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In vitro anti-HIV-1 activity in cervicovaginal secretions from pregnant and non-pregnant women

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Abstract

Objective—To evaluate whether cervicovaginal secretions inhibit HIV-1 infectivity in an *in vitro* model, and estimate concentration of immune mediators.

Study design—We enrolled mid-trimester pregnant and regularly menstruating (non-pregnant) women. Cervicovaginal lavage (CVL) was collected at 2 visits and incubated with HIV-1 and TZM-bl cells. Infectivity was compared to positive controls. Concentrations of immune mediators were compared between groups.

Results—At enrollment, CVL inhibited IIIB virus 88.2% and 82.4%, and BaL virus 72.8% and 77.9%, among pregnant (n=13) and non-pregnant women (n=9), respectively. At second visit, CVL inhibited IIIB 89.7% and 82.5%, and BaL 77.4% and 69.9% among pregnant (n=15) and non-pregnant women (n=8), respectively (all $P \leq 0.04$). Adjusting for body mass index, race, and protein content of CVL, antimicrobials were suppressed but cytokines and chemokines were not markedly different in pregnancy.

Conclusion—Cervicovaginal secretions significantly suppress HIV-1 infectivity in this model. Concentrations of certain immune mediators are altered in pregnancy.

Keywords

cervicovaginal lavage; cervicovaginal secretions; HIV; pregnancy

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Introduction

Women account for half of all people living with HIV-1 globally and for 60% of those in sub-Saharan Africa.[1] This figure represents a significant overall increase in the number of adult women infected with HIV globally since 2001. The vast majority of incident HIV worldwide is caused by heterosexual intercourse. The lower genital tract is one of the most susceptible areas in the body for HIV acquisition. During vaginal intercourse, women are twice as likely to contract HIV from their male partner as men are from a female partner.[2,3] As more women of reproductive age become HIV-infected, they may become pregnant and are at risk of transmitting HIV to their fetus. As a result of infection in women, there are now nearly 2 million children living with HIV, the vast majority of these perinatally infected.[1] A large, rigorous study following over 10,000 women done in Rakai, Uganda found that women were at significantly increased risk of HIV acquisition during pregnancy. Data from this community cohort with longitudinal data were analyzed for the incidence rate of HIV during pregnancy, and compared to the incidence rate during periods of non-pregnancy. The incidence rate was 2.3 per 100 person years in pregnancy as compared to 1.1 per 100 person years in non-pregnant women. This difference in incidence rates resulted in an incident rate ratio of HIV acquisition in pregnancy of 2.16 (95% CI 1.39-3.37) after adjusting for age, marital status, education, number of sex partners, genital ulcer disease, and condom use.[4] The data are not entirely consistent, however, as several smaller studies have not demonstrated such an association.[4-7]

The biologic reasons for a potentially increased risk of HIV acquisition during pregnancy have not been elucidated. It has been suggested that mucosal immunity in the genital tract is compromised during pregnancy. Concentrations or expression of certain antimicrobial peptides, cytokines, and chemokines have been shown to be altered under certain conditions in pregnancy, such as bacterial vaginosis[8], trichomoniasis[9], or premature rupture of membranes[10].

A number of studies have examined the use of a TZM-bl indicator assay as an *in vitro* surrogate of HIV-1 infectivity. This infectivity assay is the World Health Organization (WHO) preferred infectivity assay and is commonly used in HIV vaccine research. It is considered to be more standardized than traditional peripheral blood mononuclear cell (PBMC) infectivity assays.[11] The assay has been studied to measure the impact of genital tract secretions on prevention of HIV infectivity but its performance testing CVL from pregnant women has not been explored. [12-14] Our purpose in this study was three-fold. First, we sought to assess whether cervicovaginal lavage (CVL) fluid would suppress HIV-1 infection of target cells differentially in pregnant and non-pregnant women, second, to evaluate whether protective immune mediator concentrations were altered in pregnancy and third, determine whether cytokines, chemokines and anti-HIV molecules results vary when expressed per unit volume versus per unit protein.

