

Differential vaginal expression of interleukin-1 system cytokines in the presence of *Mycoplasma hominis* and *Ureaplasma urealyticum* in pregnant women

Kunihiko Doh¹, Parrin T. Barton², Irina Korneeva¹, Sriram C. Perni², Ann Marie Bongiovanni¹, Sara L. Tuttle², Daniel W. Skupski² and Steven S. Witkin¹

¹Division of Immunology and Infectious Diseases, Department of Obstetrics and Gynecology, Weill Medical College of Cornell University, New York, USA

²Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Weill Medical College of Cornell University, New York, USA

Objective: The genital mycoplasmas, *Ureaplasma urealyticum* and *Mycoplasma hominis*, are commonly identified in the vagina of healthy pregnant women. However, these microorganisms are the most common isolates from the amniotic fluids of women in preterm labor. The mechanisms responsible for vaginal colonization and ascent to the uterus remain undetermined. We evaluated the association between *U. urealyticum* and *M. hominis* vaginal colonization and the presence of pro-inflammatory and anti-inflammatory interleukin-1 system components in asymptomatic pregnant women of different ethnicities.

Methods: Vaginal specimens, obtained from 224 first trimester pregnant women, were assayed for interleukin-1 β (IL-1 β) and IL-1 receptor antagonist (IL-1ra) concentrations by ELISA. *U. urealyticum* and *M. hominis* vaginal colonization were identified by polymerase chain reaction (PCR).

Results: Vaginal colonization with *M. hominis* was identified in 37 (16.5%) women, and was more prevalent in black (18.9%) and Hispanic (20.9%) than in white (4.2%) women ($p = 0.01$). *U. urealyticum* was present in 84 (37.5%) women and there was no ethnic disparity in its detection. *M. hominis* colonization was associated with elevated median vaginal IL-1 β concentrations in both black women ($p = 0.02$) and Hispanic women ($p = 0.04$), and was unrelated to vaginal IL-1ra concentrations. In marked contrast, *U. urealyticum* colonization was associated with elevations in vaginal IL-1ra levels, but not with IL-1 β concentrations, in black women ($p = 0.02$) and Hispanic women ($p < 0.0001$) and marginally in white women ($p = 0.06$).

Conclusion: *M. hominis* colonization in healthy pregnant women is associated with localized pro-inflammatory immune activation, while *U. urealyticum* colonization is associated with immune suppression.

Key words: MYCOPLASMAS; PREGNANCY; VAGINAL COLONIZATION; INTERLEUKIN-1 β ; INTERLEUKIN-1 RECEPTOR ANTAGONIST

INTRODUCTION

Mycoplasma hominis and *Ureaplasma urealyticum* are frequent colonizers of the vagina during preg-

nancy^{1–4}. In most women the presence of these microorganisms is without apparent effect on pregnancy outcome. In some women, however, *M. hominis* and *U. urealyticum* ascend to the

Correspondence to: S. S. Witkin, PhD, Department of Obstetrics and Gynecology, Weill Medical College of Cornell University, 1300 York Avenue, Box 35, New York, NY 10021, USA. Email: switkin@med.cornell.edu

endometrium, either prior to or during pregnancy, where they may contribute to preterm labor and neonatal morbidity. *U. urealyticum* and *M. hominis* are the microorganisms most frequently cultured from amniotic fluids of women in preterm labor^{5,6}. A recent study demonstrated an association between *U. urealyticum* in second trimester amniotic fluids and a subsequent increased rate of premature rupture of fetal membranes (PPROM) and preterm labor and delivery⁷.

The host factors associated with *M. hominis* and *U. urealyticum* vaginal colonization and migration to the upper genital tract in some women but not in others have not been extensively examined. *Ureaplasma urealyticum* vaginal colonization in pregnant women has been related to elevated vaginal concentrations of interleukin-1 receptor antagonist (IL-1ra)⁴. IL-1ra is an anti-inflammatory cytokine and a natural competitive inhibitor of the pro-inflammatory cytokine, interleukin-1 β (IL-1 β)⁸. Anti-microbial cell-mediated immune defenses are initiated by IL-1 β production⁹.

The aim of the present study was to perform a direct comparison, in a group of pregnant women of known ethnic backgrounds, of the relationship between *M. hominis* and *U. urealyticum* vaginal colonization and vaginal IL-1 β and IL-1ra concentrations.

