Interleukin-1 Receptor Antagonist Gene Polymorphism, Vaginal Interleukin-1 Receptor Antagonist Concentrations, and Vaginal Ureaplasma urealyticum Colonization in Pregnant Women

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Received 1 July 2002/Returned for modification 28 August 2002/Accepted 16 October 2002

Ureaplasma urealyticum is the microorganism most frequently isolated from amniotic fluids of women in preterm labor. The relationship between vaginal colonization with U. urealyticum, vaginal interleukin-1 receptor antagonist (IL-1ra) levels, and the IL-1ra genotype in pregnant women was examined. Vaginal specimens, obtained with a cotton swab from 207 women in their first trimester of pregnancy, were tested for IL-1ra concentrations by enzyme-linked immunosorbent assay and for U. urealyticum and IL-1ra genotypes by PCR. U. urealyticum was detected in 85 (41.1%) women. The median IL-1ra level was 450 ng/ml in women positive for U. urealyticum, as opposed to 225 ng/ml in women negative for this microorganism (P < 0.0001). Sixty-two percent of the 16 women who were homozygous for allele 2 of the IL-1ra gene (IL-1RN*2) were colonized with U. urealyticum, as opposed to 47% of the 49 women who were IL-1RN*1/IL-1RN*2 heterozygotes and 34% of the 133 women who were IL-1RN*1 homozygotes (P < 0.05). Median IL-1ra levels were 750 ng/ml in IL-1RN*2 homozygotes, 300 ng/ml in IL-1RN*1/IL-1RN*2 heterozygotes, and 250 ng/ml in IL-1RN*1 homozygotes (P = 0.02). The vast majority of subjects had an uneventful pregnancy and delivered a healthy infant at term. The IL-1ra genotype or U. urealyticum colonization was unrelated to birth weight. Pregnant women who are colonized with U. urealyticum during the first trimester have elevated vaginal IL-1ra concentrations and a higher prevalence of the IL-1RN*2 homozygote genotype than do noncolonized women.

Susceptibility to infections or microbial colonization and differences in the magnitudes of the immune response to different stimuli vary even between seemingly healthy individuals. A major contributor to these individual variations is the polymorphic nature of genes involved in immune reactions (30). One of the most intensively studied genes in this regard is the gene coding for the interleukin-1 receptor antagonist (IL-1ra) (27).

The IL-1ra gene and the genes coding for IL-1α and IL-1β are located within a 430-kb region of chromosome 2 (23). IL-1α and IL-1β are proinflammatory cytokines that bind to IL-1 receptors on a variety of cell types and trigger recruitment and activation of phagocytic cells, vascular dilation, fever, and inflammation (6). IL-1ra, by binding to the IL-1 receptors, but without inducing signal transduction, is a competitive inhibitor of IL-1 bioactivity (2). Typically, IL-1 synthesis is rapidly induced following an infection to mobilize immune defenses. At a later period, synthesis of IL-1ra is initiated to terminate the acute proinflammatory event and prevent chronic inflammation from damaging healthy cells (17).

The gene coding for IL-1ra is polymorphic due to an 86-bp tandem repeat sequence of variable length (24). Allele 1 (IL-1RN*1) contains four repeats and is more common than allele 2 (IL-1RN*2), which contains two repeats. The other three alleles are very rare. Most individuals are either homozygous for IL-1RN*1 or are IL-1RN*1/IL-1RN*2 heterozygotes (27).

The relationship between IL-1ra genotype and IL-1ra protein concentration has been studied with inconsistent results (reviewed in reference 27). Most frequently, individuals who were IL-1RN*2 homozygous produced higher levels of IL-1ra than did individuals with other genotypes (5, 8).

In this communication, we report on variations in IL-1ra genotype among 207 pregnant women and the relationship between the individual genotypes, vaginal concentrations of IL-1ra protein, and vaginal colonization by the mycoplasma Ureaplasma urealyticum. This microorganism is the most frequent bacterial isolate in amniotic fluids from women in preterm labor with intact membranes (21). Infection of the amniotic cavity is a consequence of the ascent of U. urealyticum from the vagina to the uterus. Therefore, the delineation of factors associated with U. urealyticum vaginal colonization is relevant to efforts to decrease susceptibility to preterm birth.