Materials and Methods

We enrolled HIV-negative pregnant and non-pregnant women between the ages of 18 and 35 presenting for care at our tertiary care institution. Pregnant women were offered enrollment if they were between 14 and 26 weeks' gestation as determined by best obstetrical estimate. Non-pregnant women were offered enrollment if they had regular menses for the previous three months. Exclusion criteria were pre-gestational diabetes mellitus, chronic hypertension requiring medications, antibiotic use within two weeks of specimen collection, use of hormonal contraception, current or planned cerclage, planned termination of pregnancy, known fetal anomalies, or symptomatic vaginal discharge requiring doctor visit within two weeks of enrollment.

All participants signed written, informed consent. The study was approved by the Women and Infant's Hospital Institutional Review Board on October 6, 2008, Protocol number 08-0115. At enrollment, baseline data were collected, including demographic information, basic medical and obstetric risks, and vaginal practices. All women underwent cervicovaginal lavage collection performed in a standard manner. 10 cc of normal saline was instilled into the vaginal cavity with the stream directed toward the external os of the cervix. The fluid was allowed to pool in the posterior fornix, and then aspirated. At second study visits, CVL was collected in the same manner. Pregnant women were in the third trimester at the time of follow-up. Non-pregnant women were enrolled during the proliferative phase of the menstrual cycle and follow-up was performed when they were peri-ovulatory. On the same day as collection, CVL was centrifuged at 1500g for 10 minutes and the supernatant was frozen at -80°C until used in the TZM-bl assay.

The HIV-1 strains used, IIIB (X4), a virus that infects via the CXCR4 co-receptor and BaL (R5), which infects via the CCR5 co-receptor, thought to be a more common viral co-receptor for sexual transmission were kindly provided by Dr P. Gupta (University of Pittsburgh, PA). Virus stocks were propagated in PHA-stimulated human PBMC and stored frozen at -80°C. Details on this assay have been previously described.[15] The light intensity of each well was measured using a luminometer and expressed as Relative Light Units (RLU). Uninfected cells and cells incubated with CVL only were used to determine background luminescence. HIV-1 incubated in media alone prior to adding it to the TZM-bl cells was used as positive control. TZM-bl cells were incubated with secretions alone and media alone were used a negative controls and determination of background values. Viability of TZM-bl cells upon treatment with CVL was quantified using the CellTiter 96® Aqueous One Solution Cell Proliferation Assay (Promega) according to manufacturer's instructions.

The relative light units were expressed as median values, percent inhibition as compared to virus-only positive control set at 100%, and after adjustment for background luminescence. Comparisons were made between pregnant and non-pregnant groups by Wilcoxon rank sum test, and each group was tested against the positive control. The van Elteren test was used to compare medians adjusting for body mass index and race. Statistical significance was considered $p<0.05$.

Antimicrobial peptides, cytokines, and chemokines that have been previously shown to have an impact on HIV infectivity were measured in the CVL of pregnant and non-pregnant women. Secretory leukocyte protease inhibitor (SLPI), macrophage inflammatory protein-3-alpha (MIP)-3α, and elafin were measured using ELISA kits from R&D Systems (Minneapolis, MN) according to manufacturer's instructions. Standards for each ELISA were re-suspended in phosphate buffered saline (PBS). Samples were diluted in 1xPBS. Antimicrobials were quantified based on standard curves obtained using an ELISA reader (Dynex, Chantilly, VA). Human beta defensin (HBD)2 was assayed using ELISA test kit from PeproTech (Rocky Hill, NJ) according to manufacturer's instructions. CVL were assayed for 14 different chemokines and cytokines (BioRad, Hercules, CA), using a multiplex bead assay (Luminex Corp., Austin, TX) as previously described.[16] Total protein concentration in each CVL sample was determined using the BCA Protein Assay kit from Fisher Scientific, according to manufacturer's instructions. Concentration of each molecule was expressed as picograms/ml as well as per 10 micrograms of protein and compared between pregnant and non-pregnant women. The van Elteren test was used to compare concentrations between groups after controlling for BMI and race.

