Published in final edited form as:

Cytokine. 2013 November ; 64(2): 597–604. doi:10.1016/j.cyto.2013.08.009.

Comparison of the immune microenvironment of the oral cavity and cervix in healthy women

Carole Fakhry^{a,b,c,*}, Morgan A. Marks^{c,*}, Robert H. Gilman^d, Lilia Cabrera^e, Pablo Yori^d, Margaret Kosek^d, and Patti E. Gravitt^{c,f}

^aDepartment of Otolaryngology – Head and Neck Surgery, Johns Hopkins Medicine, Baltimore, MD, United States

^bMilton J. Dance Center Head and Neck Center, Baltimore, MD, United States

^cDepartment of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

^dDepartment of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

^eAsociación Benéfica Proyectos de Informática, Salud, Medicina, y Agricultura, Lima, Peru

^fPerdana University Graduate School of Medicine, Serdang, Malaysia

Abstract

Background—Despite similar frequencies of exposure, the low prevalence of certain sexually transmitted infections such as Chlamydia, HPV and HIV-1 in the oral cavity relative to the cervix is poorly understood. This could be explained in part by differences in host immune microenvironments between these two anatomic sites.

Objective—We compared the concentration and correlation of 27 different immune markers in paired secretion specimens collected from the oral and cervical mucosa of healthy women.

Methods—Paired oral and cervical secretion specimens were collected from thirty-nine women. The concentration of twenty-seven different immune markers was estimated using a Luminex multiplex assay. Marker concentration was normalized to total protein present in the specimen. Median immune marker concentrations were compared across anatomic sites. Unsupervised hierarchical clustering analysis was utilized to identify groups of markers that shared similar patterns of relative concentrations across anatomic sites.

Results—The oral cavity had significantly higher concentrations of eotaxin, IFN- γ , IL-2, IL-4, IL-5, IL-7, IL-9, IL-13, IL-15, PDGF-BB, TNF- α , ($p < 0.01$ for each) while the cervix had higher concentrations of proinflammatory markers such as FGF-basic, IL-1 α , IL-1 β , IL-6, IL-8, IP-10, G-CSF, GM-CSF, MCP-1, MIP-1 β , VEGF ($p < 0.01$ for each). Hierarchical cluster analysis identified two groups of immune markers comprised of T-cell related immune markers with

significantly higher concentrations in the oral cavity relative to the cervix, and a third cluster consisting of mostly inflammatory immune markers which were higher concentrations in the cervix. The oral cavity had a larger number of significant inter-marker correlations as compared to the cervix.

Conclusions—The oral cavity and cervix have significantly different immune marker profiles, which may in part explain the significantly lower burden of sexually transmitted infections such as Chlamydia, HPV, and HIV-1 in the oral cavity vs. the cervix.

Keywords

Oral; Cervical; Pro-inflammatory; T-cell; Immunoregulatory

1. Introduction

The oral cavity and anogenital tract are exposed through sexual activity to similar pathogens, yet the prevalence of certain sexually transmitted infections including Chlamydia trachomatis, human papillomavirus (HPV) and human immunodeficiency virus-1 (HIV-1) appears to be lower at the oral site [1–3]. For example, the prevalence of oncogenic HPV in the oral cavity is significantly lower than in the cervix, even among women with similar histories of oral and vaginal sexual activity [4,5]. Furthermore, the relative risk of anogenital HIV-1 transmission is three times higher than oral HIV transmission [6]. The site-specific differences in the prevalence and incidence of sexually transmitted infections, after controlling for differences in exposure, may be explained in part by distinct mucosal immunologic microenvironments of the two anatomic sites.

Host molecular factors present in the oral cavity and cervix serve to facilitate site-specific physiologic functions including food digestion and reproduction, respectively, as well as mediate innate and adaptive immunological responses. Secretory immunoglobulins (Ig) A, IgG and IgM are detectable at both sites and serve to recognize and bind foreign antigens for presentation to macrophages and dendritic cells [7,8]. Also, epithelial cells of the oral cavity and cervix contain pattern recognition receptors such as toll-like receptors (TLR), which induce production of pro-inflammatory effector molecules such as IL-1 β , IL-6, and IL-8 [9–11]. Despite similarities in innate immune responses, the oral cavity contains high levels of dendritic cells and T-cells [12,13] while the cervix has been shown to have high concentrations of non-antigen specific neutrophils and polymorphonuclear cells and relatively low levels of T-cells and dendritic cells [14]. There have been no studies that directly compared local soluble immune factors of the innate and cell-mediated immune responses across anatomic sites.

The goal of this study is to compare the concentration of immune markers of the oral cavity and cervix using paired secretion specimens collected from healthy women. Delineating baseline differences in the host immune responses of the oral and cervical compartments may help to better understand differences in the prevalence of infectious agents at both sites.

2. Materials and methods

2.1. Study population

Samples were selected from women enrolled in a study evaluating differences in HPV prevalence in populations with and without endemic soil transmitted helminth infection (Iquitos and Lima, Peru, respectively). Paired oral and cervical secretion specimens were selected from 39 women enrolled at the Lima site for this analysis. Women were eligible to be sampled if they were nonsmokers, were negative for cervical HPV DNA, had a normal pap smear, and had no evidence of sexually transmitted infections by clinical examination.

2.2. Data and specimen collection

Each participant underwent a pelvic examination which included collection of a cervical swab using a Digene sampling brush for assessment of cervical HPV status as well as a liquid-based pap smear for assessment of cervical abnormalities. An oral rinse specimen was collected by a 30-s rinse and gargle with 10 milliliters (ml) Scope mouthwash (Proctor and Gamble, Cincinnati, Ohio) for assessment of oral HPV status. Information on demographics, socioeconomic status, sexual behavior, reproductive history (including hormonal contraceptive use and date of last menstrual period), and other behaviors of interest including smoking, drinking and illicit drug use was collected from each woman using an interviewer-administered questionnaire. Phase of the menstrual cycle was estimated as the difference between the date of last menstrual period (LMP) and date of specimen collection. For women who did not report hormonal contraceptive use, phases of menstrual phase were defined as follicular (<13 days from LMP), ovulatory (13–15 days from LMP), luteal (16–30 days from LMP) or abnormal (>30 days from LMP).