MATERIALS AND METHODS

Study population. The study population consisted of 207 women in the first trimester of pregnancy being seen in the outpatient obstetrics clinic at The New York Presbyterian Hospital. Women with known pregnancy complications were excluded. The study group consisted of 67 (32.3%) African-Americans, 62 (30.0%) Hispanics, 36 (17.4%) European Caucasians, 5 (2.4%) Asians, 7 (3.4%) women of other origins, and 30 (14.5%) women whose race could not be determined. The median ages of the subjects were 23.5 years (range, 17.0 to 44.0 years) for African-Americans, 26.0 years (range, 17.0 to 42.0 years) for Hispanics, 28.0 years (range, 19.0 to 39.0 years) for European Caucasians, and 33.0 years (range, 22.0 to 40.0 years) for the other women. The difference in ages between the African-Americans and the European Caucasians was significant (P = 0.02).

Each subject underwent a complete physical examination, including routine testing for Chlamydia trachomatis, Neisseria gonorrhoeae, bacterial vaginosis, and...
other pathogens. Perinatal infections were diagnosed in 26 (12.6%) of the subjects. All were treated with the appropriate antibiotics and were negative for these microorganisms prior to collection of specimens.

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

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

Detection of U. urealyticum. An aliquot of the pellet fraction from each subject was tested for U. urealyticum by PCR employing primer pairs specific for a 224-bp region of the urease gene, as described previously (14, 29). Purified U. urealyticum was always processed and analyzed in parallel to the test samples as a positive control. H2O blanks served as negative controls. The specificity of the PCR was verified by hybridization of the amplicons to a U. urealyticum-specific internal probe and detection by enzyme-linked immunosorbent assay (ELISA).

IL-1ra gene polymorphism. DNA was extracted from the thawed and washed pellet fractions by treatment with nonionic detergent and proteinase K. Details of this treatment, as well as the subsequent PCR with primer pairs that spanned the polymorphic region of the IL-1ra gene, have been described previously (7, 11). The sizes of the amplicons were determined by electrophoresis on 1% agarose gels after visualization with ethidium bromide.

Vaginal IL-1ra protein concentrations. The thawed supernatant fractions were tested in duplicate for IL-1ra concentrations by a commercial ELISA (BioSource International, Camarillo, Calif.). The mean optical density value was converted to nanograms per milliliter by reference to a standard curve utilizing purified IL-1ra that was analyzed in parallel to each batch of test samples. The lower limit of detection of the assay was 4.0 ng/ml.

Statistics. Differences between nonrandomly distributed variables were analyzed by the nonparametric Mann-Whitney test. Randomly distributed variables were analyzed by Student’s t test. A P value of <0.05 was considered significant.

RESULTS

U. urealyticum vaginal colonization. U. urealyticum was detected in 85 (41.1%) of the pregnant women. There was no difference in the rate of colonization among the different races. Among African-Americans, 32.8% were positive for this microorganism, as were 45.2% of Hispanics and 36.1% of European Caucasians. In the present study, there was a small but significant inverse relationship between U. urealyticum colonization and the subjects’ age. The median age (range) of positive women was 24.0 years (17.0 to 44.0 years), as opposed to 27.0 years (18.0 to 44.0 years) in negative women (P = 0.04). None of the women was positive for C. trachomatis, N. gonorrhoeae, or bacterial vaginosis at the time of sample collection. U. urealyticum colonization was unrelated to parity.

IL-1ra genotype and U. urealyticum colonization. Homozygosity for the IL-1RN*1 allele was detected in 64.3% of the women; 23.7% were IL-1RN*1/IL-1RN*2 heterozygous, 7.7% were IL-1RN*2 homozygous, and 4.3% had other allelic combinations. The relationship between IL-1ra genotype and U. urealyticum colonization is shown in Table 1. Among the 16 women who were IL-1RN*2 homozygous, 10 (62.5%) were positive for U. urealyticum in the vagina. This rate was greater than the 34.6% of IL-1RN*1 homozygotes who were colonized by this microorganism (P < 0.05). The colonization rate for IL-1RN*1/IL-1RN*2 heterozygotes was intermediate between these two values (46.9%), but was not statistically different from either of the homozygote values.