MATERIAL AND METHODS

Subjects

A total of 224 consecutive first trimester black, Hispanic and white pregnant women from the outpatient obstetrics clinic at The New York Presbyterian Hospital, Weill Medical College of Cornell University, with no known pregnancy complications were studied. Ninety of the women (40.2%) were black, 86 (38.4%) were Hispanic and 48 (21.4%) were white and of European background. The median ages of the subjects were 25 years (range 15–44 years) for black participants, 26 years (range 14–42 years) for Hispanic participants and 28 years (range 19–39 years) for white participants. The difference in ages between groups was not significant ($p = 0.07$). All subjects participated in the New

York State Prenatal Care Assistance Program for low income women. Each subject underwent a complete physical examination that included testing for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* by DNA hybridization, bacterial vaginosis by clinical criteria and other microbial pathogens by standard techniques. Infections were diagnosed in 34 (15.2%) of the subjects. This included 16 women with bacterial vaginosis, eight with *C. trachomatis*, four with *N. gonorrhoeae*, and two each with syphilis, *Candida albicans* or urinary tract infections. All were treated and were asymptomatic at the time of specimen collection. Six (2.7%) women subsequently developed preterm premature rupture of fetal membranes and 14 (6.3%) delivered preterm (< 37 weeks).

This study was approved by the Institutional Review Board of The New York Presbyterian Hospital-Weill Cornell Medical Center.

Specimens

Specimens were obtained from the posterior vaginal walls with a cotton swab and deposited into a test tube containing phosphate-buffered saline. The specimens were kept at 4°C and transported to the laboratory within 3–4 h. In the laboratory as much liquid as possible was extruded from the swabs. Supernatant and pellet fractions were obtained by microcentrifugation and stored separately at –80°C until tested.

Detection of *M. hominis*

An aliquot of the pellet fraction from each subject was tested for *M. hominis* by a published polymerase chain reaction protocol employing primer-pairs specific for a 324 base pair region of the 16S ribosomal RNA gene¹⁰, except that digoxigenin-labeled dUTP was added to the reaction mixture. To insure specificity, the PCR amplicons were hybridized to a biotinylated oligonucleotide internal probe (5¹-biotin-GCC CAC CAA GAC TAT GAT GTT TAG-3¹) and the complex detected in duplicate by ELISA utilizing streptavidin-coated wells of a microtiter plate and peroxidase-labeled anti-digoxigenin antibody (Roche Diagnostics, Mannheim, Germany). Purified *M. hominis* was always processed

and analyzed in parallel to the test samples as a positive control. H₂O blanks served as negative controls.

Detection of *U. urealyticum*

An aliquot of the pellet fraction was analyzed for *U. urealyticum* by a published PCR protocol¹¹, utilizing digoxigenin-labelled dUTP and primer pairs specific for a 429 base pair region of the urease gene. Analysis was as described above for *M. hominis*, using 5'-biotin- GAG ATA ATG ATT ATA TGT CAG GAT CA-3' as the biotinylated internal probe. Purified *U. urealyticum* was always processed and analyzed in parallel to the test samples as a positive control. H₂O blanks served as negative controls.

Vaginal IL-1ra and IL-1β protein concentrations

The thawed supernatant fractions were tested in duplicate for IL-1ra and IL-1β concentrations by commercial ELISA assays (BioSource International, Camarillo, CA). The mean optical density values were converted to ng/ml (IL-1ra) or pg/ml (IL-1β) by reference to a standard curve utilizing purified cytokines. The lower limit of detection of the assay was 4 pg/ml for IL-1ra and 1 pg/ml for IL-1β.

Statistics

Differences in IL-1β and IL-1ra levels between groups were non-randomly distributed and analyzed by the non-parametric Mann-Whitney test. Comparisons between discrete variables were analyzed by Fisher's exact test. A *p* value < 0.05 was considered significant.

RESULTS

Detection of *M. hominis* and *U. urealyticum* in vaginal samples from pregnant women

M. hominis was detected in 37 (16.5%) of the women tested, while *U. urealyticum* was identified in 84 (37.5%) women. The relation between *M. hominis* and *U. urealyticum* detection and subjects' ethnicity is shown in Table 1. *M. hominis* was

Table 1 Relation between ethnicity and vaginal colonization with *M. hominis* and *U. urealyticum*

Ethnic group	No. women	No. (%) positive	
		<i>M. hominis</i>	<i>U. urealyticum</i>
Black	90	17 (18.9)	30 (33.3)
Hispanic	86	18 (20.9)	38 (44.2)
White	48	2 (4.2) ^a	16 (33.3)

^a*p* = 0.01 vs. black and Hispanic women

identified in 20.9% of the Hispanic women and 18.9% of the black women, but in only 4.2% of the white subjects (*p* = 0.01). In contrast, *U. urealyticum* colonization was unrelated to ethnicity. Hispanic women positive for *M. hominis* were considerably younger (median of 21 years of age) than Hispanic women who were negative (median of 27 years of age) for this mycoplasma (*p* = 0.001). There was no relationship between *M. hominis* colonization and age in the black and white populations, or between *U. urealyticum* colonization and any of the three ethnic groups.

