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Journal of Reproductive Immunology xxx (2008) xxx–xxx

JOURNAL OF REPRODUCTIVE IMMUNOLOGY

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Correlations of selected vaginal cytokine levels with pregnancy-related traits in women with bacterial vaginosis and mycoplasmas

Kelli K. Ryckman [a,b], Scott M. Williams [a,b,*], Jaroslaw Kalinka [c]

[a] *Department of Medicine, Vanderbilt University, Nashville, TN, USA*
[b] *Center for Human Genetics Research, Vanderbilt University, Nashville, TN 37232, USA*
[c] *Medical and Environmental Pregnancy Health Hazards Unit, Department of Perinatology, I Chair of Gynecology and Obstetrics, Medical University of Lodz, Poland*

Received 18 July 2007; received in revised form 6 November 2007; accepted 14 February 2008

Abstract

The aim of this study was to examine correlations between vaginal inflammatory cytokines (IL-1α, IL-1β, IL-6 and IL-8) and pregnancy-related traits (gestational age, birth-weight, BMI, weight gain during pregnancy and vaginal pH). Differences in correlation coefficients were examined among bacterial vaginosis (BV) status and the presence or absence of mycoplasmas. A total of 105 women between the 22nd and 34th week of pregnancy were enrolled in this study. There was a strong negative correlation between IL-1α and weight gain during pregnancy ($r = -0.877$, $p < 0.001$) and a strong positive correlation between IL-6 and BMI ($r = 0.670$, $p = 0.024$) in women with normal vaginal flora and mycoplasmas. These correlations were not present in women who had normal flora and no mycoplasmas. In women with BV and no mycoplasmas, there were significant correlations of gestational age with IL-6 ($r = 0.727$, $p = 0.027$) and IL-8 ($r = 0.689$, $p = 0.040$); however, these correlations were not significant in women with mycoplasmas. Our findings support the conclusion that correlations between inflammatory cytokines and pregnancy-related traits are dependent on context, suggesting that expression is labile. In particular, BMI and gestational age correlation differs depending on BV status and the presence or absence of BV-related mycoplasmas such as *Mycoplasma hominis* and *Ureaplasma urealyticum*.

Keywords: Bacterial vaginosis; *Ureaplasma urealyticum*; *Mycoplasma hominis*; Inflammatory cytokine levels; Coordinated protein expression; Pregnancy

1. Introduction

Bacterial vaginosis (BV) is considered one of the most prevalent vaginal disorders in adult women and is found in approximately 15–40% of pregnant women (Hillier et al., 1995; Goldenberg et al., 1996; Meis et al., 1998). BV is characterized by alterations in the normal vaginal flora, such as a decrease in lactobacilli and overgrowth of *Gardnerella vaginalis*, *Prevotella* and *Mobiluncus* species (Koumans and Kendrick, 2001). There is strong evidence that BV is associated with severe adverse pregnancy outcomes, such as preterm delivery (PTD), premature rupture of membranes (PROM), amniotic fluid infection and low birth-weight (Silver et al., 1989; Martius and Eschenbach, 1990; Kurki et al., 1992; Hillier et al., 1995).

* Corresponding author at: Center for Human Genetics Research, Vanderbilt University, 519 Light Hall, Nashville, TN 37232, USA. Tel.: +1 615 322 8036; fax: +1 615 343 8619.
E-mail address: smwilliams@chgr.mc.vanderbilt.edu (S.M. Williams).

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doi:10.1016/j.jri.2008.02.001

Recent evidence suggests that myoplasmas such as *Mycoplasma hominis* (*M. hominis*) and *Ureaplasma urealyticum* (*U. urealyticum*), which are found more frequently in women with BV, may also contribute to poor pregnancy outcomes. *M. hominis* is found in 58–76% of women with BV and has been associated with preterm birth (Hill, 1993; Usui et al., 2002) *U. urealyticum* is found in 62–92% of women with BV and is associated with low birth-weight (Hill, 1993; Cassell et al., 1993; Gonzalez et al., 2006); however, the Vaginal Infections and Prematurity Study Group did not find any association with *U. urealyticum* and poor pregnancy outcome, including low birth-weight, PTD or PROM (Carey et al., 1991). It is unclear if these microorganisms are found independently or are part of a flora that occurs in conjunction with other BV-related microorganisms to increase the risk for negative pregnancy outcomes.

