Candida-Specific Systemic Cell-Mediated Immune Reactivities in Human Immunodeficiency Virus–Positive Persons with Mucosal Candidiasis

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Oropharyngeal candidiasis (OPC), as opposed to vulvovaginal candidiasis (VVC), is a common opportunistic infection in human immunodeficiency virus (HIV)–positive persons that correlates with reduced CD4 T cell counts. Although cell-mediated immunity (CMI) by CD4 Th1-type cells is considered to be the predominant host defense against mucosal candidiasis, the immune factors associated with susceptibility to OPC in HIV-positive persons are not well understood. This study investigated Candida-specific systemic CMI in HIV-positive persons with OPC and/or VVC. Reductions in delayed skin test reactivity to Candida antigen were observed in HIV-positive persons with CD4 cell counts <200 cells/µL, irrespective of the presence of mucosal infection. Likewise, despite the correlate of OPC with reduced CD4 cell counts in HIV-positive persons, differences in Candida-specific peripheral blood mononuclear cell proliferation and Th1/Th2 cytokine production between HIV-positive and HIV-negative persons were not consistent in a manner to suggest that deficiencies in Candida-specific systemic CMI account solely for the susceptibility to OPC.

Mucosal candidiasis, especially oropharyngeal, is a common opportunistic infection in human immunodeficiency virus (HIV)–positive persons and frequency correlates with reduced CD4 cell counts [1–5]. In fact, in the era before highly active antiretroviral therapy (HAART), ~95% of HIV-positive persons experienced an episode of oropharyngeal candidiasis (OPC) sometime during progression to AIDS [3, 6]. In the post-HAART era, although the incidence of OPC has declined, OPC continues to be a significant problem [7–10]. Although previous studies similarly suggested an increased prevalence of vulvovaginal candidiasis (VVC) in HIV-positive women [11–13], more recent, case-controlled studies show that the incidence of symptomatic VVC is not significantly higher in HIV-positive women, compared with HIV-negative women who are at risk for exposure to HIV [14–16]. Furthermore, in contrast to OPC, cases of VVC, if present, have not correlated with lower CD4 cell counts or systemic immunosuppression [17, 18].

Cell-mediated immunity (CMI) is considered to be the predominant host defense mechanism against mucosal candidiasis, as evidenced not only by the high incidence of OPC in HIV-positive persons with reduced CMI [1, 19, 20] but also by a similar prevalence under other conditions of T cell immunosuppression (i.e., transplantation, corticosteroid therapy, and treatment for lymphoma [2, 21, 22]). Furthermore, individuals with chronic mucocutaneous candidiasis (CMC) have reduced Candida-specific CMI [20]; however, the underlying immune factors specifically associated with susceptibility to OPC and/or VVC have not been identified. Experimentally, Th1-type responses have been associated with resistance to gastrointestinal and systemic candidal infections in animals, whereas Th2-type responses are associated with susceptibility to infection [23–29]. In contrast, most experimental studies in animals show that Th1-type CMI in the peripheral circulation is not associated with protection against vaginal Candida infections [30–32]. Similarly, in the clinical setting, recurrent VVC (RVVC) often occurs in the presence of normal levels of Candida-specific systemic Th1-type CMI [33–36]. Thus, the natural host defense mechanisms that protect against vaginal Candida albicans infections remain elusive, but point toward local mechanisms.

Host defense against OPC is somewhat clearer and suggests a role for both systemic and local CMI. The correlation of reduced CD4 cells in blood with the appearance of OPC strongly suggests a role for systemic CMI [1, 4, 18]. At the local level, salivary cytokine analysis showed predominantly Th2-
type cytokines in HIV-positive persons, whereas HIV-negative persons had a Th0/Th1 cytokine profile [37].

There appears to be a limited protective role for antibody in either infection, because normal or elevated levels of Candida-specific serum or mucosal antibodies have been reported in HIV-positive persons with OPC and in HIV-negative persons with RVVC [38–41].

There have been several reports suggesting that progression to AIDS in HIV-positive persons is associated with a general immunological shift from Th1- to Th2-type reactivity [42–49]. Although this paradigm has been challenged [27, 50], a shift in Candida-specific Th reactivity to a predominant Th2 type may have significant impact on the incidence of mucosal candidiasis during HIV infection by creating a state of susceptibility at the systemic and/or local level. Candida-specific systemic Th1/Th2-type reactivity has not been examined in HIV-positive individuals with either OPC or VVC, although several studies have included Candida in a panel of recall antigens tested in HIV-positive persons [51–54]. The purpose of this study was to examine Candida-specific Th1/Th2 reactivity in the peripheral circulation of HIV-positive persons with mucosal candidiasis, to better understand the varied susceptibility to mucosal candidiasis during HIV disease.