M. hominis and *U. urealyticum* colonization and vaginal cytokine levels

The relation between *M. hominis* or *U. urealyticum* vaginal colonization and vaginal concentrations of IL-1ra and IL-1β was analyzed. *M. hominis* colonization was associated with elevated vaginal IL-1β levels in the subjects as a whole (*p* = 0.007) and in both blacks subjects (*p* < 0.02) and Hispanic subjects (*p* = 0.04), and a markedly decreased IL-1ra to IL-1β ratio. The number of white women who were *M. hominis*-positive was too few to analyze. In contrast, there was no relation between the presence of *M. hominis* and vaginal IL-1ra concentration (Table 2).

U. urealyticum colonization was associated with elevated vaginal IL-1ra levels in the study population as a whole (*p* < 0.0001), as well as in black women (*p* < 0.02) and Hispanic women (*p* = 0.002) (Table 3). IL-1ra was also elevated in whites women that were positive for *U. urealyticum*, but this did not reach statistical significance (*p* = 0.06). *U. urealyticum* colonization was unrelated to vaginal IL-1β levels in all groups; the

Table 2 Relation between *M. hominis* vaginal colonization, ethnicity and vaginal concentrations of IL-1 β and IL-1ra

Ethnic group	Microorganism	Median IL-1 β (range)	Median IL-1ra (range)
All	<i>M. hominis</i> (+)	19.8pg/ml (0-440)	350 ng/ml (35-1800)
	<i>M. hominis</i> (-)	9.2 pg/ml (0-448) ^a	275 ng/ml (0-1900)
Black	<i>M. hominis</i> (+)	22.6 pg/ml (13.7-184) ^b	337 ng/ml (75-1800)
	<i>M. hominis</i> (-)	9.6 pg/ml (12.8-204)	250 ng/ml (10-1900)
Hispanic	<i>M. hominis</i> (+)	17.7 pg/ml (0-440) ^c	425 ng/ml (35-1800)
	<i>M. hominis</i> (-)	7.8 pg/ml (0-448)	287 ng/ml (0-1900)

^a*p* = 0.007, ^b*p* = 0.02, ^c*p* = 0.04 vs. *M. hominis* (-)

Table 3 Relation between *U. urealyticum* vaginal colonization, ethnicity and vaginal concentrations of IL-1 β and IL-1ra

Ethnic group	Microorganism	Median IL-1 β (range)	Median IL-1ra (range)
All	<i>U. urealyticum</i> (+)	12.7 pg/ml (0-372)	550 ng/ml (35-2000) ^a
	<i>U. urealyticum</i> (-)	9.2 pg/ml (0-364)	250 ng/ml (10-1800)
Black	<i>U. urealyticum</i> (+)	15.2 pg/ml (0-204)	374 ng/ml (60-1900) ^b
	<i>U. urealyticum</i> (-)	11.8 pg/ml (0-148)	237 ng/ml (10-1800)
Hispanic	<i>U. urealyticum</i> (+)	10.7 pg/ml (0-372)	672 ng/ml (35-1800) ^c
	<i>U. urealyticum</i> (-)	8.8 pg/ml (0-242)	250 ng/ml (10-1800)
White	<i>U. urealyticum</i> (+)	21.8 pg/ml (4-148)	340 ng/ml (90-2000) ^d
	<i>U. urealyticum</i> (-)	9.2 pg/ml (0-364)	200 ng/ml (0-1800)

^a*p* < 0.0001, ^b*p* = 0.02, ^c*p* = 0.002, ^d*p* = 0.06

IL-1ra to IL-1 β ratio substantially increased in the presence of *U. urealyticum*.

There were no significant differences in the vaginal concentrations of IL-1 β and IL-1ra between the different ethnic groups. Among the 17 women positive for both mycoplasmas the median vaginal concentration of IL-1 β was elevated (31.2 pg/ml) as compared to those positive only for *U. urealyticum* (*p* = 0.02). Similarly, median vaginal IL-1ra levels were higher (550 ng/ml) as compared to those positive only for *M. hominis* (*p* = 0.006).

There was no apparent relationship between *U. urealyticum* and *M. hominis* vaginal colonization. Among black subjects, *U. urealyticum* was detected in 7 of 17 (41.2%) women who were positive for *M. hominis*. Similarly, among Hispanic subjects, 10 of the 18 women (55.6%) with *M. hominis* were also positive for *U. urealyticum*. There was no relationship between detection of *M. hominis* or *U. urealyticum* and a prior treated bacterial vaginosis or any other infection.

***M. hominis* and *U. urealyticum* colonization and pregnancy outcome**

The 14 women in our study who delivered preterm (6.3%), were too few to determine if there was an association between pregnancy outcome and mycoplasmal vaginal colonization. However, the rate of preterm birth was highest when *M. hominis* was present either alone or in conjunction with *U. urealyticum*. A preterm birth was documented in 7.3% of women who were positive only for *U. urealyticum*, 11.1% positive only for *M. hominis*, 11.8% positive for both microorganisms and 6.7% who were negative for both mycoplasmas.

