of HIV+ individuals. Similar to that reported for other mucosal sites, *Candida*-specific IgA antibodies were either normal or elevated in saliva of HIV+ individuals with OPC [79]. Thus, as was found in vaginitis, the local presence of *Candida*-specific antibodies do not appear to protect HIV+ individuals from OPC.

Innate resistance at the oral mucosa has not been studied with respect to cellular function (i.e., PMNL, macrophages, NK cells). Clinical observations, however, show that OPC is extremely common in neutropenic individuals (J. Sobel, personal communication). On the other hand, considerable data exists regarding the effects of salivary-associated antimicrobial compounds. Specifically, with respect to *Candida*, it has been shown that anticandidal compounds are present in saliva of normal individuals, and reduced or absent in HIV+ individuals [80]. Our laboratory recently tested the effects of oral epithelial cells against *C. albicans*. Results showed that similar to vaginal epithelial cells, human oral epithelial cells or cell lines also have the ability to inhibit the growth of *C. albicans in vitro* (Fidel, unpublished observations). Thus, in addition to soluble compounds, oral epithelial cells may represent an innate host defense mechanism. At present, it is unknown if the anti-*Candida* activity of oral epithelial cells is modulated under immunocompromised conditions.

Taken together, based on the increased incidence of OPC in the presence of decreased blood CD4+ cells and the infection-associated Th2-type salivary cytokine profile in HIV+ individuals, it would seem that both systemic and local CMI play a role in protection against OPC. Furthermore, although it remains unclear what role antibodies or conventional innate resistance mechanisms have in protection, there is some evidence that epithelial cells may play a role in innate resistance against infection. In any event, based on these data, there is no evidence that would dispute OPC as a strict opportunistic infection.

Table 2. Current concepts regarding host defenses for protection against mucosal candidiasis.

	Mucosa			
	Vaginal		Oral	
	Local	Systemic	Local	Systemic
T cell	??	--	++	++
Antibody	+/-	--	--	--
Innate	++	--	++	??

CONCLUSION

From these data it has become evident that the host defense mechanisms governing protection against oral and vaginal *C. albicans* infections are distinct and different. While there is a correlation between the incidence of OPC and CD4+ T cell systemic immunosuppression or reduced *Candida*-specific systemic CMI, no such correlation exists for VVC or RVVC. Furthermore, while no characteristic differences were detected in vaginal-associated cytokines in RVVC patients compared to controls, there was a distinct Thl/Th2 dichotomy in salivary cytokines between HIV- individuals (Th0) and HIV+ individuals with or without OPC (Th2). Thus, while both local and systemic CMI appear important for protection against OPC, neither seem to be effective against VVC or RVVC. This is supported by the presence or absence of systemic cell infiltration at the oral and vaginal mucosa, respectively, in response to experimental *C. albicans* infection. Finally, although vaginal T cells are phenotypically distinct and potentially compartmentalized at least in animals, there is little evidence to date that they have a role in host defense against experimental vaginal *C. albicans* infection, although immunoregulatory mechanisms may preclude their function. On the other hand, epithelial cells at both sites may play some role in innate resistance against infection or serve as a potential mechanism to retain the organism in a commensal state. These defenses though are expected to be weak and easily overwhelmed by large numbers of organisms. A summary of the current concepts regarding host defense mechanism(s) for protection against OPC and VVC is given in Table 2. It will be interesting to determine if deficiencies in epithelial cell anti-*Candida* activity occur in those with OPC or RVVC or play a role in susceptibility to infection. In any event, the distinct differences in host defense against *C. albicans* at the oral and vaginal mucosa has begun to suggest that, unlike OPC, vaginal candidiasis does not represent a strict opportunistic infection. Intensive efforts to elucidate the anti-*Candida* host defense mechanisms at each site will be required to fully realize this concept and to develop site-specific immune-based strategies to prevent or treat these clinically relevant mucosal *C. albicans* infections.

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