Cervical and oral secretion specimens were collected at enrollment for cytokine analysis using Merocel ophthalmic sponges (Medtronic, Minneapolis, MN). For cervical samples, sponges were placed at 6 o'clock on the cervical os for 30 s. For oral specimens, sponges were placed under the tongue for 30 s. After collection, specimens were stored at 4 °C for a maximum of 8 h and then stored at –80 °C at the Universidad Cayetano Peruana Heredia in Lima until the time of protein extraction. After protein extraction, all specimens were shipped on dry ice to Johns Hopkins Bloomberg School of Public Health (JHSPH), Baltimore, Maryland for cytokine analysis.

2.3. HPV detection and genotyping

All HPV DNA testing was performed on cervical and oral specimens stored in STM and PBS, respectively (Digene, Columbia, MD) at the JHBSPH. DNA was extracted using the QIAamp DNA Blood and Media Kit (Qiagen, Courtaboeuf, France) according to manufacturer's instructions with modification. Extracted DNA was tested using the Roche HPV Linear Array® PCR assay (Roche Diagnostics, Indianapolis, IN). The HPV Linear Array® is based on the PGMY09/ 11 primer system that allows for high efficiency amplification of >40 types of HPV [15,16]. The quality and validity of the extracted DNA specimen was assessed by inclusion of β -globin gene-specific primers in the PCR reaction. Only specimens with detectable β -globin were used in this analysis. All oral rinse and cervical swab specimens had detectable β -globin.

2.4. Specimen processing and immune marker measurement

Protein for immune marker analysis was extracted from ophthalmic sponges using previously described methods [8]. Briefly, sponges were thawed at room temperature for 10 min. Sponges were then inserted into a microcentrifuge tube containing a 0.2 µm filter (SpinX, Costar), equilibrated by adding 300 µl of extraction buffer (10 mg/ml aprotinin in phosphate buffered saline with 10% sodium azide) and incubated for 30 min at 4 °C, followed by centrifugation at 4 °C for 30 min at 14,000 rpm. After centrifugation, specimens were stored at -20 °C until the time of immune marker measurement.

At the time of immune marker measurement, specimens were thawed and diluted 1:3 in protein extraction buffer. The following cytokines, chemokines, and growth factors were measured using a magnetic bead-based multiplex assay according to the manufacturer's protocol (Bio-Rad, Hercules, CA): Fgfbasic (Fgfbasic), eotaxin-1 (eotaxin), granulocyte colony stimulating factor (GCSF), granulocyte macrophage colony stimulating factor (GMCSF), interferon-γ (IFN-γ), interleukin-1β (IL-1β, interleukin-1 receptor antagonist (IL-1ra), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), interleukin-7 (IL-7), interleukin-8 (IL-8), interleukin-9 (IL-9), interleukin-10 (IL-10), interleukin-12p70 (IL-12p70), interleukin-13 (IL-13), interleukin-15 (IL-15), interleukin-17 (IL-17), inflammatory protein-10 (IP-10), monocyte chemotactic protein (MCP-1), macrophage inflammatory protein-1α (MIP-1α, macrophage inflammatory protein-1β MIP-1β, platelet derived growth factor BB (PDGF-BB), regulated upon activation, normal T cell expressed and secreted (RANTES), tumor necrosis factor α (TNF-α, and vascular endothelial growth factor (VEGF). Plates were read using a Luminex instrument (Bio-Rad, Hercules, CA). An 8-point standard curve was created and fit using a 5-parameter logistic model. This assay has a reported lower limit of detection of 1–20 pg/ml depending on the cytokine target with an intra-assay coefficient of variation <20% [17].

Each oral/cervical secretion pair from a given individual was tested on the same assay plate to maximize internal validity of the comparisons. Furthermore, each oral/cervical secretion pair was tested in duplicate on a separate assay plate to demonstrate reproducibility.

2.5. Total protein normalization

Prior work conducted by our group demonstrated a strong correlation of immune marker concentration with specimen volume [18]. Therefore, to minimize sampling bias, immune marker concentrations were normalized to the concentration of total protein present in the extracted secretion specimen. The total concentration of protein was measured in each specimen using a bicinchoninic acid (BCA) assay per manufacturer's protocol (Pierce, Rockford IL). This assay has a dynamic range of 20–20,000 µg/ml and has a 14.7% mean coefficient of variation for repeat testing across 14 different human and non-human purified protein targets. Specimens were diluted 1:10 in PBS and run in duplicate. Colorimetric detection of test specimens was normalized to background specimens that contain extraction buffer only. Total protein concentration was estimated using an 8-point standard curve and is expressed as µg/ml. The ratio of immune marker concentration to total protein concentration was then calculated and expressed as (ng of immune marker)/(mg of total protein).

All specimens were tested in duplicate across two testing batches. For oral secretions, the coefficient of variation (CV) values for inter-batch duplicates was <10% across all markers tested and the inter-class correlation coefficient (ICC) values were >0.60 (Supplemental Table S1). For cervical secretions, the CV values for inter-batch duplicates was <10% for a majority of markers tested with the exception of IL-4 (95.4%) and IL-9 (47.2%). Furthermore, ICC values in cervical secretions was >0.60 for a majority of markers with the exception of eotaxin (ICC:0.38), GMCSF (ICC:0.25), IL-4 (ICC:0.0), IL-9 (ICC:0.0), IL-17 (ICC:0.41). In order to retain the internal validity of the matched paired design of this study we present the primary results from a single plate.