It is well established that inflammatory cytokines such as interleukin-1α (IL-1α), interleukin-1β (IL-1β), interleukin-6 (IL-6) and interleukin-8 (IL-8) play critical roles in regulating the response to infections (Kelso, 1998). IL-1 is produced by many different cells, including macrophages, and is important in B-cell growth and the production of immunoglobulin. IL-1 plays a crucial role also in inflammation and fever. IL-6 is produced by activated Th2 cells and other somatic cells, and promotes the replication of B-cells and production of immunoglobulin. IL-6 does not induce the production of other cytokines; however, it does act synergistically with IL-1α to promote T-cell activation. IL-8 is a chemokine produced by macrophages and other somatic cells, and acts as a chemoattractant for neutrophils and T cells. Because of their clear roles in inflammation and infection, these cytokines are good candidates to investigate when examining the pathogenesis of BV and mycoplasma infection.

We (Wasiela et al., 2005) and others (Platz-Christensen et al., 1993; Imseis et al., 1997; Mattsby-Baltzer et al., 1998; Sturm-Ramirez et al., 2000; Cauci et al., 2003) have found significantly higher levels of IL-1α and IL-1β in women with BV, but have found no difference in the concentration of IL-6. We (Wasiela et al., 2005), but not others (Imseis et al., 1997; Mattsby-Baltzer et al., 1998), have found IL-8 concentrations are significantly (∼2 times) higher in women with BV. Strong positive correlations between levels of IL-1β with IL-1α and IL-8, and correlations between IL-6 and IL-8, have been observed in women with BV, and often these correlations are stronger in women with BV than in healthy controls (Cauci et al., 2003; Wasiela et al., 2005). A weak correlation ($r = 0.31$, $p = 0.017$) between IL-1β and IL-6 in women with BV has been observed (Wasiela et al., 2005).

Several inflammatory cytokine levels have been found to associated with the presence of mycoplasmas, specifically *M. hominis* and *U. urealyticum*. For example, the presence of *M. hominis* associates with an increase in vaginal IL-1β levels, but not with posterior vaginal interleukin-1 receptor antagonist (IL-1rα) levels nor intra-amniotic fluid levels of IL-1β, IL-1rα and IL-6 (Doh et al., 2004; Perni et al., 2004). *U. urealyticum* associates with an increase in posterior vaginal IL-1rα levels, but not with vaginal IL-1β concentrations or intra-amniotic fluid levels of IL-1β, IL-1rα or IL-6 (Doh et al., 2004; Perni et al., 2004). In addition, vaginal levels of IL-8 but not IL-1α, IL-1β or IL-6 are higher in women with mycoplasma infection, specifically the presence of either *M. hominis* or *U. urealyticum* (Wasiela et al., 2004).

To determine the role these cytokines play in both BV and mycoplasma infection, we conducted an exploratory analysis of the correlations between selected vaginal inflammatory cytokines (IL-1α, IL-1β, IL-6 and IL-8) and pregnancy-related traits measured at enrollment such as pH, body mass index (BMI), weight gain and gestational age. Examining the correlation patterns among vaginal cytokines, and between cytokines and pregnancy-related traits, also gives insight into the regulation of the inflammatory response. Differences in these correlations were examined by BV status and between women with and without the presence of mycoplasmas, specifically *M. hominis* and *U. urealyticum*.

2. Materials and methods

2.1. Study participants

The study population was comprised of 120 women at 22–34 weeks gestation recruited from patients at the clinical hospital at the Department of Perinatology, Medical University of Lodz, Poland, between May 2001 and December 2002. Only singleton pregnancies were qualified for inclusion in the survey. The exclusion criteria were: antibiotic therapy less than 4 weeks prior to examination, vaginal bleeding and serious maternal diseases.

This prospective cohort study was approved by the Biomedical Ethics Committee of the Medical University of Lodz, Poland. (Decision No. RNN/215/00). Each participant provided written informed consent to participate in the study. A standard questionnaire covering medical, socio-economic, demographic and constitutional aspects, as well as smoking, was administered to every subject and verified based on medical records. Routine ultrasound examination of fetal biometry was

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performed. The gestational age at the time of sampling was based on the time of last menstruation and verified by early ultrasound (CRL-crown-rump length) of the fetus. Weight gain was determined relative to pre-pregnancy weight and weight at enrollment. BMI was calculated at enrollment.

In a previous report (Wasiela et al., 2005) some of the same analyses were performed, such as comparison of cytokine levels by BV status and the presence of mycoplasmas. Also, cytokine correlations were examined by BV status; however, in the present paper we have excluded individuals with *Chlamydia trachomatis* (11 women) because the infection could affect vaginal cytokine levels and thus bias the results of this study. Also excluded were individuals with zero values for 3 or more cytokines (4 women) because this could indicate poor sample quality. Therefore, our final study group comprised of 105 women. We expanded our analysis also to include correlations between cytokine levels and pregnancy-related traits.