Subjects and Methods

Subjects. A cohort of 251 subjects, 60% of whom were women and >80% of whom were minorities, was established, including 173 HIV-positive and 85 HIV-negative persons enrolled at the Detroit Medical Center and Louisiana State University Health Sciences Center (New Orleans). The presence of HIV in blood was identified by standard ELISA and Western blot assays, whereas actual virus load was quantified by polymerase chain reaction, all conducted by the Clinical Immunology Laboratory at Louisiana State University Health Sciences Center or Damon Laboratories at the Detroit Medical Center. HIV-positive persons were stratified according to CD4 cell counts and the presence of clinical OPC or VVC. Of the HIV-positive persons, control subjects (HIV-OPC+ VVC-; n = 108) were defined as those with no clinical evidence of any form of mucosal candidiasis and no repeated episodes of such infections by history (questionnaire and interview with research nurse). HIV-OPC+ or HIV-VVC+ patients (n = 46 and 19, respectively) were defined as those who had active clinical evidence of OPC or VVC (confirmed by signs or symptoms and positive culture at that site; n = 41 and 13, respectively) or were culture negative at the time of testing but were on maintenance antifungal therapy as a result of repeated confirmed episodes of mucosal candidiasis in the past 12 months (n = 5 and 6, respectively). Seven women had OPC and VVC simultaneously. HIV-OPC+ or HIV-VVC+ persons were also stratified by the presence of recurrent infection (defined as >3 episodes per year). Nineteen HIV-OPC+ and 3 HIV-VVC+ persons satisfied these criteria. Fifty percent of the HIV-positive persons enrolled into the study were receiving HAART. In this study, HAART was defined as ≥3 antiretroviral medications, whereas mono or dual therapy without a protease inhibitor was defined as non-HAART.

HIV-negative subjects (n = 85) were confirmed as such by standard HIV testing and were stratified further by high-risk behavior for exposure to HIV (i.e., injection drug user [IDU], sexual contact with an IDU, or other high-risk sexual practices; n = 48) or low-risk behavior for HIV exposure (n = 37). HIV-negative persons enrolled were required to have no history of repeated episodes of mucosal candidiasis. During initial prescreening, 4 HIV-negative women were excluded from immunological analyses because of either symptomatic VVC or a history of repeated episodes of VVC.

Diagnosis of mucosal candidiasis and detection of mucosal yeast colonization. The diagnosis of OPC was made on the basis of the clinical appearance of reddened, atrophic areas (erythematous) or white curdlike plaques on the oral mucosa (pseudomembranous). Nine of the 46 OPC+ cases (~20%) were defined as erythematous [55]. In either case, OPC was confirmed by an oral smear that was potassium hydroxide (KOH) positive and by a positive swab culture. Symptomatic VVC was defined as having signs and symptoms associated with vaginitis (i.e., puritis, edema, burning, and discharge), a KOH-positive vaginal smear, and a positive swab culture. Lateral swab cultures were plated on Sabouraud-dextrose (SAB) and Chromagar (CHROMagar Microbiology) biochemical reactive media and were incubated for 48 h at 35°C (SAB) or 37°C (CHROMagar Microbiology). Initial speciation was screened for by color on Chromagar. Green colonies were confirmed as C. albicans by germ-tube test. Nongreen colonies were speciated by API biochemical tests (BioMérieux). All cases of mucosal candidiasis were identified as being caused by C. albicans.

CD4 cell counts. CD4 cell counts per microliter of blood were determined at the Damon Laboratories or the Clinical Immunology Laboratory at the Louisiana State University Health Sciences Center, by use of standard flow cytometry.

Delayed skin test reactivity. Delayed cutaneous skin tests were conducted by injecting 0.1 mL of Candida antigen (Allermed Laboratories) intradermally at a site on the forearm. Injection of physiological saline was used as a negative control. Induration was measured at 48–72 h, with ≥5 mm defined as a positive reaction.

Immediate hypersensitivity testing. Immediate type hypersensitivity scratch test reactions were carried out by placing a drop (10 μL) of Candida antigen (ALK) on the forearm and by drawing a scratch across with the bevel of a sterile needle. Physiological saline was used as a negative control, and histamine was used as a positive control. Induration was measured at 20 min, with erythema defined as a positive reaction or ≥2-fold larger area of erythema than that for the negative control, if present.