2.6. Statistical analysis

All protein adjusted values were log-transformed. Immune marker values that were below the limit of detection and completely outside the dynamic range (therefore reported as undetectable by the Luminex instrument) were assigned half the lowest measured value in our sample [19]. Immune marker values that were below the manufacturer's published limit of detection, with a concentration that could be estimated from the standard curve were assigned the imputed value.

Differences in the median concentration of immune markers between paired oral and cervical secretion specimens were initially assessed using Wilcoxon rank-sum tests for paired observations. Differences in the proportion of oral and cervical specimens that were determined to be completely undetectable by Luminex assay were compared using a chi-squared test. A *p*-value of <0.05 was considered statistically significant.

Markers were classified according to presumed primary function as either pro-inflammatory, immunoregulatory, or growth factors *a priori*. Markers identified as "pro-inflammatory" included IL-1 β , IL-1ra, eotaxin, IL-6, IL-8, IP-10, MCP-1, MIP-1 α , MIP-1 β , RANTES, TNF- α . Markers identified as "immunoregulatory" were IFN- γ , IL-2, IL-4, IL-5, IL-7, IL-9, IL-10, IL-12p70, IL-13, IL-15, and IL-17. Markers considered "growth factors" were Fgfbasic, GCSF, GMCSF, pdgfb, and VEGF.

A ratio of the concentration of each marker in oral vs. cervical specimen ($[C]_{\text{oral}}/[C]_{\text{cervical}}$) was created and hierarchical cluster analysis was then utilized to explore differences in the patterns of these ratio values across all markers. Clustering was assessed using the Euclidian minimal distance method for each marker and a heatmap with dendograms was generated (Partek, St. Louis, MI). The validity of the observed clusters was evaluated based on their recognized known relationship to the *a priori* identified groups as described above (e.g. proinflammatory, immunoregulatory, growth factors).

Spearman's rank correlation was estimated for each pair of immune markers separately for oral and cervical secretions in order to identify patterns of co-expression. A *p*-value of <0.01, after Sidak's correction for multiple comparisons, was considered statistically significant [20].

3. Results

3.1. Study population

Thirty-nine women were selected for this study (Table 1). The median age of these women was 35 years (interquartile range: 30, 42). A majority of these women reported one lifetime (53.1%) and one recent (84.6%) sexual partner. A majority of the participants reported vaginal sex within the last 6 months (92.3%). Oral sex within the last 6 months was less frequently reported (20.5%). These women were non-smokers and one (2.6%) reported current alcohol use. Nearly half of the women (41.0%) reported using hormonal contraception (HC). All women had a normal Pap smear. By design, all cervical samples were undetectable for HPV. Oral HPV testing was performed after the cytokine analyses were complete and three specimens were detectable for HPV 55.

3.2. Differences in median marker concentration by site

Median concentrations of oral and cervical immune markers were compared in Table 2. The oral cavity as compared to the cervix had higher concentrations of eotaxin, IFN- γ , IL-2, IL-4, IL-5, IL-7, IL-9, IL-10, IL-13, IL-15, pdgfb, TNF- α ($p < 0.01$ for each). Conversely, the cervix had higher concentrations of FgfBasic, GCSF, GMCSF, IL-1ra, IL-1 β , IL-6, IL-8, IP-10, MCP-1, MIP-1 β , VEGF ($p < 0.01$ for each) as compared to specimens from the oral cavity. Similar results were observed among specimens tested in a duplicate batch (Supplemental Table 2). These results were the same after exclusion of individuals who were detectable for oral HPV infection (data not shown).

We then considered the relative concentration of each immune marker in the oral cavity vs. the cervix ($[C]_{\text{oral}}/[C]_{\text{cervix}}$) (Fig. 1). Immune markers which have a higher concentration in the oral cavity relative to the cervix ($[C]_{\text{oral}}/[C]_{\text{cervix}} > 1.0$) were observed to be immunoregulatory (IFN- γ , IL-2, IL-4, IL-5, IL-7, IL-9, IL-10, IL-13, IL-15). In contrast, markers with lower concentrations in the oral cavity as compared to the cervix ($[C]_{\text{oral}}/[C]_{\text{cervix}} < 1.0$) were observed to be growth factors and/or proinflammatory in nature (Fgfbasic, GMCSF, GCSF, IL-1 β , IL-1ra, IP-10, IL-6, IL-8, MCP-1, MIP-1 β , VEGF).

Hierarchical cluster analysis of these ratio values identified three distinct clusters of immune markers (Fig. 2). The first cluster contained primarily immunoregulatory markers with consistently higher concentrations in the oral cavity vs. the cervix (eotaxin, IL-2, IL-4, and IL-5). The second cluster contained a combination of immunoregulatory, pro-inflammatory, and growth factors with similar concentrations in both anatomic sites (IFN- γ , IL-7, IL-9, IL-10, IL-12, IL-13, IL-15, MIP-1 α , pdgfb, RANTES, and TNF- α). The third cluster contained mostly pro-inflammatory markers and growth factors that had a lower concentration in the oral cavity as compared to the cervix (Fgfbasic, GCSF, GMCSF, IL-1 β , IL-1ra, IL-6, IL-8, IL-17, IP-10, MCP-1, MIP-1 β , and VEGF).

3.3. Differences in inter-marker correlations by anatomic site

The majority of immune markers in the oral cavity (69.6%, 229 of 329) were strongly and significantly correlated with one another ($p < 0.01$) (Fig. 3a) when considering the mean concentrations for each marker. In contrast, only 25 of 329 (7.6%) immune markers in the

cervical secretion specimens were correlated with one another (Fig. 3b). The significant inter-marker correlations observed in the cervix were primarily observed within immunoregulatory markers and between immunoregulatory and pro-inflammatory markers.