2.2. Bacteriological examination

For the assessment of biocenosis in the lower genital tract, vaginal and cervical swabs were collected from the pregnant women under study. Bacteriological tests of cervical swabs were made to screen for *M. hominis* and *U. urealyticum*. For isolation and identification of genital mycoplasmas, the commercially available Mycoplasma DUO kit (Sanofi Diagnostics, Pasteur, Marnes la Coquette, France) was used. Groups were established as having neither *M. hominis* nor *U. urealyticum* (68 women) or having one or both mycoplasma (37 women). BV was diagnosed by Gram stain of vaginal smear according to Spiegel's criteria (Spiegel et al., 1983). Based on microbiological results, three groups were distinguished: grade I, normal vaginal flora (47 women); grade II, intermediate vaginal flora (31 women); and grade III, BV (27 women). Measurements from the lateral vaginal wall were taken to obtain pH at enrollment using pH paper (Merck).

2.3. Cytokine measurements

Cervicovaginal fluids were obtained by Dacron swabs from the posterior fornix. The Dacron swabs were then placed in a glass probe containing 2 mL phosphate-buffer saline solution and stored at −70 °C. Samples were analyzed for the concentration of selected cytokines by a commercially available standard enzyme-linked immunosorbent assay kit according to the manufacturer's protocol (Endogen). The sensitivities of the assay for IL-1β and IL-6 were <1 pg/mL, and for IL-1α and IL-8 it was <2 pg/mL. For data analysis purposes, half of the sensitivity level was recorded for any reading that was below detectable levels.

2.4. Statistical analysis

Continuous data are reported as mean ± standard deviation, or as median (range) if the distribution was not normal or the variances between groups were not equal. Differences in cytokine concentrations and baseline traits among BV status were evaluated by one-way analysis of variance (ANOVA) or by the Kruskal–Wallis test for traits that were not normally distributed. Differences in traits by presence or absence of mycoplasmas were evaluated with Student's *t*-test, or the Mann–Whitney *U*-test if the distributions were not normal. Calculations were performed in STATA (version 9.2 for Windows, Stata Corporation, TX, USA).

Spearman's rank correlation coefficients were calculated for pair-wise combinations of cytokine concentrations and pregnancy-related traits. Student's *t*-test was performed on the Fisher *r*-to-*z* transformations of the Spearman correlation coefficients to determine heterogeneity between BV statuses and the presence and absence of mycoplasma. Correlation calculations were performed with the JMP-IN® software package (Sall et al., 2005). Graphical representations of correlations among BV status and between the presence and absence of mycoplasmas is similar to previous work by Asselbergs et al. (2007) and Reilly et al. (1994). In the figures, solid lines indicate a significant positive correlation and dotted lines indicate a significant negative correlation. The thickness of the line indicates the strength of the correlation. We considered *p*-values less than 0.05 to be significant.

3. Results

3.1. Baseline characteristics

The only clinically measured trait that differed by BV status was pH, which was highest in women with BV (Table 1). Vaginal IL-1α levels significantly increased with increasing BV scores ($p < 0.001$). Women with intermediate flora had vaginal levels of IL-1α that were about one and a half times that of women with normal flora (medians of 19.8 and 11.9 pg/mL, respectively) and the concentration was about six times higher in women with BV (median, 74.9 pg/mL). The vaginal concentra-

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Table 1
Baseline characteristics by BV status

	Normal ($n=47$)	Intermediate ($n=31$)	BV ($n=27$)	p-Value
Age (years)	27.3 ± 4.9	27.8 ± 5.1	26.1 ± 3.7	0.366
BMI	25.0 ± 3.2	24.3 ± 3.0	24.1 ± 3.5	0.467
GA (weeks)	28.3 ± 4.6	28.3 ± 3.6	29.7 ± 3.9	0.309
Vaginal pH	5.0 (4.0–5.8)	5.0 (4.0–7.0)	5.2 (4.4–7.0)	**0.017**
WG (kg)	9.8 ± 4.2	9.3 ± 4.2	8.6 ± 3.9	0.491
BW (g)	3132.0 ± 624.9	3162.8 ± 579.3	3099.3 ± 594.0	0.926
Vaginal IL-1α (pg/mL)	11.9 (0.7–296.7)	19.8 (1.0–457.8)	74.9 (1.0–392.2)	**<0.001**
Vaginal IL-1β (pg/mL)	2.3 (0.5–332.4)	11.7 (0.5–433.8)	36.2 (0.5–296.7)	**<0.001**
Vaginal IL-6 (pg/mL)	6.4 (0.5–80.7)	5.5 (0.5–76.6)	9.4 (0.5–62.3)	0.554
Vaginal IL-8 (pg/mL)	399.1 (2.4–1065.5)	390.3 (16.2–1010.2)	656.7 (1.0–1075.0)	0.195