DISCUSSION

The delineation of immune factors associated with vaginal colonization by individual microorganisms in healthy pregnant women has received scant research attention. Since both *M. hominis* and *U. urealyticum* have been implicated in

pregnancy-related pathology it is important to understand the factors involved in their colonization of the vagina. The results of the present study suggest that *M. hominis* and *U. urealyticum* differ in their immune responses in the vagina of pregnant women. Specifically, *M. hominis* vaginal colonization was associated with elevated vaginal IL-1 β concentrations and unchanged IL-1ra levels, while the reverse was true for *U. urealyticum* colonization.

Differences between *M. hominis* and *U. urealyticum* in relation to vaginal colonization and pathogenic effects, and which may reflect their differential effect on induction of IL-1 system components, have been noted by other investigators. Carriage of the specific genotype of the polymorphic IL-1ra gene that is associated with elevated IL-1ra production appears to influence the rate of *U. urealyticum* vaginal colonization⁴, but not *M. hominis* colonization¹². In both asymptomatic pregnant and non-pregnant women, *U. urealyticum* vaginal colonization is much more prevalent than is *M. hominis* colonization^{3,13-15}. This might reflect the capability of *U. urealyticum*, but not *M. hominis*, to inhibit the local pro-inflammatory immune response. Conversely, there is an increase in the rate of vaginal *M. hominis* colonization, but not *U. urealyticum* colonization, in association with bacterial vaginosis, or *Trichomonas vaginalis* or *C. trachomatis* infections^{16,17}. Immune system alterations induced by pathogenic microorganisms may alter local immune defense mechanisms and thereby selectively enhance the likelihood of *M. hominis* colonization and proliferation. It has also been suggested that *M. hominis* magnifies the effects of other sexually transmitted infections¹⁷. Our observation that *M. hominis* is associated with a pro-inflammatory immune response, i.e. elevated vaginal IL-1 β concentration, in the vagina of healthy asymptomatic women suggests that the enhanced proliferation of this mycoplasma in conjunction with other infections results in increased vaginal inflammation. It might also be relevant that the ability of *M. hominis* but not *U. urealyticum*, to induce the release of high levels of histamines, a potent inducer of inflammation, from rat mast cells has been noted¹⁸.

The influence of *M. hominis* and *U. urealyticum* on neonatal pathology also appears to differ. Tracheal aspirates of preterm infants are frequently colonized with *U. urealyticum*, without any apparent effect on the rate of bronchopulmonary dysplasia¹⁹ or chronic lung disease²⁰. In one study, the presence of *U. urealyticum* in tracheal aspirates was actually associated with a reduced rate of cystic periventricular leukomalacia²¹. Colonization of the placenta by *U. urealyticum* was shown to be unrelated to an increased rate of cerebral damage in preterm infants, while the presence of placental *M. hominis* was associated with a three-fold increased risk of echolucency²². The present data support the suggestion by Dammann *et al.* that *U. urealyticum* may be a marker for decreased inflammatory capability²².

Our previous study⁴ and the present investigation noted no racial differences between white and non-white subjects in *U. urealyticum* colonization rates. In the present study *M. hominis* was predominantly detected in non-white women. Racial disparities in microbial vaginal colonization have been reported previously^{17,23,24}. A prior study of microbial colonization in black and Hispanic women with a concurrent sexually transmitted infection found *M. hominis* colonization to be associated with the black race²¹. There are two major differences between this investigation and our study. Beside the absence of sexually transmitted diseases in our population, the Hispanic women analyzed in our study were from Puerto Rico and other Caribbean islands, while in the previous study the Hispanics were of Mexican ancestry.

The rate of adverse pregnancy outcome in this cohort of patients was not outside the expected range for this population. The number of patients was insufficient to be able to comment on whether mycoplasmal vaginal colonization was associated with an increased rate of any adverse pregnancy outcome. In addition, our PCR analysis was only qualitative and not quantitative.

Thus, the present study suggests that *M. hominis* colonization may be recognized by the immune system in the vagina of pregnant women and results in the induction of pro-inflammatory immunity, while *U. urealyticum* colonization is

associated with induction of the anti-inflammatory cytokine, IL-1ra. Further investigations are needed, however, to definitively differentiate between a direct effect of these microorganisms on vaginal cytokine levels or whether *M. hominis* and/or *U. urealyticum* colonization is increased under conditions when immunity in the vaginal milieu is altered due to the presence of other infectious agents or non-infectious factors. Conditions that inhibit pro-inflammatory immunity

may lead to increased colonization/proliferation by *U. urealyticum*, and thereby enhance their capacity to ascend to the endometrium and cause clinical disease.

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