Results

A total of 31 subjects were enrolled in the study, 20 pregnant and 11 non-pregnant. Thus, we obtained 13 usable samples from women pregnant in the second trimester, 15 in the third trimester, and 17 samples from non-pregnant women at various stages of the menstrual cycle. In the latter group, sample numbers were too low to allow cycle dependent stratification. Pregnant women were slightly older, but of similar race, with the majority being Caucasian. Nearly all participants had completed high school. Pregnant women were more likely to be married (Table 1). Using the TZM-bl assay, we investigated whether anti-HIV activity in CVL is similar in pregnancy as we have shown in non-pregnant women[15]. As seen in Tables 2 and 3, when compared to non-pregnant samples, CVL from pregnant subjects collected at enrollment and second visits markedly suppressed infectivity of both X4 and R5 viral strains. These studies indicated further that inhibitory capacity was slightly lower, but not dramatically so, in the presence of IIIB (Figure 1A and C) and Bal (Figure 1B and D) virus in CVL collected at Enrollment or at Return visit. Within the limits of the sample size, no difference was detected in infectivity between visits for a given viral strain, or for viral inhibition between pregnant and non-pregnant women, (not shown).

To understand whether immune parameters in CVL change with pregnancy, an analysis was carried out in which CVL from non-pregnant and pregnant women were analyzed for antimicrobial, cytokine and chemokine levels. As seen in Tables 4 and 5, data are expressed both as pg/ml (shaded boxes) and pg/10μg protein (clear boxes). Data are presented in both ways owing to our finding of an elevation in protein concentration that occurs in CVL during pregnancy. Overall protein concentration was significantly higher among pregnant women, median 139.8 μg/ml (range: 48.3-514.2) in CVL compared to 22.6 μg/ml(range: 1.0-1139.5) (p=0.03) in CVL from non-pregnant women after controlling for race and body mass index.

As seen in Tables 4 and 5, when cytokines and chemokines are expressed as pg/ml, and analyzed by Wilcoxon Rank-sum test and van Elteren test, Regulated on Activation, Normal T Expressed and Secreted (RANTES), Interleukin (IL)-1α, and IL-1 receptor antagonist (RA) are significantly higher in CVL from pregnant when compared to non-pregnant women. The remainder of cytokines and chemokines were unchanged with the exception of granulocyte macrophage colony stimulating factor (GM-CSF), which increased in return visit samples (Table 5). As a part of these studies key antimicrobials were analyzed. When expressed as pg/ml, no differences were seen in the concentrations of Elafin, SLPI, HBD2 or MIP3α.

In contrast to our CVL findings expressed per unit volume (pg/ml), we found that when results were expressed based on CVL protein content that marked changes were seen in a number of compounds. For example, as shown in Table 4, IL-6, monocyte chemoattractant protein (MCP)-1, MIP-1α, Elafin, HBD-2 and MIP-3α in CVL from pregnant women were significantly lower than that seen in non-pregnant women. In contrast, as seen in Table 5, RANTES, Eotaxin, Fractalkine, IL-6, MCP-1, Elafin, SLPI, HBD2 and MIP-3α in CVL from pregnant women were lower when compared to non-pregnant CVL. Overall, unlike Elafin, HBD2, and MIP-3α, which were not decreased when expressed as pg/ml, all three were significantly decreased in pregnant women after controlling for protein content.

Comment

In this study, we found that genital secretions collected from both pregnant and non-pregnant women suppress HIV-1 infectivity in an *in vitro* model and to a similar degree. To our knowledge, this is the first comparison of intrinsic anti-HIV activity and concentrations

of anti-HIV antimicrobials in the CVL of second and third trimester pregnant and non-pregnant women. Our data suggest that genital secretions contain anti-viral factors including SLPI, Elafin, MIP-3α and HBD2 that inhibit HIV infection and/or replication

Our approach to studying HIV-1 infection among pregnant women is novel because it focuses on the point-of-entry into the body, the genital tract. Given that the majority of incident HIV occurs through heterosexual contact, recent studies have begun focusing on ways to understand and prevent infection at the level of the genital tract. Vaginal microbicides seem like a logical choice for prevention but thus far, their effectiveness has not been proven. Research such as ours highlights the potential to develop preventive therapies using natural immune mediators, possibly in the form of microbicides. The recent publication of tenofovir vaginal gel as an effective means of HIV-1 prevention is highly promising.[17] However, use of this product in pregnant women has yet to be established and would mean exposing a fetus to a medication without a current medical indication for use. The recent cessation of a large clinical trial of tenofovir gel has made this area even more controversial[18].