3.4. Differences in marker concentration by hormonal contraceptive use

Concentrations of immune markers were stratified by hormonal contraceptive (HC) use for each anatomic site (Table 3). Overall, marker concentrations measured in the cervix were consistently lower in HC users as compared to non-HC users. Specifically, HC users had lower median concentrations of IL-6 ($p = 0.002$), IP-10 ($p = 0.033$), and MCP-1 ($p = 0.001$) as compared to non-HC users. Conversely, cervical concentration of GMCSF was higher among HC-users as compared to non-users ($p = 0.009$). By contrast, concentrations of immune markers in the oral cavity did not differ by HC use. Lastly, among non-HC users with a normal menstrual cycle, the concentrations of immune markers in the cervix overall did not vary by phase of menstrual cycle (data not shown), with the exception of IL-10 (87.4 pg/ml vs. 47.5 pg/ml; $p = 0.04$) and Fgfbasic (336.9 pg/ml vs. 183.1 pg/ml; $p = 0.02$) which were significantly higher in the follicular than luteal phase. Immune markers in the oral cavity did not differ by phase of menstrual cycle ($p > 0.05$).

4. Discussion

The oral cavity and cervix have a strikingly different distribution of immune markers. In paired oral and cervical secretion specimens collected from healthy women, we observed higher concentrations of T-cell associated markers in the oral cavity while in the cervix we noted higher concentrations of pro-inflammatory markers associated with the innate immune response. The higher concentration of T- and B-cell specific immune markers in the oral cavity relative to the cervix may be driven by several factors.

The elevated concentrations of immunoregulatory and T-cell associated cytokines observed in oral secretions may in part be driven by the close proximity of the oral cavity to lymphoid tissue of the tonsil. The palatine and lingual tonsils are secondary lymphoid organs that are adjacent to the oral cavity and contain a high density of antigen presenting cells (e.g. monocytes, dendritic cells, and antigen-specific T- and B-cells) [21]. In contrast, the cervix and adjacent subsites do not have similar definitive secondary lymphoid organs. Indeed, the oral mucosa and submucosa have been shown to have high concentrations of dendritic cells and lymphocyte subsets [22,23] while histopathologic examination of the cervix has shown high concentration of non-antigen specific inflammatory immune cells such as neutrophils [14].

The higher concentration of cytokines produced by T-cells and dendritic cells (e.g. IFN- γ , IL-2, IL-7 and IL-10) that was observed in oral secretions may be representative of oral tolerance [24]. The oral cavity is exposed to a vast array of environmental antigens such as food and airborne particulate matter. These antigens can induce host inflammatory responses resulting in food and environmental allergic reactions. Indeed, the oral cavity had significantly higher concentration of immune markers such as eotaxin, IL-13, and IL-15 as compared to the cervix which are produced by eosinophils, basophils and mast cells in response to challenge with environmental allergens. To counter this, a process known as

“oral tolerance” is established throughout the gastrointestinal tract to regulate the immune response to these antigens, thereby limiting potential immunocytotoxicity [25]. Oral tolerance is believed to be driven by an interplay between antigen presenting dendritic cells and T-cell subsets, particularly regulatory T-cells. The elevated concentration of IL-2, IL-7, and IFN- γ , markers of T-cell activity, in conjunction with IL-10, a broad marker of immune regulation produced by a variety of cell types including T-cells and macrophages and dendritic cells, suggest a tightly controlled immunologic environment. Conversely, the anogenital tract, which is exposed to a completely different subset of environmental challenges, may lack an analogous mechanism and therefore may not require such high levels of immunomodulatory markers.

The differences in the composition and diversity of commensal bacterial populations in the oral cavity and the cervix may also affect the immunologic profiles of each anatomic site. The female reproductive tract contains a diverse set of bacterial species, mostly comprised of lactic acid producing Lactobacilli, while the oral cavity contains bacteria from a variety of taxa including Firmicutes, Proteobacteria, and Actinobacteria [26–28]. Commensal bacterial species are established on mucosal surfaces soon after birth and aid in a variety of physiological functions including host defense. For example, experiments using germ-free mice show an increased susceptibility and virulence to a variety of pathogenic bacterial and viral infections such as Leishmania and Influenza [29,30]. Maturation of the host immune response is believed to be driven by the colonization of both commensal and pathogenic organisms [31]. Lactobacilli that colonize the female reproductive tract have been shown to induce T-regulatory cells function and downregulate dendritic cell function whereas certain oral pathogens such as *P. Gingivalis* induce a skewed T-cell response leading to tolerance [32–34]. Therefore, anatomical site-specific diversity of commensal bacterial populations may contribute to specific immunologic phenotypes observed at each site.

Sex steroid hormones such as estrogens and progesterone have been shown to modulate host immune responses. The effect of exogenous exposures (e.g. hormonal contraception) upon host immune response is dose-dependent and mediated through specific nuclear and cell membrane-bound hormone receptors [35,36] located on B-and T-cells, and macrophages [37] which are modulated by physiologic feedback mechanisms. Therefore, HC use may affect the distribution and density of these receptors on different immune cell subsets. In this study, differences in immune marker concentration were observed between HC users than non-HC users in the cervix only. This suggests a higher concentration of hormone receptor – expressing immune cell subsets in the cervix than the oral cavity and that immune cell subsets in the cervix are more responsive to exogenous hormonal exposures than in the oral cavity. This reinforces the hypothesis that the oral cavity and cervix are two distinct immune micro-environments.

Higher concentrations of markers associated with the adaptive and cell-mediated immune response are found in the oral cavity than in the cervix. This may explain the reduced prevalence of HPV and other STIs. Prior work has shown that the oral mucosa contain significantly elevated concentrations of IgA as compared to the genital mucosa [38]. The higher concentration of T-cell associated markers such as those related to immunoglobulin production and subsequent T-cell responses is in part driven by Th2 type T-cell activity

which is characterized by effector molecules such as IL-2 and IL-4, both of which were shown to be in relatively high concentrations in oral secretions in this study. Additionally, establishment and persistence of a cervical HPV infection has been shown to be driven in part by delayed or diminished CD8 + T-cell responses characterized by lower concentrations of IL-2 and IFN γ [39,40]. Overall, the elevated concentrations of T-cell associated immune markers in the oral cavity as compared to the cervix are consistent with described immunological mechanisms that regulate the replication of HPV.