Traits are presented as mean ± S.D., or as median (range) if the distribution was skewed. Bolded values indicate $p<0.05$. p-Values were calculated using ANOVA or the Kruskal–Wallis test if the distribution was skewed. BMI, body mass index; GA, gestational age; WG, weight gain; BW, birth-weight; IL-1α, interleukin-1 alpha; IL-1β, interleukin-1 beta; IL-6, interleukin-6; IL-8, interleukin-8.

tions of IL-1β also significantly differed by BV status ($p<0.001$), with the intermediate group having concentrations 5 times higher than those of the normal flora group (medians of 11.7 and 2.3 pg/mL, respectively). Women with BV had vaginal IL-1β concentrations that were almost sixteen times higher than women with normal flora (median of 36.2 pg/mL). Vaginal concentrations of IL-6 and IL-8 did not differ with respect to BV status.

Birth-weight ($p=0.025$) and pH ($p<0.001$) differed with presence or absence of mycoplasmas, with pH being higher and birth-weight being lower in women with mycoplasmas (Table 2). Vaginal IL-1β, IL-6 and IL-8 levels were significantly higher in women with mycoplasmas compared to those without (Table 2).

When examining the differences between women stratified by both BV status and presence or absence of mycoplasmas, pH differed across all groups ($p=0.005$) (Table 3). Women with normal flora but without mycoplasmas had the lowest pH (median of 4.9), and those with intermediate flora and mycoplasmas had the highest pH (median of 5.8). Vaginal IL-1α, IL-1β and IL-8 concentrations differed significantly across groups ($p=0.005$, $p<0.001$ and $p=0.032$, respectively). Vaginal IL-1α concentrations were lowest in women with normal flora but without mycoplasmas (median of 10.9 pg/mL), while the highest concentrations were found in women with BV and the absence of mycoplasmas (median of 130.6 pg/mL). Vaginal IL-1β concentrations were lowest in women with normal flora and mycoplasmas (median of 0.5 pg/mL). The highest vaginal IL-1β concentrations were found in women with BV and mycoplasmas (median of 44.6 pg/mL). Vaginal IL-8 concentrations were lowest in women with intermediate flora and no mycoplasmas, and highest in women with intermediate flora and mycoplasmas (medians 252.5 and 732.1 pg/mL, respectively). Vaginal IL-6 concentrations were not significantly different between groups.

Table 2
Baseline characteristics by presence of or absence of mycoplasma

	No mycoplasma ($n=68$)	Any mycoplasma ($n=37$)	p-Value
Age (years)	27.7 ± 4.9	26.0 ± 4.2	0.081
BMI	24.3 ± 3.0	25.0 ± 3.6	0.304
GA (weeks)	28.5 ± 4.2	29.1 ± 4.1	0.467
Vaginal pH	5.0 (4.0–6.5)	5.3 (4.5–7.0)	**<0.001**
WG (kg)	9.2 ± 3.9	9.6 ± 4.5	0.641
BW (g)	3227.6 ± 523.8	2946.8 ± 692.3	**0.025**
Vaginal IL-1α (pg/mL)	19.6 (0.7–457.8)	42.8 (1.0–392.4)	0.130
Vaginal IL-1β (pg/mL)	6.7 (0.5–433.8)	21.0 (0.5–296.7)	**0.028**
Vaginal IL-6 (pg/mL)	5.1 (0.5–80.7)	11.9 (0.5–76.6)	**0.034**
Vaginal IL-8 (pg/mL)	306.6 (2.4–1065.5)	656.7 (1.0–1075.0)	**0.004**

Traits are presented as mean ± S.D., or as median (range) if the distribution was skewed. Bolded values indicate $p<0.05$. p-Values were calculated using ANOVA or the Mann–Whitney U-test if the distribution was skewed. BMI, body mass index; GA, gestational age; WG, weight gain; BW, birth-weight; IL-1α, interleukin-1 alpha; IL-1β, interleukin-1 beta; IL-6, interleukin-6; IL-8, interleukin-8.