Our study did not demonstrate that anti-HIV activity in the CVL of pregnant women during the second and third trimester is different from that seen in CVL from non-pregnant women. There are a number of reasons that could account for this. For example, since the epidemiologic finding of increased risk of HIV acquisition in pregnancy controlled for behavioral factors[4], it suggests that the underlying mechanism may be biological rather than behavioral. Alternatively, while past studies included women throughout pregnancy, our study analyzed CVL exclusively from women between 14 and 38 weeks (2^{nd} and 3^{rd} trimester). Whether susceptibility to HIV infection and other sexually transmitted infections is elevated early in pregnancy remains to be determined and is under investigation by our group.

Another contributing factor to increased risk of HIV infection is the bacterial content in the lower genital tract of pregnant women. While much needs to be learned about the effect of sex hormones and pregnancy on the vaginal ecosystem, it has been shown that early disruption of the immune milieu in pregnancy is associated with infectious complications of pregnancy later in gestation.[19] Our study excluded women with symptomatic vaginal discharge. In doing so, we may have eliminated that portion of the population at risk, and who might have compromised lower genital tract immune protection. Further studies are needed to determine whether baseline bacterial populations differ between women in our study and the Rakai cohort and whether subsequent infectious risk is the same in different study populations.

An alternative explanation is that we used two laboratory-adapted viral strains, one that is a CXCR4-tropic virus and one that is CCR5-tropic. Therefore, we cannot exclude the possibility that other viral strains, especially primary or transmitted/founder viruses, may infect differently depending on the biological conditions during pregnancy that differ from that in the non-pregnant lower genital tract.[20] In previous studies, our group found that innate anti-HIV activity in CVL of healthy non-pregnant HIV-infected and un-infected women varied with the viral strain analyzed.[15] Further studies are needed to define the extent to which CVL from pregnant women inhibit different viral strains. Our study wasn't designed to test this but it is possible that certain viral strains may be more infectious during pregnancy than in the non-pregnant milieu.

Antimicrobial peptides, cytokines, and chemokines are important effectors of innate immune protection within the female lower genital tract. Some recruit immune cells and others directly target microbes. Many of these molecules are known to have potent anti-HIV

activity.[21-25] Previous work by our group showed that CVL from healthy HIV-infected and uninfected non-pregnant women inhibit infection of HIV target cells.[15] We reported a positive correlation with certain antimicrobials including MIP-3α and HBD2 in CVL and anti-HIV-1 activity. We suspect that similar antimicrobial peptides function to inhibit infectivity in the CVL of pregnant women as well. In the present study, we found that concentrations of MIP-3α and HBD2 were the same as that measured in CVL from non-pregnant women when expressed per unit volume. Whether these molecules are responsible for the anti-HIV activity seen in CVL from pregnant women remains to be determined given that more than 20 antimicrobials have been identified in CVL .[16] Previously, we found that MIP-3α and HBD2 levels in CVL vary with stage of the menstrual cycle, thereby contributing to a window of vulnerability when women are more likely to be at risk of infection by HIV and other STI. We reported that levels of HBD2 range from a low of 600 pg/ml at midcycle to 1600 pg/ml during the early proliferative and late secretory phase of the menstrual cycle. [26] Our finding that these values are comparable to those measured in our samples during pregnancy (640-1100pg/ml) suggests that pregnancy levels are comparable to that seen during the menstrual cycle when innate protection against HIV and other STI is elevated. Further studies are needed to identify the key immune factors responsible for protection during pregnancy.

An unexpected finding in the present study was the pronounced increase in protein in the CVL from pregnant women. As seen in Tables 4 and 5, when results are expressed in terms of protein content, antimicrobials as well as a number of cytokine and chemokine concentrations are diminished during pregnancy relative to that seen in CVL from non-pregnant women. Our results are presented in both ways for completeness. What remains incompletely elucidated is why protein levels increase sharply with pregnancy. Whether these proteins alter immune function is an unanswered question. One source of protein in CVL during pregnancy may be due to altered genital tract secretion resulting from hormone levels that are distinct from that seen in the non-pregnant women. Alternatively, the protein content of the cervical mucous plug may be high in protein content. Whatever the reason, further studies are needed to understand the underlying mechanisms of elevated protein levels in CVL during pregnancy and whether immune protection is altered as a result of these changes.