This study has some limitations. First, significant diversity in immune and epithelial cell composition has been shown across subsites of the oral cavity. Our current study sampled from the sublingual region that has been shown to have a significantly higher concentration of certain immune cells such as Langerhans dendritic cells as well as a higher percentage of non-keratinized epithelia as compared to other regions of the mouth [23]. Therefore, the immune marker profiles measured from oral secretions in this study may not be fully representative of the total immunologic microenvironment of the oral cavity. Second, while the women selected in this study were generally homogenous with regard to specific demographic, behavioral, and lifestyle factors, nearly half of the women reported use of hormonal contraception. However, the differences in immune marker concentration by anatomic site were similar among HC and non-HC users (Table 3).

5. Conclusions

The oral cavity is considered a hostile environment for the establishment of infectious agents due to the presence of both mechanical and molecular mechanisms related to digestion. In the first study to directly compare the local immunologic profiles of the oral cavity and cervix from healthy women using paired secretion specimens, we show that the oral cavity contains significantly higher concentrations of immunoregulatory factors that are related to the adaptive and cell-mediated immune response as compared to the cervix. These findings provide additional information to better understand the differences in the etiology and natural history of pathogenic agents that are capable of colonizing both the oral cavity and female reproductive tract.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

References

1. Edwards S, Carne C. Oral sex and the transmission of viral STIs. *Sex Transm Infect.* 1998; 74:6–10. [PubMed: 9634307]
2. Kent CK, Chaw JK, Wong W, Liska S, Gibson S, Hubbard G, et al. Prevalence of rectal, urethral, and pharyngeal chlamydia and gonorrhea detected in 2 clinical settings among men who have sex with men: San Francisco, California, 2003. *Clin Infect Dis.* 2005; 41:67–74. [PubMed: 15937765]
3. Schlecht NF, Burk RD, Nucci-Sack A, Shankar V, Peake K, Lorde-Rollins E, et al. Cervical, anal and oral HPV in an adolescent inner-city health clinic providing free vaccinations. *PLoS ONE.* 2012; 7:e37419. [PubMed: 22624027]
4. Brown B, Blas MM, Cabral A, Carcamo C, Gravitt PE, Halsey N. Oral sex practices, oral human papillomavirus and correlations between oral and cervical human papillomavirus prevalence among female sex workers in Lima. *Peru Int J STD AIDS.* 2011; 22:655–8.

5. Schlecht NF, Rojas M, Lorde-Rollins E, Nucci-Sack A, Strickler HD, Burk RD, et al. Burden of cervical, anal, and oral HPV in an inner-city pre-vaccine adolescent population. *J Urban Health*. 2012
6. Rothenberg RB, Scarlett M, del Rio C, Reznik D, O'Daniels C. Oral transmission of HIV. *Aids*. 1998; 12:2095–105. [PubMed: 9833850]
7. Alexander R, Mestecky J. Neutralizing antibodies in mucosal secretions: IgG or IgA? *Curr HIV Res*. 2007; 5:588–93. [PubMed: 18045115]
8. Nardelli-Haeffliger D, Wirthner D, Schiller JT, Lowy DR, Hildesheim A, Ponci F, et al. Specific antibody levels at the cervix during the menstrual cycle of women vaccinated with human papillomavirus 16 virus-like particles. *J Natl Cancer Inst*. 2003; 95:1128–37. [PubMed: 12902442]
9. Daud II, Scott ME, Ma Y, Shiboski S, Farhat S, Moscicki AB. Association between toll-like receptor expression and human papillomavirus type 16 persistence. *Int J Cancer*. 2011; 128:879–86. [PubMed: 20473890]
10. Hans M, Hans VM. Toll-like receptors and their dual role in periodontitis: a review. *J Oral Sci*. 2011; 53:263–71. [PubMed: 21959652]
11. Schaefer TM, Desouza K, Fahey JV, Beagley KW, Wira CR. Toll-like receptor (TLR) expression and TLR-mediated cytokine/chemokine production by human uterine epithelial cells. *Immunology*. 2004; 112:428–36. [PubMed: 15196211]
12. Cutler CW, Jotwani R. Dendritic cells at the oral mucosal interface. *J Dent Res*. 2006; 85:678–89. [PubMed: 16861283]
13. van Loon LA, Krieg SR, Davidson CL, Bos JD. Quantification and distribution of lymphocyte subsets and Langerhans cells in normal human oral mucosa and skin. *J Oral Pathol Med*. 1989; 18:197–201. [PubMed: 2570142]
14. Nicol AF, Fernandes AT, Grinsztejn B, Russomano F, ESJR, Tristao A, et al. Distribution of immune cell subsets and cytokine-producing cells in the uterine cervix of human papillomavirus (HPV)-infected women: influence of HIV-1 coinfection. *Diagn Mol Pathol*. 2005; 14:39–47. [PubMed: 15714063]
15. Gravitt PE, Peyton CL, Alessi TQ, Wheeler CM, Coutlee F, Hildesheim A, et al. Improved amplification of genital human papillomaviruses. *J Clin Microbiol*. 2000; 38:357–61. [PubMed: 10618116]
16. Coutlee F, Gravitt P, Kornegay J, Hankins C, Richardson H, Lapointe N, et al. Use of PGMY primers in L1 consensus PCR improves detection of human papillomavirus DNA in genital samples. *J Clin Microbiol*. 2002; 40:902–7. [PubMed: 11880413]
17. Bio-Rad Laboratories I. Bio-Plex suspension array system tech note. Hercules, CA: 2005.
18. Marks MA, Eby Y, Howard R, Gravitt PE. Comparison of normalization methods for measuring immune markers in cervical secretion specimens. *J Immunol Methods*. 2012; 382:211–5. [PubMed: 22677266]
19. Bio-Rad Laboratories I. Bio-plex pro human cytokine, chemokine, and growth factor assays technical. Bulletin. 2009:1–4.
20. UCLA. Academic technology services SCG. Sidak-Holm: Adjusted *p*-values. [cited 17.07.2011]; <http://www.ats.ucla.edu/stat/stata/code/sidak_holm.htm>
21. Nave H, Gebert A, Pabst R. Morphology and immunology of the human palatine tonsil. *Anat Embryol*. 2001; 204:367–73. [PubMed: 11789984]
22. Cutler CW, Teng YT. Oral mucosal dendritic cells and periodontitis: many sides of the same coin with new twists. *Periodontol 2000*. 2007; 45:35–50. [PubMed: 17850447]
23. Daniels TE. Human mucosal Langerhans cells: postmortem identification of regional variations in oral mucosa. *J Invest Dermatol*. 1984; 82:21–4. [PubMed: 6228611]
24. Castro-Junior AB, Horta BC, Gomes-Santos AC, Cunha AP, Silva Steinberg R, Nascimento DS, et al. Oral tolerance correlates with high levels of lymphocyte activity. *Cell Immunol*. 2012; 280:171–81. [PubMed: 23399844]
25. Mowat AM, Parker LA, Beacock-Sharp H, Millington OR, Chirido F. Oral tolerance: overview and historical perspectives. *Ann NY Acad Sci*. 2004; 1029:1–8. [PubMed: 15806729]
26. Huse SM, Ye Y, Zhou Y, Fodor AA. A core human microbiome as viewed through 16S rRNA sequence clusters. *PLoS ONE*. 2012; 7:e34242. [PubMed: 22719824]