Peripheral blood lymphocyte cultures. Peripheral blood mononuclear cells (PBMC) from each subject were collected from venous blood by density gradient centrifugation by using Ficoll-Paque (Pharmacia Biotech) and were cultured separately with the mitogen phytohemagglutinin (PHA; 20 μg) and 2 Candida antigens, heat-killed blastospores (HKB; 5 × 10^6 cells/mL), or soluble cytoplasmic substances (SCS; 125 μg/mL), as described elsewhere [33]. Controls included cells cultured in medium alone.

Proliferation. Proliferation was detected by 3H-thymidine incorporation, as described elsewhere [33].PHA responses were measured on day 3 of culture, whereas Candida-specific responses were measured on day 6 of culture. Results were expressed by proliferation index (fold increase in counts per minute [cpm] in cultures with antigen versus that in cultures with medium alone), net cpm.
Correlation of CD4 cell counts with the presence of OPC.

We first established that the cohort under investigation had a similar correlation of OPC with reduced CD4 cell counts. Results illustrated in figure 1 show that the mean CD4 cell counts of HIV-OPC+ individuals were significantly lower than those of HIV+OPC− VVC− persons (P = .0086) and HIV-negative persons (P = .0001). CD4 cell counts were generally higher in those with erythematous OPC, compared with those with pseudomembranous OPC, with a 2-fold difference in the mean number of cells (307 vs. 153 cells/μL; P < .005). In contrast, mean CD4 cell counts in HIV+VVC+ persons, although significantly lower compared with those of HIV-negative persons (P < .0001), were not different from those for HIV−OPC− VVC− persons (P = .14).

Delayed skin test reactivity. Results of delayed cutaneous skin test reactivity to Candida antigen are shown in figure 2. Rates of positive responses between HIV-negative low- and high-risk persons were not significantly different. Compared with rates of positive responses by HIV-negative persons with low- or high-risk behavior, HIV-positive persons with CD4 cell counts <200 cells/μL had a significant decrease in positive responses (P < .01), irrespective of mucosal Candida infection status, although a trend toward reduced responsiveness was evident in those with OPC and/or VVC, irrespective of CD4 cell counts. No distinct pattern of responses was observed in those with recurrent infection or those with pseudomembranous versus erythematous candidiasis (data not shown).

Immunologic study.

PBMC proliferation. PBMCs were cultured in vitro with 2 Candida antigens and were evaluated for proliferation by 3H-thymidine incorporation. Median responses are shown in figure 3. Responses by PBMC to either Candida antigen were largely similar among all groups of individuals, irrespective of HIV status, CD4 cell counts, or infection status. The only exceptions were reduced responses to Candida HKB (figure 3a) by PBMC from the subset of HIV+OPC+ persons with CD4 cell counts ≥200 cells/μL, compared with HIV-negative persons with low- or high-risk behavior (P < .01), and from HIV−OPC− persons with CD4 cell counts <200 or ≥200 cells/μL, compared with the subset of HIV+OPC+ VVC− persons with CD4 cell counts ≥200 cells/μL (P < .01). Results did not differ if expressed as net 3H-thymidine incorporation (cpm of unstimulated cultures subtracted from that of stimulated cultures), on a per CD4 cell basis (net cpm or proliferation index), or qualitatively as a percentage of positive responses (>3.0 proliferation index; data not shown). There was also no distinct pattern of responses in the subset of those with VVC and OPC (responses indicated by arrows in the figure), erythematous or pseudomembranous OPC, or with recurrent OPC or VVC (data not shown). All subjects, irrespective of HIV or infection status, had positive responses (>3.0 proliferation index) to the mitogen PHA (data not shown).
there was no distinct pattern for any cytokine production from those with VVC and OPC (specific responses are indicated by arrows in the figure), recurrent infection, or those with erythematous or pseudomembranous OPC (data not shown).

**Discussion**

The prevalence of OPC, unlike many other oral conditions (i.e., Kaposi’s sarcoma, cytomegalovirus infection, and aphthous ulcers), remains a significant problem in both men and women with HIV, despite the use of HAART, and continues to correlate predominantly with diminished CD4 cell counts [1, 4, 7, 9]. On the other hand, although VVC can be common in healthy HIV-negative immunocompetent women, it is a lesser problem than is OPC in HIV-positive women and is not correlated with reduced CD4 cell counts [14–16, 18]. Indeed, in the present study, the number of cases of OPC was relatively small, compared with the total number of female patients (7 of 30), and cases of VVC were identified less frequently than were cases of OPC in the time that the cohort was enrolled (19 vs. 46). In addition, the cohort of HIV-positive persons evaluated was consistent with previous studies regarding the occurrence of OPC with reduced CD4 cell counts and the lack thereof with VVC [1, 6]. Also, when cases of OPC were stratified according to type, patients with erythematous OPC had higher CD4 cell counts, which supports previous reports suggesting that this variety of candidiasis occurs earlier in HIV disease progression [55, 59].