Because the body needs to tolerate the foreign paternal antigens in a developing fetus, maternal immunity is altered during pregnancy. This has been shown in previous studies of genital immunity in pregnancy and could be a plausible explanation for the epidemiologic phenomena demonstrating an increased risk of HIV acquisition in pregnancy.[27,28] This is an area of controversy because there are a number of elements of maternal immunity that remain intact throughout gestation so while maternal immunity may be altered, it is not likely to be suppressed per se.[29] The difference in concentration in these antimicrobial peptides between people more or less susceptible to infection in this *in vitro* model is the topic of planned future work. To the best of our knowledge, this is the first study to demonstrate that secretions from the lower genital tract of pregnant women are capable of potent anti-HIV activity. It further suggests that immune mediators play a role in protecting pregnant and non-pregnant women. Determining which factor or factors are responsible for preventing HIV infection could lead to therapeutic approaches in the form of a vaginal microbicide utilizing a woman's own immunity. Future work will examine the differences between pregnant and non-pregnant women with this goal in mind.

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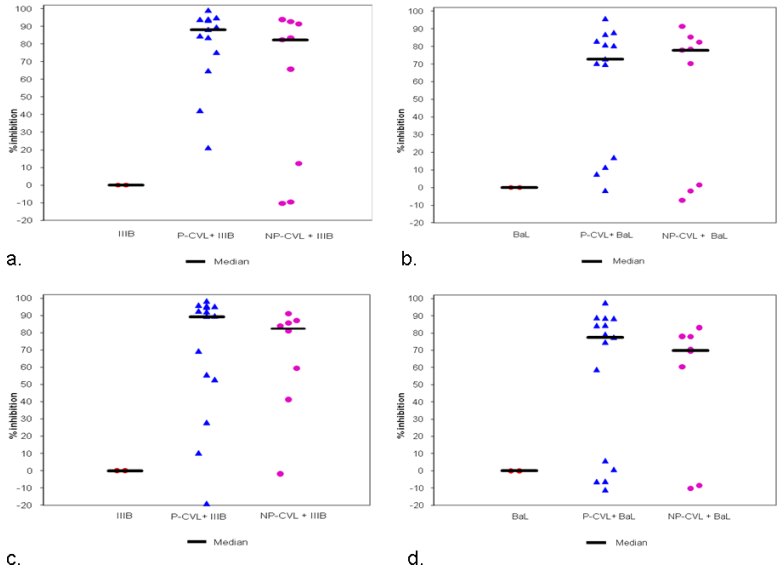

Figure 1. Median percent inhibition by pregnancy status

Each data point in the graph represents one individual patient sample. In the positive control (virus only) column, each point represents replicate wells and no inhibition of infectivity is noted. When CVL from pregnant and non-pregnant women is added, infectivity is inhibited.

a: Enrollment visit using IIIB virus

b: Enrollment visit using BaL virus

c: Second study visit using IIIB virus

d: Second study visit using BaL virus

P-CVL: pregnant women cervicovaginal lavage fluid, NP-CVL: non-pregnant women cervicovaginal lavage fluid

