Published in final edited form as:
J Reprod Immunol. 2008 June ; 78(1): 76–79.

Vaginal IL-8 levels are positively associated with *Candida albicans* and inversely with lactobacilli in HIV-infected women

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Abstract

IL-8/ CXCL8 is induced during infections, but has not been reported for *Candida albicans* colonization of the female genital tract. Cervicovaginal lavage (CVL) samples were collected from 406 HIV-infected women. IL-8 levels were evaluated by ELISA and compared with levels of *C. albicans* detected by potassium hydroxide (KOH) and PCR. Levels of lactobacilli, *Gardnerella vaginalis* and *Mycoplasma hominis* were also determined by PCR. IL-8 was significantly higher in samples from women with *Candida*, and regression analysis showed a positive association between IL-8 and *Candida*. In contrast, there was an inverse relationship between lactobacilli and IL-8. *G. vaginalis* and *M. hominis* were not significantly associated with IL-8. This study has shown an association between *C. albicans* and levels of IL-8 in mucosal genital fluid.

Introduction

IL-8/CXCL8 is a chemokine that recruits neutrophils to areas of inflammation and/or infection (Harada et al., 1994; Roebuck, 1999). IL-8 expression is induced in a variety of immune and non-immune cell types by microbial products or through pro-inflammatory cytokine cascades (Mukaida et al., 1998). Measurement of IL-8 may therefore be useful as a biomarker of inflammation at mucosal sites, including the female genital tract. For example, high vaginal IL-8 concentrations were associated also with amniotic fluid infections in women with preterm labor (Hitti et al., 2001) and in women with bacterial vaginosis (Spandorfer et al., 2001; Wennerholm et al., 1998). Vaginal IL-8 increases were noted also after sexual activity, after exposure to some microbicides and during menses (Al-Harthi et al., 2001; Fichorova, 2004).

Candida albicans is generally considered to be a commensal organism of the female lower genital tract, but can be present at higher levels in some women and induce vaginitis (Sobel, 2000). The relationship between *Candida* and IL-8 levels in women's genital tract samples has not been well studied. Heat-killed *Candida albicans* or zymosan, a yeast extract, induced *in vitro* the expression of IL-8 by vaginal epithelial cells (Pivarcsi et al., 2005), although live organisms do not induce IL-8 (Steele and Fidel, 2002). Experimentally-induced vaginal yeast

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infections have been associated with increased levels of neutrophils in the vagina, though IL-8 levels were not assessed (Fidel et al., 2004).

Genital tract inflammation can have adverse effects on reproductive health and on susceptibility to sexually transmitted diseases such as HIV (Fichorova, 2004). In this study, we have explored the relationship between IL-8 and *C. albicans*. IL-8 was assessed in 406 cervicovaginal lavage (CVL) samples collected from HIV-seropositive women enrolled in the Women's Interagency HIV Study (WIHS). The characteristics of this national multi-site cohort have been previously reported (Sha et al., 2005). Briefly women had a median plasma HIV RNA level of 4.57 log10 copies/ml (range 3.62–6.54) and a median CD4 cell count of 321 cell/mm^3 (range 0–1787). No woman was receiving highly active antiretroviral therapy. Vaginal swabs were obtained from women regardless of symptoms prior to collection of CVL fluid. KOH smears revealed characteristic hyphae of yeast in 67 evaluations, 322 were negative for yeast, and data were not available for 17 evaluations. Two hundred and three women had bacterial vaginosis (BV) based on a Nugent gram stain score of 7–10 and 203 did not (Nugent score 0–3). Trichomoniasis (having a positive in either wet mount, culture, or Pap smear) was found in 57 samples. HSV and cervicitis/pelvic inflammatory (PID) were diagnosed in 19 and 11 persons, respectively.

Levels of IL-8 in CVL were measured by ELISA (Biosource International, Camarillo, CA). 189 CVL samples had detectable IL-8 (median level of 136pg/ml; range of 6–6904pg/ml), while 217 CVL samples had less than the level of detection (<5pg/ml). TNF-α, IL-10 and IL-12 in CVL were assessed also by ELISA but were detectable in <10 samples. IL-8 levels in the CVL obtained from KOH (yeast)-positive women were significantly higher than in yeast-negative samples (Table 1). In contrast, there were no significant differences between IL-8 levels when CVL samples were compared from either BV-positive versus BV-negative ($p=0.68$, Mann Whitney U test), Trichomonas-positive versus Trichomonas-negative ($p=0.24$), HSV-positive versus HSV-negative ($p=0.75$) or cervicitis/PID-positive versus negative ($p=0.81$). There remained a significant difference in IL-8 levels ($p<0.001$, Mann Whitney U test) between yeast-positive and yeast-negative samples when samples positive for either BV, Trichomonas, HSV or cervicitis/PID were eliminated from the analysis (203, 57, 19 and 11 samples, respectively, were eliminated from each analysis).