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Table 3
Baseline characteristics by the combination of BV score and presence or absence of mycoplasmas

	No mycoplasma			Any mycoplasma			*p*-Value
	Normal ($n=36$)	Intermediate ($n=23$)	BV ($n=9$)	Normal ($n=11$)	Intermediate ($n=8$)	BV ($n=18$)	
Age (years)	27.3 ± 4.8	28.9 ± 5.3	26.2 ± 3.4	27.0 ± 5.5	24.8 ± 2.9	26.0 ± 3.9	0.250
BMI	24.4 ± 2.9	24.4 ± 3.2	23.3 ± 3.0	26.6 ± 3.9	23.9 ± 2.5	24.4 ± 3.7	0.278
GA (weeks)	28.3 ± 4.8	28.5 ± 3.5	29.0 ± 3.8	28.5 ± 4.2	27.5 ± 4.0	30.1 ± 4.1	0.682
Vaginal pH	4.9 (4.0–5.8)	5.0 (4.0–6.5)	5.2 (4.4–6.5)	5.0 (4.5–5.8)	5.8 (4.7–7.0)	5.2 (4.7–7.0)	**0.005**
WG (kg)	9.2 ± 3.8	9.4 ± 4.5	9.0 ± 2.4	11.9 ± 4.6	9.1 ± 3.4	8.4 ± 4.5	0.374
BW (g)	3168.5 ± 530.6	3246.5 ± 554.8	3402.2 ± 413.4	3008.0 ± 900.7	2841.7 ± 607.8	2947.8 ± 621.5	0.275
Vaginal IL-1α (pg/mL)	10.9 (0.7–296.7)	21.9 (1.0–457.8)	130.6 (5.0–318.3)	24.4 (1.0–234.2)	15.9 (1.0–392.4)	49.6 (1.0–392.2)	**0.005**
Vaginal IL-1β (pg/mL)	3.4 (0.5–332.4)	9.3 (0.5–433.8)	36.2 (0.5–149.7)	0.5 (0.5–26.8)	30.5 (0.5–143.8)	44.6 (0.5–296.7)	**<0.001**
Vaginal IL-6 (pg/mL)	6.8 (0.5–80.7)	3.0 (0.5–62.7)	3.2 (0.5–28.6)	6.0 (0.5–59.7)	24.6 (2.7–76.6)	10.9 (0.5–62.3)	0.114
Vaginal IL-8 (pg/mL)	354.4 (2.4–1065.5)	252.5 (16.2–931.8)	383.8 (43.5–856.0)	579.7 (46.2–843.7)	732.1 (94.3–1010.2)	688.4 (1.0–1075.0)	**0.032**

Traits are presented as mean ± S.D., *z* or as median (range) if the distribution was skewed. Bolded values indicate $p<0.05$. *p*-Values were calculated using ANOVA or the Kruskal–Wallis test if the distribution was skewed. BMI, body mass index; GA, gestational age; WG, weight gain; BW, birth-weight; IL-1α, interleukin-1 alpha; IL-1β, interleukin-1 beta; IL-6, interleukin-6; IL-8, interleukin-8.

3.2. Correlations by BV status

In women with normal flora, there were four significant positive correlations: IL-8 with IL-1α ($r=0.508$), IL-1β ($r=0.396$) and IL-6 ($r=0.347$), and IL-1α with IL-1β ($r=0.379$) (Fig. 1A; Supplemental Table 1). In women with intermediate flora, there were five significant positive correlations, the strongest being identical to those found in normal flora: IL-8 with IL-1α ($r=0.520$), IL-1β ($r=0.607$) and IL-6 ($r=0.625$) and IL-1α with IL-1β ($r=0.606$) (Fig. 1B; Supplemental Table 1). The additional significant correlation present in women with intermediate but not normal flora was between IL-8 and pH. In women with BV, there were three significant positive correlations: IL-1α with IL-1β ($r=0.686$) and BMI ($r=0.439$), and IL-6 with IL-8 ($r=0.603$) (Fig. 1C; Supplemental Table 1). The only significant difference in correlation coefficients between women with normal flora and women with BV was the correlation between IL-1α and BMI ($p=0.015$). This was also the only significant difference in correlation coefficients between women with intermediate flora and BV ($p=0.037$). There were no significant differences in correlation coefficients between women with normal flora and those with intermediate flora.