Table 1

Demographic characteristics of all enrolled participants

	Pregnant (n=13)	Non-pregnant (n=9)
Age in years	24 (18-33)	21 (18-35)
Gestational age in weeks	23.5 (15-26)	Not applicable
Race		
Black/African-American	2 (11.1)	2 (18.2)
White	12 (66.7)	7 (63.6)
Other/More than one race	4 (22.2)	2 (18.2)
Insurance		
Private	6 (30.0)	6 (54.6)
Medicaid	8 (40.0)	0
Other	4 (20.0)	0
Uninsured	2 (10.0)	5 (45.5)
Marital status		
Single	10 (50.0)	9 (81.8)
Married	10 (50.0)	2 (18.2)
Education level		
<High school	2 (10.0)	0
High school/equivalent	8 (40.0)	1 (9.1)
Some college	8 (40.0)	8 (72.7)
College graduate	2 (10.0)	2 (18.2)
Employment		
Unemployed	8 (40.0)	2 (18.2)
Employed full-time	6 (30.0)	4 (36.4)
Employed part-time	5 (25.0)	5 (45.5)
Other	1 (5.0)	0
Income level		
<$10,000	2 (11.1)	4 (36.4)
$10,000-24,999	8 (44.4)	1 (9.1)
$25,000-49,999	4 (22.2)	3 (27.3)
$50,000+	4 (22.2)	3 (27.3)
Body Mass Index (kg/m^2)	25.9 (20.1-40.5)	24.4 (20.6-39.8)

Data presented as median (range) or n(%)

Table 2

HIV-1 infectivity in the presence of CVL as compared to positive control at enrollment

	Pregnant (n=13) Median (Range)	**P***	**Non-pregnant (n=9) Median (Range)**	**P***
Illb % inhibition	88.2% (21.3 – 99.1)	0.0002	82.4% (-10.4 – 93.9)	0.02
BaL % inhibition	72.8% (-1.7 – 95.7)	0.0005	77.9% (-7.2 – 91.3)	0.04

* p value for comparison to no inhibition (positive control) by signed rank test

Table 3

HIV-1 infectivity in the presence of CVL as compared to positive control at return visit

	Pregnant (n=15) Median (Range)	**P**	**Non-pregnant (n=8) Median (Range)**	**P**
Illb % inhibition	89.7% (-19.1 – 98.3)	0.0002	82.5% (-1.8 – 91.1)	0.02
BaL % inhibition	77.4% (-11.1 – 97.5)	0.004	69.9% (-10.3 – 83.1)	0.04

* p value for comparison to no inhibition (positive control) by signed rank test

Table 4

Immune mediators by pregnancy status at enrollment (n=22)

	Median (Range)			
	Pregnant (n=13)	**Non-pregnant (n=9)**	**P[1]**	**P[2]**
Gestational age in weeks	23.5 (15-26)			
RANTES				
(pg/ml)	6.0 (4.2-92.2)	5.1 (4.2-17.9)	**0.03**	0.05
(pg/10μg protein)	0.8 (0.2-2.8)	1.9 (0.2-50.6)	0.1	0.1
Eotaxin				
(pg/ml)	28.4 (0-136.1)	18.0 (0-92.3)	0.2	0.1
(pg/10μg protein)	2.5 (0-6.7)	8.1 (0-66.7)	0.06	0.2
Fractalkine				
(pg/ml)	86.7 (44.2-1475.7)	44.2 (44.2-351.1)	0.1	0.09
(pg/10μg protein)	8.5 (1.6-37.4)	29.7 (1.8-440.2)	0.1	0.09
G-CSF				
(pg/ml)	12.1 (3.5-2795.6)	6.7 (3.5-24447.5)	0.1	0.1
(pg/10μg protein)	1.7 (0.5-73.9)	7.2 (0.7-214.5)	0.2	0.6
IL-1α				
(pg/ml)	56.2 (9.9-1812.2)	6.5 (2.7-1838.5)	**0.02**	**0.03**
(pg/10μg protein)	4.0 (0.8-35.2)	4.4 (1.9-26.9)	0.9	0.7
IL-1RA				
(pg/ml)	24588.8 (1991.5-84614.6)	776.4 (0.1-57124.8)	**0.03**	**0.03**
(pg/10μg protein)	1283.0 (289.3-5908.1)	405.1 (0.3-2133.9)	0.06	0.2
IL-6				
(pg/ml)	6.3 (4.5-103.9)	4.9 (4.0-751.6)	0.2	0.2
(pg/10μg protein)	0.9 (0.3-3.5)	4.4 (0.8-39.8)	**0.01**	**0.03**
IL-8				
(pg/ml)	243.4 (0-4443.1)	32.9 (0-36052.4)	0.3	0.3
(pg/10μg protein)	13.0 (0-143.5)	6.3 (0-316.4)	0.7	0.9
IP-10				
(pg/ml)	165.0 (0-933.5)	0 (0-2970.4)	0.2	0.1
(pg/10μg protein)	10.1 (0-132.3)	0 (0-27.0)	0.1	0.1
MCP-1				
(pg/ml)	18.2 (4.2-94.6)	8.5 (4.2-890.5)	0.8	0.5
(pg/10μg protein)	0.9 (0.6-2.6)	7.4 (2.0-48.2)	**0.002**	**0.001**
MIP-1α				
(pg/ml)	30.2 (13.4-108.2)	19.6 (2.6-455.0)	0.3	0.2
(pg/10μg protein)	2.2 (0.9-8.3)	7.8 (1.2-176.0)	**0.04**	0.09
MIP-1β				