Since microscopic methods may be less sensitive than molecular methods for detection of *Candida*, we quantified also the number of *Candida albicans* organisms in CVL using real-time polymerase chain reaction (rtPCR) with primers and amplification methods (Hsu et al., 2003) and DNA isolation methods (Sha et al., 2005) described previously. We found that this method sensitively detects *C. albicans*, but not *C. glabrata*, and is reported not to detect *C. krusei*, *C. parapsilosis* or *C. tropicalis*.

Candida was detected in 74 CVL samples by rtPCR (median of 1.3×10^6 organisms per CVL; range 1.6×10^4 –3.2×10^8), but was not detected in 332 samples. Fifty of these samples were positive for yeast by the KOH method. Of the 67 samples that had hyphae identified by the KOH smear, 50 had Candida detected by rtPCR. The rtPCR assay is suspected to be more sensitive than KOH smear, but is limited by its inability to detect non-*albicans Candida spp.* IL-8 was significantly higher in rtPCR-positive samples (median 62pg/ml IL-8) than in negative samples (Table 1).

Regression analysis was performed using log transformed data for both IL-8 and numbers of *Candida*, and samples that had undetectable levels of IL-8 or *Candida* were assigned values of 5pg/ml and 100 organisms, respectively, for the analysis. Since numbers of *Mycoplasma hominis*, *Gardnerella vaginalis* and lactobacilli had been previously measured in these samples (Sha et al., 2005), regression analysis for these log-transformed variables versus IL-8 was also performed.

Increasing numbers of *Candida albicans* were associated significantly with IL-8 (p=0.001), while decreasing numbers of lactobacilli were associated significantly with IL-8 (p=0.006) (Table 2). In contrast, neither *M. hominis* nor *G. vaginalis* was significantly associated with IL-8.

In conclusion, this study has shown a significant positive association in the lower genital tract between *Candida albicans* and IL-8 in HIV-infected women. This association has not been previously reported, although one study reported that a positive *Candida* culture was associated with vaginal IL-8 during menses (Al-Harthi et al., 2001). The current study also found a negative relationship between lactobacilli and IL-8. Hitti et al. (2001) reported that lower levels of H_2O_2-producing lactobacilli were associated with increased IL-6 in women in preterm labor, but IL-8 was not significantly elevated in that group. This study did not observe a significant difference in IL-8 between the BV-positive and BV-negative groups, while previous studies did (Spandorfer et al., 2001; Wennerholm et al., 1998). A possible explanation for this could be differences in the study groups: HIV-seropositives were assessed in the current study, while HIV-seronegative women undergoing *in vitro* fertilization or with twin-pregnancies were assessed in the other two studies.

Limitations of our study include the fact that the WIHS did not collect either genital tract symptom data at the time of gynecological examination or reliable measures of neutrophil numbers. This compromised the ability to correlate IL-8 to this important parameter of inflammation. Thus, it is possible that women diagnosed with vaginitis were asymptomatic. Another limitation of the study is that IL-8 levels in the CVL were not adjusted for total protein, as performed in some studies, due to insufficient amounts of the samples. The findings presented here help elucidate the relationships between yeast and IL-8. Further study is needed to determine if these associations occur also in HIV-uninfected women.

Acknowledgements

This work was supported by NIH grants UO1-AI-34993 and P01HD40539. Data in this manuscript were collected by the Women's Interagency HIV Study (WIHS) Collaborative Study Group with centers (Principal Investigators) at New York City/Bronx Consortium (Kathryn Anastos); Brooklyn, NY (Howard Minkoff); Washington DC Metropolitan Consortium (Mary Young); The Connie Wofsy Study Consortium of Northern California (Ruth Greenblatt); Los Angeles County/Southern California Consortium (Alexandra Levine); Chicago Consortium (Mardge Cohen); Data Coordinating Center (Stephen Gange). The WIHS is funded by the National Institute of Allergy and Infectious Diseases, with supplemental funding from the National Cancer Institute and the National Institute on Drug Abuse (UO1-AI-35004, UO1-AI-31834, UO1-AI-34994, UO1-AI-34989, UO1-AI-34993, and UO1-AI-42590). Funding is also provided by the National Institute of Child Health and Human Development (grant UO1-HD-32632) and the National Center for Research Resources (grants MO1-RR-00071, MO1-RR-00079, MO1-RR-00083).

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Table 1

Median IL-8 levels of CVL samples grouped by KOH exam or Candida PCR.

	Positive (range, N)	Negative (range, N)	p[a]
Yeast by KOH	78pg/ml (<5–7081, 67)	<5pg/ml (<5–4371, 322)	0.0001
Candida by PCR	62pg/ml (<5–7081, 74)	<5pg/ml (<5–4371, 332)	0.0001

[a] Mann Whitney U test

Table 2

Regression analysis of associations of IL-8 levels with microorganisms[a]

Microorganisms[b]	Regression coefficient	P
C. albicans	0.28	0.001
Lactobacilli	−0.14	0.006
M. hominis	0.04	0.5
G. vaginalis	−0.05	0.7

[a] IL-8 levels (determined by ELISA) and microorganism counts were log transformed for analysis

[b] Levels of organisms in CVL samples determined by rtPCR