27. Ravel J, Gajer P, Abdo Z, Schneider GM, Koenig SS, McCulle SL, et al. Vaginal microbiome of reproductive-age women. *Proc Natl Acad Sci USA*. 2011; 108(Suppl 1):4680–7. [PubMed: 20534435]
28. Zaura E, Keijser BJ, Huse SM, Crielaard W. Defining the healthy “core microbiome” of oral microbial communities. *BMC Microbiol*. 2009; 9:259. [PubMed: 20003481]
29. Ichinohe T, Pang IK, Kumamoto Y, Peaper DR, Ho JH, Murray TS, et al. Microbiota regulates immune defense against respiratory tract influenza A virus infection. *Proc Natl Acad Sci USA*. 2011; 108:5354–9. [PubMed: 21402903]
30. Smith K, McCoy KD, Macpherson AJ. Use of axenic animals in studying the adaptation of mammals to their commensal intestinal microbiota. *Semin Immunol*. 2007; 19:59–69. [PubMed: 17118672]
31. Round JL, O’Connell RM, Mazmanian SK. Coordination of tolerogenic immune responses by the commensal microbiota. *J Autoimmun*. 2010; 34:J220–5. [PubMed: 19963349]
32. Jotwani R, Pulendran B, Agrawal S, Cutler CW. Human dendritic cells respond to *Porphyromonas gingivalis* LPS by promoting a Th2 effector response in vitro. *Eur J Immunol*. 2003; 33:2980–6. [PubMed: 14579266]
33. Pulendran B, Kumar P, Cutler CW, Mohamadzadeh M, Van Dyke T, Banchereau J. Lipopolysaccharides from distinct pathogens induce different classes of immune responses in vivo. *J Immunol*. 2001; 167:5067–76. [PubMed: 11673516]
34. Pulendran B, Palucka K, Banchereau J. Sensing pathogens and tuning immune responses. *Science*. 2001; 293:253–6. [PubMed: 11452116]
35. Cutolo M, Sulli A, Capellino S, Villaggio B, Montagna P, Seriolo B, et al. Sex hormones influence on the immune system: basic and clinical aspects in autoimmunity. *Lupus*. 2004; 13:635–8. [PubMed: 15485092]
36. Straub RH. The complex role of estrogens in inflammation. *Endocr Rev*. 2007; 28:521–74. [PubMed: 17640948]
37. Marks MA, Gravitt PE, Burk RD, Studentsov Y, Farzadegan H, Klein SL. Progesterone and 17beta-estradiol enhance regulatory responses to human papillomavirus type 16 virus-like particles in peripheral blood mononuclear cells from healthy women. *Clin Vaccine Immunol*. 2010; 17:609–17. [PubMed: 20130130]
38. Kim JS, Nag P, Landay AL, Alves M, Cohn MH, Bremer JW, et al. Saliva can mediate HIV-1-specific antibody-dependent cell-mediated cytotoxicity. *FEMS Immunol Med Microbiol*. 2006; 48:267–73. [PubMed: 16978244]
39. Garcia-Pineros AJ, Hildesheim A, Herrero R, Trivett M, Williams M, Atmetlla I, et al. Persistent human papillomavirus infection is associated with a generalized decrease in immune responsiveness in older women. *Cancer Res*. 2006; 66:11070–6. [PubMed: 17108147]
40. Stanley M. HPV – immune response to infection and vaccination. *Infect Agent Cancer*. 2010; 5:19. [PubMed: 20961432]

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.cyto.2013.08.009>.

Fig. 1.
Ratio of marker concentration $[C]$ in oral vs. cervical secretion specimens.

Fig. 3a.

Fig. 3b.

Fig. 3.
Fig. 3a. Inter-marker correlations of immune markers measured in oral secretions.
Fig. 3b. Inter-marker correlations of immune markers measured in cervical secretions.

Table 1

Subject characteristics.