	Median (Range)			
	Pregnant (n=13)	**Non-pregnant (n=9)**	**P[1]**	**P[2]**
(pg/ml)	20.4 (0-585.3)	7.3 (0-660.8)	0.3	0.1
(pg/10μg protein)	1.9 (0-11.4)	2.4 (0-72.7)	0.6	0.6
TNF-α				
(pg/ml)	0 (0-145.8)	0 (0-13.0)	0.3	0.1
(pg/10μg protein)	0 (0-2.8)	0 (0-0.1)	0.2	0.1
GM-CSF				
(pg/ml)	0.2 (0-91.8)	0 (0-6.1)	0.2	0.3
(pg/10μg protein)	0.1 (0-1.8)	0 (0-1.2)	0.1	0.1
Elafin				
(pg/ml)	32310 (7510-79160)	35930 (12270-89800)	0.7	0.5
(pg/10μg protein)	3749.1 (146.1-7503.0)	25718.6 (162.5-181990.0)	**0.02**	**0.03**
SLPI				
(pg/ml)	58426 (0-224000)	18304 (3404-230000)	0.4	0.4
(pg/10μg protein)	2790.8 (0-21060.9)	9500.4 (1842.9-77655.4)	0.06	0.07
HBD2				
(pg/ml)	710 (100-8220)	1110 (30-9650)	0.6	0.3
(pg/10μg protein)	35.1 (8.8-1164.9)	211.1 (39.3-4265.4)	**0.03**	**0.03**
MIP-3α				
(pg/ml)	24 (4-660)	40 (4-3420)	1.0	0.8
(pg/10μg protein)	1.4 (0.1-24.5)	22.5 (0.8-42.7)	**0.01**	**0.04**

RANTES: Regulated on Activation, Normal T Expressed and Secreted; G-CSF: granulocyte-colony stimulating factor; IL: interleukin; RA: receptor antagonist; IP: interferon-inducible protein; MCP: monocyte chemoattractant protein; TNF: tumor necrosis factor; GM: granulocyte macrophage; SLPI: Secretory leukocyte protease inhibitor; HBD: human beta defensin; MIP: macrophage inflammatory protein

[1]P-value by Wilcoxon rank-sum test for difference between groups.

[2]P-value by van Elteren test for difference between groups, adjusting for overweight BMI & White race.