Vaginal U. urealyticum colonization and vaginal IL-1ra protein concentration. IL-1ra protein was detected in the vaginas of 95.2% of the women tested. The median IL-1ra protein concentration in the vagina, determined by ELISA, was strongly related to U. urealyticum colonization at that site. Among the 85 (41.1%) women colonized with U. urealyticum (as determined by PCR), the median vaginal IL-1ra protein concentration was 450 ng/ml (range, 10 to 2,000 ng/ml). This was exactly twice the median level (225 ng/ml; range, 0 to 1,900 ng/ml) identified in the 122 women who were not colonized by this microorganism (P < 0.0001). There was no relationship between total vaginal protein and IL-1ra concentration.

There was a marginal relationship between race and vaginal IL-1ra concentrations. Hispanic women had a median vaginal IL-1ra protein level of 603 ng/ml (range, 10 to 1,900 ng/ml). This was greater than the median level of 421 ng/ml (range, 0 to 1,900 ng/ml) observed for African-Americans (P = 0.03) and somewhat greater than the median level of 412 ng/ml (range, 0 to 2,000 ng/ml) observed for European Caucasians (P = 0.07). There was no statistically significant relationship between IL-1ra levels and a woman’s age or parity.

Vaginal IL-1ra protein concentration and IL-1ra genotype. The relationship between concentrations of IL-1ra protein in the vagina (ELISA) and the IL-1ra genotype (PCR) is shown as follows. Women who were IL-1RN*2 homozygous had higher levels of IL-1ra protein in their vaginas than did women who were IL-1RN*1 homozygous (P = 0.01) or IL-1RN*1/IL-1RN*2 heterozygous (P = 0.02). The median vaginal IL-1ra protein levels were 750 ng/ml (range, 35 to 2,000 ng/ml) for IL-1RN*2 homozygotes, 300 ng/ml (range, 0 to 1,900 ng/ml) for IL-1RN*1/IL-1RN*2 heterozygotes, and 250 ng/ml (range, 0 to 1,900 ng/ml) for IL-1RN*1 homozygotes. For women with other genotypes, the median vaginal IL-1ra concentration was 287 ng/ml (range, 35 to 1,500 ng/ml).

Pregnancy outcome. An uneventful pregnancy with a healthy full-term baby was observed in 186 (90.0%) of the women studied. Preterm premature rupture of membranes occurred in 14 (6.8%) women. Five (2.4%) women had a preterm delivery prior to 32 weeks, and two (1.0%) women gave birth to a baby with intrauterine growth restriction. There was no relationship between any of these adverse events with U. urealyticum colonization, vaginal IL-1ra protein levels, or IL-1ra genotype (data not shown).

There was no relationship between IL-1ra genotype and birth weight. The mean birth weights were 3,334 g in babies...
whose mothers were IL-1RN*1 homozygous, 3,449 g when the mother was IL-1RN*1/IL-1RN*2 heterozygous, and 3,322 g when the mother was IL-1RN*2 homozygous. Similarly, birth weight was unrelated to the presence or absence of U. urealyticum colonization, with means of 3,293 g in positive women and 3,410 g in negative women.

**DISCUSSION**

In the present study, 41.1% of 207 pregnant women were colonized in their vagina with *U. urealyticum*. Other investigators have also identified this mycoplasma as being a frequent lower-genital-tract isolate during pregnancy (3, 13, 18). Also, similar to other investigations (3, 13, 25), the presence of this microorganism in the vagina did not influence pregnancy outcome or birth weight in our study. The ascension of *U. urealyticum* from the vagina to the pregnant uterus is an uncommon event, and our sample size might not have been sufficient to detect an influence of vaginal colonization on pregnancy outcome. In addition, our analysis was not quantitative, and it has been reported that only high levels of *U. urealyticum* vaginal colonization were associated with adverse pregnancy outcome (1).