Variable	n (%) (N = 39)
<i>Marital status</i>	
Married	11 (28.2)
Single	2 (5.1)
Living with partner	23 (58.9)
Separated	3 (7.7)
<i>Lifetime # of sexual partners</i>	
1	21 (53.1)
2	14 (35.9)
3+	4 (10.3)
<i>Recent sexual partners</i>	
0	6 (15.4)
1	33 (84.6)
<i>Time since last sexual intercourse</i>	
7 days	23 (58.9)
>7 days	16 (41.0)
<i>Recent oral sex^a</i>	
No	30 (76.9)
Yes	8 (20.5)
No-response	1 (2.6)
<i>Recent vaginal sex^a</i>	
No	1 (2.6)
Yes	36 (92.3)
Non-response	2 (5.1)
<i>Ever smoker</i>	
No	39 (100)
Yes	0 (0)
<i>Current smoker</i>	
No	39 (100)
Yes	0 (0)
<i>Current alcohol use</i>	
No	38 (97.4)
Yes	1 (2.6)
<i>Current hormonal contraception use</i>	
No	7 (18.0)
Yes	16 (41.0)
No-response	16 (41.0)
<i>Menstrual cycle stage^b</i>	
Follicular	2 (5.0)
Ovulatory	1 (3.0)

Variable	<i>n</i> (%) (<i>N</i> = 39)
Luteal	8 (21.0)
Abnormal	12 (31.0)

^aLast 6 months.

^bAmong those not reporting hormonal contraceptive use.

Table 2

Differences in concentration of immune markers of oral cavity vs. cervix.

Immune marker	Median concentration [IQR] (Ng immune marker/ mg total protein)		p-Value ^a	Outside lower limit of detection N (%)		p-Value
	Oral	Cervical		Oral	Cervical	
Pro-inflammatory factors						
eotaxin	99.5 (14.9, 330.3)	22.2 (11, 60.3)	<0.001	14 (35.9)	19 (48.7)	0.252
IL-1β	22.2 (20.1, 27.1)	601.8 (134.3, 897.8)	<0.001	33 (84.6)	2 (5.1)	<0.001
IL-1ra	2981 (1998.2, 6002.9)	147,266 (36,316, 219,696)	<0.001	0 (0)	0 (0)	–
IL-6	121.5 (66.7, 200.3)	403.4 (270.4, 1, 339.4)	<0.001	6 (15.4)	0 (0)	0.011
IL-8	181.3 (121.5, 244.7)	10,938 (4915, 14,765)	<0.001	1 (2.6)	0 (0)	0.314
IP-10	897.8 (665.1, 1, 480.3)	1, 635.9 (897.8, 3640.9)	0.002	0 (0)	0 (0)	–
MCP-1	121.5 (99.5, 164)	270.4 (181.3, 812.4)	<0.001	0 (0)	0 (0)	–
MIP-1α	49.4 (33.1, 66.7)	60.3 (24.5, 164)	0.241	8 (20.5)	9 (23.1)	0.784
MIP-1β	81.5 (49.4, 109.9)	992.3 (403.4, 1808)	<0.001	0 (0)	0 (0)	–
RANTES	121.5 (81.5, 164.0)	73.7 (54.6, 181.3)	0.387	0 (0)	0 (0)	–
TNF-α	298.9 (181.3, 445.9)	90 (60.3, 109.9)	<0.001	3 (7.7)	6 (15.4)	0.288
Immunoregulatory factors						
IFN-γ	1635.9 (992.3, 2440.6)	492.7 (365, 601.8)	<0.001	1 (2.6)	0 (0)	0.314
IL-2	66.7 (12.2, 134.3)	9.9 (7.4, 22.2)	<0.001	11 (28.2)	23 (58.9)	0.006
IL-4	33.1 (22.2, 49.4)	8.2 (6.0, 9.9)	<0.001	0 (0)	0 (0)	–
IL-5	121.5 (90, 181.3)	16.4 (13.5, 24.5)	<0.001	3 (7.7)	29 (74.4)	<0.001
IL-7	134.3 (90, 200)	44.7 (36.6, 66.7)	<0.001	3 (7.7)	4 (10.3)	0.692
IL-9	66.7 (22.2, 148.4)	44.7 (36.6, 60.3)	0.209	7 (17.9)	0 (0)	0.006
IL-10	73.7 (54.6, 109.9)	54.6 (40.4, 90)	0.042	5 (12.8)	2 (5.1)	0.235
IL-12	200.3 (134.3, 270.4)	200.3 (109.9, 270.4)	0.558	0 (0)	0 (0)	–
IL-13	200.3 (121.5, 244.7)	81.5 (66.7, 99.5)	<0.001	0 (0)	0 (0)	–
IL-15	16.4 (14.9, 18.2)	11.0 (9.0, 13.5)	<0.001	36 (92.3)	38 (97.4)	0.305
IL-17	90.0 (54.6, 181.3)	90.0 (66.7, 121.5)	0.665	5 (12.8)	0 (0)	0.021
Growth factors						
Egfbasic	164.0 (99.5, 298.9)	244.7 (181.3, 330.3)	0.002	4 (10.3)	0 (0)	0.040
GCSF	221.4 (134.3, 365.0)	14,764.8 (3,640.9, 24,343)	<0.001	1 (2.6)	0 (0)	0.314

Immune marker	Median concentration [IQR] (Ng immune marker/mg total protein)		Outside lower limit of detection N (%)			
	Oral	Cervical	<i>p</i> -Value ^a	Oral	Cervical	<i>p</i> -Value
GMCSF	9.0 (7.4, 298.9)	4447.1 (4023.9, 5431.7)	<0.001	23 (58.9)	0 (0)	<0.001
pdgfb	445.9 (330.3, 812.4)	181.3 (134.3, 244.7)	<0.001	0 (0)	0 (0)	–
VEGF	148.4 (121.5, 221.4)	1635.9 (812.4, 2697.3)	<0.001	0 (0)	0 (0)	–

^a *P*-value generated using Wilcoxin Rank Sum for paired obs.