Table 5

Immune mediators by pregnancy status at return visit 1 (n=23)*

	Median (Range)			
	Pregnant (n=15)	**Non-pregnant (n=8)**	**P[1]**	**P[2]**
Gestational age in weeks	34 (28-38)	--	--	--
RANTES				
(pg/ml)	6.0 (4.2-47.2)	5.1 (4.2-28.3)	0.2	0.1
(pg/10μg protein)	0.9 (0.1-2.1)	3.1 (1.3-42.3)	**0.004**	**0.01**
Eotaxin				
(pg/ml)	20.9 (0-133.6)	17.1 (0-48.9)	0.4	0.3
(pg/10μg protein)	2.9 (0-12.6)	6.0 (0-41.1)	**0.04**	0.1
Fractalkine				
(pg/ml)	44.2 (0-625.2)	44.2 (0-360.4)	0.8	0.2
(pg/10μg protein)	9.0 (0-22.5)	37.1 (0-451.4)	**0.02**	0.1
G-CSF				
(pg/ml)	8.5 (0-14202.9)	6.7 (3.5-54.2)	0.1	0.3
(pg/10μg protein)	1.2 (0-205.3)	4.5 (1.1-67.9)	0.08	0.5
IL-1α				
(pg/ml)	49.1 (5.9-2193.1)	6.9 (2.7-41.3)	**0.01**	**0.01**
(pg/10μg protein)	2.7 (0.7-31.7)	4.3 (0.7-33.8)	0.7	0.3
IL-1RA				
(pg/ml)	40898.3 (408.4-85612.1)	442.7 (0-14440.9)	**0.002**	**0.01**
(pg/10μg protein)	3012.9 (161.5-7003.0)	272 (0-5929.3)	**0.01**	**0.01**
IL-6				
(pg/ml)	4.5 (2.9-70.8)	4.2 (3.5-29.9)	0.5	0.3
(pg/10μg protein)	0.8 (0.1-2.2)	3.3 (1.2-40.8)	**0.006**	**0.04**
IL-8				
(pg/ml)	118.0 (0-4100.3)	25.8 (0-778.1)	0.1	0.4
(pg/10μg protein)	11.4 (0-281.9)	13.7 (0-104.3)	0.8	0.7
IP-10				
(pg/ml)	165.0 (0-2141.8)	0 (0-144.3)	0.06	**0.01**
(pg/10μg protein)	8.0 (0-82.9)	0 (0-19.4)	0.1	0.09
MCP-1				
(pg/ml)	7.6 (2.8-103.0)	6.5 (4.2-20.9)	0.7	0.7
(pg/10μg protein)	0.8 (0.1-2.7)	3.3 (1.3-42.4)	**0.006**	**0.008**
MIP-1α				
(pg/ml)	30.2 (0-103.5)	21.1 (0-66.6)	0.2	0.2
(pg/10μg protein)	3.9 (0-17.3)	8.7 (0-77.3)	0.09	0.3
MIP-1β				

	Median (Range)			
	Pregnant (n=15)	**Non-pregnant (n=8)**	**P[1]**	**P[2]**
(pg/ml)	13.6 (0-267.8)	10.5 (0-103.3)	0.4	0.09
(pg/10μg protein)	2.4 (0.1-7.7)	7.7 (0-13.8)	0.2	0.6
TNF-α				
(pg/ml)	0 (0-59.9)	0 (0-23.2)	1.0	0.2
(pg/10μg protein)	0 (0-1.6)	0 (0-3.1)	0.8	0.8
GM-CSF				
(pg/ml)	1.2 (0-27.6)	0 (0-5.6)	0.2	**0.04**
(pg/10μg protein)	0.2 (0-1.0)	0 (0-0.8)	0.5	0.06
Elafin				
(pg/ml)	29390 (12270-81700)	28405 (18240-54900)	0.5	0.6
(pg/10μg protein)	3025.2 (186.9-12345.1)	15375 (4184.5-186122.4)	**0.02**	**0.04**
SLPI				
(pg/ml)	31616 (0-68672)	22759 (6586-98452)	1.0	0.5
(pg/10μg protein)	2551.5 (0-21898.6)	11764.9 (2069.4-146571.4)	**0.02**	0.1
HBD2				
(pg/ml)	900 (10-8740)	640 (80-5140)	0.8	0.8
(pg/10μg protein)	36.8 (3.5-1239.4)	451 (69.5-2110.4)	**0.03**	**0.004**
MIP-3α				
(pg/ml)	4 (4-104)	16 (4-5140)	0.1	0.2
(pg/10μg protein)	1.6 (0.1-15.3)	16.3 (1.1-2110.4)	**0.01**	**0.04**

RANTES: Regulated on Activation, Normal T Expressed and Secreted; G-CSF: granulocyte-colony stimulating factor; IL: interleukin; RA: receptor antagonist; IP: interferon-inducible protein; MCP: monocyte chemoattractant protein; TNF: tumor necrosis factor; GM: granulocyte macrophage; SLPI: Secretory leukocyte protease inhibitor; HBD: human beta defensin; MIP: macrophage inflammatory protein

[1]P-value by Wilcoxon rank-sum test for difference between groups.

[2]P-value by van Elteren test for difference between groups, adjusting for overweight BMI & White race.