Similar to *U. urealyticum* vaginal colonization, IL-1ra appears to be a normal constituent of the vagina in first-trimester pregnant women. This is consistent with its presence in amniotic fluid (20) as well as in sera from healthy nonpregnant individuals (8, 12). Those asymptomatic pregnant women who were colonized with *U. urealyticum* in their vaginas had higher vaginal concentration of IL-1ra protein than did noncolonized women. There are at least three possible explanations for this observation. One possibility is that individuals with elevated vaginal IL-1ra levels have a less-efficient antimicrobial local immune response that increases the likelihood that colonizing microorganisms can persist. However, the observed association of *U. urealyticum* with IL-1RN*2* homozygosity makes this unlikely. In studies of nonpregnant individuals, IL-1RN*2* has been associated with enhanced antimicrobial immunity due to increased production of both IL-1ra and IL-1 (9, 22, 26). A second possibility is that *U. urealyticum* selectively induces IL-1ra production to facilitate its colonization. Although this has not been directly examined with human vagina-derived cells, several studies have demonstrated that *U. urealyticum* induces proinflammatory cytokine production both in vivo and in vitro (16, 19). A third, most likely, possibility is that *U. urealyticum* invokes a proinflammatory immune reaction in the vaginas of pregnant women and that elevated IL-1ra levels reflect its subsequent increased production to prevent development of chronic inflammation. Intrauterine infection in nonhuman primates has been shown to result in increased IL-1ra levels in the amniotic fluid (28). However, recent studies in our laboratory of women positive for vaginal *Mycoplasma hominis* suggest that an elevation of vaginal IL-1ra concentration is not induced by this mycoplasmal organism (unpublished data). Further studies are required to delineate the mechanism of IL-1ra induction concomitant with *U. urealyticum* colonization.

The distribution of IL-1ra genotypes in our population, with only 7.7% being IL-1RN*2* homozygous, was similar to those in other investigations worldwide (27). The increased vaginal concentration of IL-1ra protein in IL-1RN*2*-positive individuals parallels findings of increased levels of this protein in plasma in IL-1RN*2*-positive healthy individuals (8, 9). Since only 7.7% of women were IL-1RN*2* homozygous, while 41.1% were colonized with *U. urealyticum*, vaginal colonization by this microorganism clearly is not dependent on the presence of this unique genotype. However, the higher rate of colonization in the IL-1RN*2* homozygous women suggests that possession of this genotype might increase susceptibility to vaginal persistence by *U. urealyticum* and, perhaps, by other microorganisms as well. IL-1RN*2* homozygosity has clearly been associated with several autoimmune disorders, such as rheumatoid arthritis, inflammatory bowel diseases, and alopecia areata (reviewed in reference 27). Whether *U. urealyticum* colonization is increased in the presence of autoimmunity individuals with IL-1RN*2* homozygosity and/or is involved in the pathogenesis of these or other autoimmune disorders has not been investigated. The possible mechanism relating IL-1RN*2* homozygosity to *U. urealyticum* colonization also remains undetermined. Interestingly, both the development of autoimmune disorders and the production of IL-1ra are higher in females than in males (4, 15).

The results reported here are contrary to the observation of an inverse relationship between the IL-1RN*2* genotype and *U. urealyticum* vaginal colonization in nonpregnant women in Brazil (10). Possible explanations for this discrepancy might be differences in *U. urealyticum* strains or the presence of additional vaginal microorganisms or unique complicating ethnic and environmental variables between the two populations.

The possible influence of IL-1ra gene polymorphism on the outcome of high-risk pregnancies also deserves further study. We have recently observed that fetuses of Hispanic descent who were positive for the IL-1RN*2* allele were at increased risk for preterm premature rupture of membranes and preterm delivery (7). The absence of a similar effect in African-American or European Caucasian fetuses highlights the variability of the influence of this polymorphism, depending on ethnic, phenotypic, infectious, and environmental factors that may differ among individuals and populations.

**ACKNOWLEDGMENT**

This study was supported by National Institutes of Health grant no. HD41676.

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Editor: B. B. Finlay