Irrespective of the discordant prevalence rates of OPC and VVC during HIV disease, the specific immunological factors predisposing to infection are not clear. This includes any explanation for the correlate of reduced CD4 cell counts in OPC. Although several studies have included *Candida* antigen in the panel of recall antigens to monitor or evaluate immune reactivity during HIV disease [51–54] and have reported reduced responsiveness during progression to AIDS [51, 53], no studies have evaluated *Candida*-specific responses in those with or without mucosal candidiasis.

To this end, the rates of positive delayed cutaneous skin test reactivity to *Candida* antigen were reduced in HIV-positive persons with CD4 cell counts <200 cells/μL, irrespective of *Candida* infection status. The correlation of reduced skin test reactivity with progressive immunodeficiency, as measured by reduced CD4 cell counts, is consistent with published reports [60]; however, this reduction in skin test reactivity clearly could not be used as an indicator of susceptibility to OPC and/or VVC.

Rates of immediate skin test reactivity to *Candida* showed no statistically significant differences between any of the groups. Thus, hypersensitivity to *Candida* by IgE antibodies and mast cell degranulation also does not appear to be responsible for the susceptibility to OPC in these patients. Although IgE was never investigated previously, these results are consistent with the relatively intact nature of humoral immunity during HIV disease [38, 39]. It is interesting that there was no association...
between immediate hypersensitivity to *Candida* antigen and VVC in HIV-positive women, as is often seen in a subset of women with RVVC [56–58].

Proliferation responses of PBMC to either of 2 *Candida* antigens, assessed quantitatively or qualitatively, were relatively similar, irrespective of risk behavior, HIV status, CD4 cell counts, or *Candida* infection status (recurrent or type), although a general trend toward reduced PBMC responses in most of those with CD4 cell counts <200 cells/μL was observed, which is consistent with reduced rates of positive delayed skin test
reactivity. The only exceptions were reduced responses to one antigen, HKB, in HIV+ OPC+ persons. Taking into account the large sample size, one would expect more profound differences if susceptibility to symptomatic Candida infection were due to a general inability of PBMC to respond to Candida antigen, although, notably, the only differences were in those with OPC.

Cytokine responses by PBMC to Candida antigens showed that most individuals had a Th1/Th0-type cytokine response.
In the present study, a decrease in IFN-γ production, with a Th2-type cytokine profile, was observed in HIV-positive persons, irrespective of CD4 cell counts. This suggests a potential role for epithelial cells in innate host defense against Candida albicans at mucosal sites [60, 61]. Although this is the first evidence of its kind for OPC, the concept presented here suggesting a stronger role for local immunity in cases of VVC is similar to that suggested by studies of women with RVVC [33] and by studies that use an experimental murine vaginitis model [32, 62]. The question remains, however, as to which factors account for the widely reported correlation of reduced CD4 cell counts with OPC [1–4]. One possible explanation is that systemic CD4 T cells are not involved in protection against Candida infection at the oral mucosa and that the correlation of reduced, yet largely functional, CD4 T cells to OPC is merely an indicator of a deficiency or dysfunction in independent immune mechanisms at the oral mucosa. Alternatively, the systemic-derived CD4 cells may, in fact, play a role in protection at the oral mucosa but, to provide protection, must be maintained at a certain threshold in the peripheral circulation. Hence, as circulating CD4 cell counts decline, there are simply not enough CD4 cells capable of keeping Candida "in check" at the mucosal surface. This is supported by the ~95% rate of OPC that is observed in HIV-positive persons at some time during disease progression. Below the threshold, local immune mechanisms must take over exclusively. Additional evidence for a local immune role comes from individuals, albeit small in number, who acquire OPC with higher levels of CD4 cells and from those who acquire recurrent OPC. A site-specific deficiency in immune function would also explain the general lack of susceptibility to acute or recurrent candidal infections at other mucosal or systemic sites in those with OPC. Studies to examine local immunity in those with OPC will not only help elucidate local immune deficiencies associated with OPC but also will help define those factors associated with erythematous versus pseudomembranous types of OPC.

References