Table 3
Comparison of median immune marker concentration[C] among HC vs. non-HC users in cervical and oral secretions.

Cytokine	Cervical		Oral			
	HC users (N = 16)	Non-HC users (N = 7)	p	HC users (N = 16)	Non-HC users (N = 7)	p
Pro-inflammatory factors						
eotaxin	16.0 (10.7, 52.7)	73.2 (11.2, 97.9)	0.316	134.3 (18.3, 255.2)	94.4 (14.5, 411.2)	0.593
IL-1β	491.3 (220.5, 925.2)	787.6 (132.8, 971.7)	0.764	23.6 (22.3, 28.2)	22.4 (20.8, 25.2)	0.316
IL-1ra	112,083.6 (35,560.8, 242,074.3)	195,633.8 (35,882.3, 251,198.8)	1.0	2192.9 (1626.2, 3190.7)	2591.5 (2542.7, 15108.3)	0.071
IL-6	276.7 (149.8, 476.8)	1352.9 (731.4, 6667.5)	0.002	119.1 (53.4, 177.5)	106.5 (65.9, 175.2)	0.894
IL-8	9946.7 (4989.1, 17,051.6)	14,603.3 (8308.2, 16,026.5)	0.789	185.1 (96.0, 246.4)	175.4 (130.1, 256.5)	0.640
IP-10	1151.7 (673.8, 2184.2)	4934.5 (932.6, 34,303.4)	0.033	809.2 (667.8, 1114.3)	1489.2 (615.8, 1887.5)	0.161
MCP-1	188.3 (135.6, 244.9)	1416.6 (781.3, 1745.9)	0.001	125.3 (91.9, 155.7)	115.7 (97.3, 172.1)	1.0
MIP-1α	58.8 (23.6, 200.9)	143.9 (81.4, 191.3)	0.349	48.5 (24.1, 64.7)	42.4 (32.9, 71.9)	0.894
MIP-1β	881.0 (355.0, 4319.9)	1418.0 (889.8, 1837.2)	0.548	74.7 (44.9, 103.3)	69.6 (49.1, 116.3)	0.947
RANTES	72.0 (64.7, 94.1)	85.3 (42.1, 147.1)	0.947	128.9 (77.2, 161.9)	110.2 (82.7, 164.7)	0.789
TNF-α	86.3 (32.6, 102.5)	101.3 (92.9, 125.5)	0.204	275.1 (141.3, 417.8)	252.9 (187.4, 434.8)	0.947
Immunoregulatory factors						
IFN-γ	470.6 (316.7, 577.7)	541.3 (390.3, 661.8)	0.316	1654.1 (747.7, 2344.9)	1395.5 (949.6, 2404.3)	0.841
IL-2	8.2 (7.0, 10.8)	21.6 (7.6, 26.0)	0.181	51.6 (11.0, 117.0)	47.1 (9.8, 136.9)	0.789
IL-4	9.0 (6.5,11.6)	8.1 (6.8, 9.2)	0.422	33.4 (19.2, 41.3)	32.5 (21.7, 48.1)	0.947
IL-5	17.3 (12.1, 22.2)	22.4 (15.7, 37.1)	0.285	125.6 (72.0, 151.6)	112.3 (86.4, 176.4)	0.947
IL-7	41.6 (27.0, 51.6)	44.4 (37.5, 82.1)	0.285	141.9 (84.2, 179.3)	118.3 (89.6, 197.2)	0.841
IL-9	40.9 (27.8, 54.9)	55.2 (52.0, 83.8)	0.061	70.0 (8.1, 115.8)	59.9 (21.4, 156.6)	0.738
IL-10	50.2 (30.4, 66.5)	63.2 (40.4, 90.9)	0.161	78.0 (55.8, 103.5)	67.9 (51.1, 161.1)	0.789
IL-12	193.5 (94.2, 216.8)	194.4 (102.3, 316.4)	0.349	208.7 (158.2, 251.1)	204.0 (108.3, 398.2)	0.738
IL-13	80.6 (44.7, 94.5)	85.5 (71.7, 136.7)	0.316	213.2 (134.4, 242.5)	197.0 (124.2, 319.3)	0.789
IL-15	12.7 (9.3, 15.2)	11.4 (9.3, 12.5)	0.462	16.6 (16.2, 19.3)	16.4 (15.2, 18.5)	0.504
IL-17	113.8 (88.0, 136.9)	74.2 (56.8, 103.4)	0.053	95.5 (28.8, 144.7)	67.8 (48.2, 173.5)	0.947
Growth factors						
Fgfbasic	303.4 (236.3, 376.9)	201.9 (168.3, 274.2)	0.109	163.0 (39.4, 239.4)	150.8 (112.7,309.5)	0.548
GCSF	4105.2 (1795.4, 16,058.6)	12,990.8 (10,239.4, 27,364.4)	0.082	213.2 (116.9, 322.5)	225.0 (177.0, 385.3)	0.548

Cytokine	Cervical		Oral			
	HC users (N = 16)	Non-HC users (N = 7)	p	HC users (N = 16)	Non-HC users (N = 7)	p
GMCSF	5608.3 (4407.2, 6483.4)	3827.6 (2910.3, 4487.3)	0.009	8.1 (7.6, 9.8)	7.8 (7.1, 1467.0)	0.841
pdgfb	148.9 (116.3, 227.0)	217.2 (167.3, 332.0)	0.142	464.5 (253.4, 720.5)	416.1 (376.2, 818.1)	0.504
VEGF	1788.3 (1255.1, 2382.7)	1001.2 (422.8, 2633.3)	0.385	144.0 (101.5, 211.2)	160.5 (118.2, 245.4)	0.504

* *p*: *P*-value generated using Wilcoxin Rank Sum Test;
HC: Hormonal contraceptive.