3.3. Correlations by presence or absence of mycoplasmas

In women without mycoplasmas, there were eight significant positive correlations and the largest correlations were IL-1α with IL-1β ($r=0.639$) and IL-8 ($r=0.459$) and IL-8 with IL-1β ($r=0.481$) and IL-6 ($r=0.532$) (Fig. 2A; Supplemental Table 2). In women with mycoplasmas there were two negative and three positive significant correlations, the strongest being IL-1α with IL-1β ($r=0.504$) and weight gain during pregnancy ($r=-0.492$), and IL-8 with weight gain ($r=-0.427$) (Fig. 2B; Supplemental Table 2). The most significant heterogeneity in correlation coefficients between women with and without mycoplasmas were for IL-1α with weight gain ($p=0.003$) and IL-8 with weight gain during pregnancy ($p=0.001$) (Fig. 3; Supplemental Table 2).

In these analyses, the presence of *M. hominis* and *U. urealyticum* were grouped as either the presence of any mycoplasma or the absence of both, because in preliminary analysis when comparing women with only *M. hominis* to those with only *U. urealyticum*, there was only a single significant difference in cytokine correlation between women with *M. hominis* and *U. urealyticum* ($p=0.04$ heterogeneity of the correlation coefficients

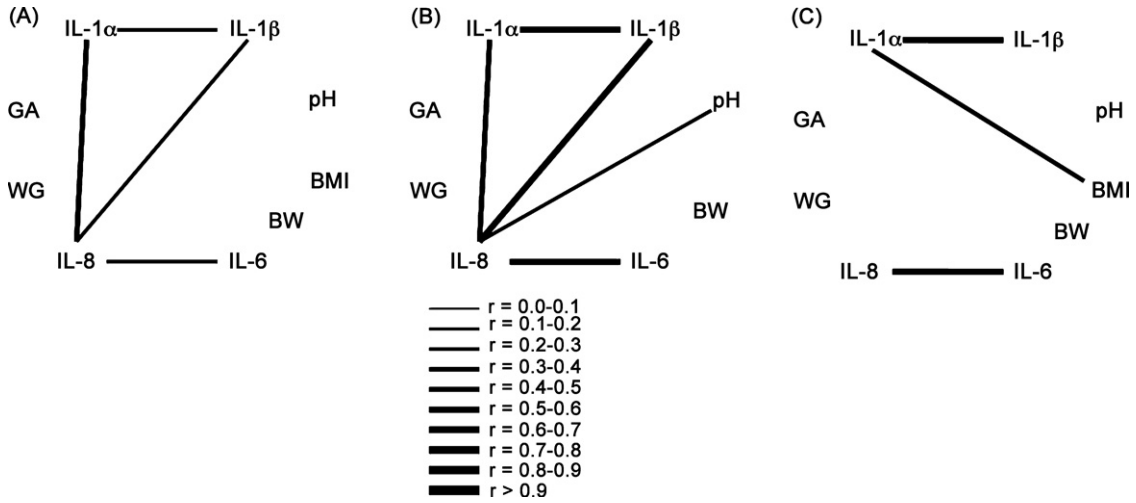

Fig. 1. Significant correlations in women with normal flora (A), intermediate flora (B) and BV (C). Solid lines indicate a significant positive correlation and dotted lines indicate a significant negative correlation. The thickness of the line indicates the strength of the correlation.

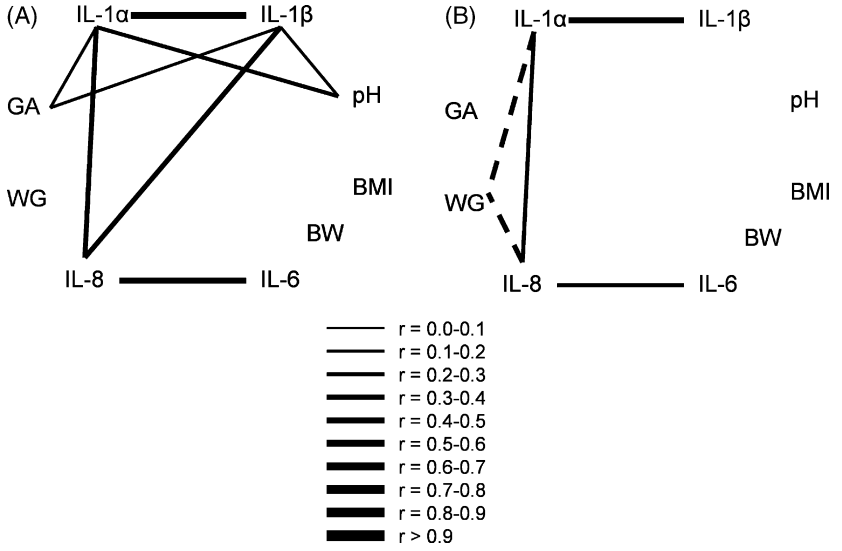

Fig. 2. Significant correlations in women without mycoplasmas (A) and with mycoplasmas (B). Solid lines indicate a significant positive correlation and dotted lines indicate a significant negative correlation. The thickness of the line indicates the strength of the correlation.

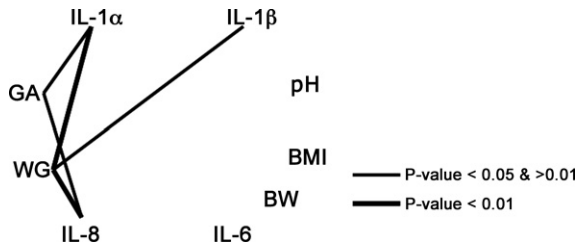

Fig. 3. Significant differences between women with and without mycoplasmas. Lines indicate a significant difference in correlation coefficients. Thicker lines indicate a p-value <0.01 and thinner lines indicate a p-value <0.05.

between IL-1α and IL-1β). A single difference was within expectation for the number of tests performed (26).

3.4. Correlations by both BV status and presence or absence of mycoplasmas

The graphical representations of the correlations between vaginal cytokine levels and pregnancy-related traits divided among six groupings based on BV status and the presence or absence of mycoplasmas is shown in Supplementary Figs. 1 and 2. There were four significant differences among correlations between the

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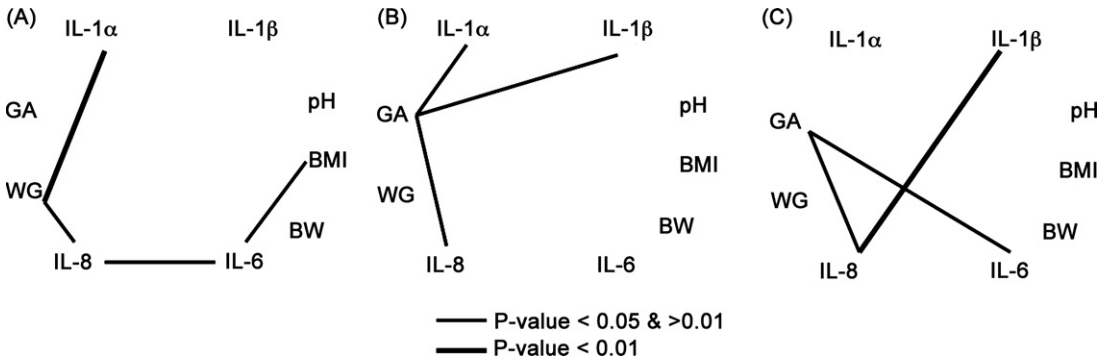

Fig. 4. Significant differences between the presence and absence of mycoplasmas in women with normal flora (A), intermediate flora (B) and BV (C). Lines indicate a significant difference in correlation coefficients. Thicker lines indicate a p-value <0.01 and thinner lines indicate a p-value <0.05.

presence or absence of mycoplasmas in women with normal flora: IL-1α with weight gain ($p < 0.001$), IL-6 with IL-8 ($p = 0.016$) and BMI ($p = 0.030$), and IL-8 with weight gain ($p = 0.049$) (Fig. 4A, Supplemental Table 3). Women with intermediate flora showed significant heterogeneity between the presence and absence of mycoplasmas for gestational age with IL-1α ($p = 0.010$), IL-1β ($p = 0.018$) and IL-8 ($p = 0.035$) (Fig. 4B; Supplemental Table 3). Three correlations between the presence and absence of mycoplasmas in women with BV were significantly heterogeneous: gestational age with IL-6 ($p = 0.010$) and IL-8 ($p = 0.019$), and IL-1β with IL-8 ($p = 0.003$) (Fig. 4C; Supplemental Table 3).

4. Discussion

In this exploratory analysis, we have examined correlations between several cytokines and pregnancy-related traits to determine how the correlations were affected by BV status and presence or absence of micro-organisms-related to BV. The goal of this study was to assess the coordinated expression of vaginal inflammatory cytokine levels and pregnancy-related traits. Our analyses identified several factors that strongly correlate in women with normal flora, intermediate flora and BV. It is poorly understood how the presence or absence of mycoplasmas, *M. hominis* and *U. urealyticum*, affects adverse pregnancy outcomes and inflammatory cytokine levels outside the context of BV (Taylor-Robinson, 2007). Therefore, we examined the correlation coefficients and differences in these correlations in women stratified by both BV status and presence of mycoplasmas. The patterns of correlation were noticeably heterogeneous as a function of the microflora present in these women, suggesting that different flora motivate different coordinated inflammatory processes.

There were a total of 26 pair-wise combinations examined for correlations subdivided by BV status and the presence of mycoplasmas. Therefore, based on a significance of 0.05, it is expected that 1.3 of these correlations would be significant in each group. There were more correlations than expected for each group, except for women with intermediate BV flora and mycoplasmas. Also, there were more differences in correlation coefficients than expected for all of the comparisons, except for those among women stratified by BV status. The largest proportion of correlation differences were between women with or without mycoplasmas regardless of BV status. This suggests that, while there were strong correlations in women with and without BV, there are few differences between groups in these correlations. Mycoplasmas, however, appear to play an important role in disrupting the coordinated expression between inflammatory cytokines and pregnancy-related traits.

Obesity has been associated previously with an increase in plasma concentrations of IL-1α (Yudkin et al., 1999; Di et al., 2007). In our data, correlations between vaginal levels of IL-1α and BMI were not significantly different when women were stratified by mycoplasma status only or by mycoplasma and BV status. However, correlations between vaginal levels of IL-1α and BMI were significantly stronger in women with BV than in women with intermediate or normal vaginal flora. BMI did not significantly differ between women with normal, intermediate or BV vaginal flora. Therefore, this correlation may indicate that BMI associates with an increased inflammatory response (IL-1α) caused by infection with BV.

Weight gain during the first 22–34 weeks of pregnancy was negatively correlated with IL-1α and IL-8 in women with mycoplasmas; however, these correla-

Please cite this article in press as: Ryckman, K.K., et al., Correlations of selected vaginal cytokine levels with pregnancy-related traits in women with bacterial vaginosis and mycoplasmas, J. Reprod. Immunol. (2008), doi:10.1016/j.jri.2008.02.001

tions were not present in women without mycoplasmas. This could suggest that women infected with mycoplasmas gained weight more slowly or it could indicate that the increased inflammatory response initiated by the mycoplasma infection is causing or contributing to a decreased ability to gain weight during pregnancy.

Generally, correlations between inflammatory cytokines were stronger in women with intermediate flora compared to normal flora and in women with BV compared to those with intermediate flora (Fig. 1). The opposite was the case in women subdivided by presence of mycoplasma only; women without mycoplasmas generally had stronger correlations between the inflammatory cytokines (Fig. 2). This suggests that, while inflammatory cytokines increase in both women with BV and those infected with mycoplasmas, only in the presence of BV is this response correlated among the cytokines. Strong correlations between IL-6 and IL-8 have been observed previously in women with and without BV; however, these studies did not examine this in conjunction with both BV status and mycoplasmas (Cauci et al., 2003; Wasiela et al., 2005). Our results indicate that mycoplasma status is important for understanding these correlations.

A study examining vaginal IL-1β, IL-6 and IL-8 concentrations in women during pregnancy who were not infected with BV found that IL-6 and IL-8 concentrations significantly dropped during the mid-trimester of pregnancy (14–28 weeks) and returned to pre-pregnancy levels during the third trimester (>28 weeks) (Donders et al., 2003). IL-1β was found in decreasing amounts throughout pregnancy; however, this difference was not significant (Donders et al., 2003). In our study, we observed only one significant correlation between vaginal cytokine concentrations and gestational age at enrollment in women without mycoplasmas, regardless of BV status, or in women with BV in the absence of mycoplasmas. That our results appear to differ could be because, in the previous study, a wide range of gestational ages were analyzed whereas, in our study, there was a relatively narrow window of gestational ages examined. However, it is interesting that our results support the previous study when considering only women without mycoplasmas.

Some limitations of the present study include: (1) the fact that we did not correct for multiple testing. We did not do this because the study was designed primarily as an exploratory analysis (2) The sample sizes were small for some of the comparisons. Nonetheless, this study demonstrates that, in order to fully understand the impact of BV on vaginal inflammatory cytokine levels and pregnancy-related traits, other microbiologic information such as the presence or absence of mycoplasmas must be considered.

In summary, clinical outcomes mediated by changing vaginal cytokine levels must be considered in the context of multiple infections. Also, it is important to consider correlation patterns to understand better the underlying biological response to infection and its impact on pregnancy. Our analyses can be used to guide future studies on the relationship of inflammatory cytokines and pregnancy-related traits in relation to the microbiology of vaginal flora.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jri.2008.02.001.

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