Influence of Fetal Gender on the Concentration of Interleukin-1 Receptor Antagonist in Amniotic Fluid and in Newborn Urine

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ABSTRACT. IL-1 receptor antagonist (IL-1ra) is a cytokine that blocks the effects of IL-1 by binding to IL-1 receptors without inducing signal transduction. Amniotic fluid contains high concentrations of IL-1ra. The purpose of this study was 1) to analyze whether factors related to the mother or the fetus influence amniotic fluid IL-1ra concentration, and 2) to study whether the fetus is a source of IL-1ra. Two hundred two specimens of amniotic fluid, as well as 21 urine samples from newborn infants, were analyzed. Women carrying a female fetus had a higher concentration of amniotic fluid IL-1ra than those carrying a male fetus (female $136.4 \pm 6.1 \mu\text{g/L}$, $n = 83$; male $74.7 \pm 3.7 \mu\text{g/L}$, $n = 119$; $p < 0.0001$, unpaired two-sided t test). Length of gestation, presence or absence of labor signs, or elevated IL-1 β in amniotic fluid did not affect the concentration of IL-1ra in amniotic fluid. Urine of infants taken during the first 48 h of life contained a high concentration of IL-1ra ($91.1 \pm 17.5 \mu\text{g/L}$). The urinary IL-1ra concentration was higher in female newborns than in male newborns (females $124.0 \pm 25.2 \mu\text{g/L}$, $n = 11$; males $54.9 \pm 19.1 \mu\text{g/L}$, $n = 10$; $p = 0.04$). We conclude that 1) the concentration of IL-1ra in amniotic fluid and newborn urine is dependent on the gender of the fetus and of the newborn and 2) fetal urine is a major source of amniotic fluid IL-1ra. The higher IL-1ra in female-bearing gestations may contribute to the better resistance of female fetuses against preterm birth and perinatal infections. (*Pediatr Res* 35: 130-134, 1994)

Abbreviations

IL-1ra, IL-1 receptor antagonist
PROM, premature rupture of the membranes

IL-1 indicates two structurally related cytokines, IL-1 α and IL-1 β , that mediate many physiologic responses to infection and inflammation (1). IL-1 α and IL-1 β act through the same cell surface receptors and share biologic activities (1). IL-1 induces fever, sleep, anorexia, and hypotension (1). It also increases the synthesis of collagenases and prostaglandins, and stimulates the production of several inflammatory mediators (2-5).

IL-1ra is a cytokine that is structurally related to IL-1 α and IL-1 β (6). IL-1ra opposes the actions of IL-1 by competing with IL-1 for binding to IL-1 receptors without inducing signal trans-

duction (6). IL-1ra has been shown to block various IL-1-induced responses on many cells (6), including inflammatory changes caused by IL-1 (7). Exogenous administration of IL-1ra reduces mortality in animal models of sepsis or endotoxemia (8-12).

Preterm labor is strongly associated with intraamniotic infection (13). Bacterial products such as endotoxin stimulate the production of IL-1 by decidual macrophages (14). IL-1 stimulates the production of prostaglandins, major inducers of uterine contractions, by amnion and decidua (15, 16). The concentration of this cytokine is elevated in amniotic fluid in women in preterm labor with microbial invasion of the amniotic cavity (17). In addition, administration of IL-1 to animals induces preterm labor (18). For these reasons, IL-1 has been proposed to act as a mediator in preterm labor occurring in the setting of infection (19). IL-1ra, on the other hand, inhibits the IL-1-stimulated prostaglandin production by amnion cells (20, 21). It also prevents preterm delivery induced in mice by IL-1 (22).

Human amniotic fluid has been reported to contain high IL-1ra concentrations (20). This may be of importance in the defense of the fetoplacental unit against inflammatory conditions mediated by IL-1 (20-22). No patient-related factor is known to affect IL-1ra concentration in amniotic fluid. The source of amniotic fluid IL-1ra is likewise unknown. The purpose of this study was 1) to analyze whether factors related to the mother or the fetus influence the concentration of IL-1ra in amniotic fluid, and 2) to study whether the fetus could be a source of IL-1ra.

SUBJECTS AND METHODS

Informed consent was obtained from the pregnant patients and the parents of the infants participating in the study. All the amniocenteses were performed for diagnostic purposes.

Amniotic fluid samples. Altogether 202 pregnant women participated in the study; one sample was obtained from each patient. Patient characteristics are shown in Table 1. One hundred thirty-one samples were obtained at a gestational age less than 37 wk. Twenty-nine samples were from women in labor; 173 were from women without signs of labor. Five patients were in preterm labor (*i.e.* labor at gestational age less than 37 completed wk). One hundred eighty-three samples were obtained transabdominally. Nineteen samples were recovered from the vaginal pool; all of these were from patients in labor at term.

Urine samples. Urine samples of 10 preterm neonates (gestational ages between 27 and 37 wk) and of 11 term neonates were analyzed for IL-1ra. All the samples were collected during the first 48 h of life. The diagnostic categories of the infants were as follows, with the number of patients in each group shown in parentheses: prematurity (gestational age less than 37 wk) ($n = 4$), respiratory distress syndrome ($n = 4$), meconium aspiration ($n = 5$), intrauterine asphyxia ($n = 4$), infant of diabetic mother

Received May 24, 1993; accepted August 18, 1993.

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Supported by the Foundation for Maternal and Infant Care and the Sigrid Juselius Foundation.

Table 1. Characteristics of patients from whom amniotic fluid samples were obtained

Diagnosis	Total number of pregnant women	Number of women carrying male/female fetus	Gestation $\pm$ SD at time when sample was taken (d)
Normal	49	32/17	264 $\pm$ 22
PROM	17	9/8	220 $\pm$ 18
Preeclampsia	32	20/12	240 $\pm$ 17
Intrahepatic cholestasis of pregnancy	20	14/6	253 $\pm$ 6
Diabetic mother	25	14/11	259 $\pm$ 9
SGA*	14	5/9	249 $\pm$ 11
Multipara†	14	7/7	245 $\pm$ 8
Miscellaneous‡	31	18/13	250 $\pm$ 28

* SGA, small for gestational age.

† One sample of amniotic fluid was obtained from each pregnant woman.

‡ This category includes the following cases (*n*): fetal asphyxia (1), fetal macrosomia (3), fetal chromosomal anomaly (3), maternal hypertension (6), polyhydramnios (3), isoimmunization (8), maternal drug exposure (3), and other maternal disease (4).

(*n* = 1), congenital cytomegalovirus infection (*n* = 1), hydrocephalus (*n* = 1), and congenital anomaly (*n* = 1).

Western blot analysis of IL-1ra protein in amniotic fluid. Proteins were separated by PAGE in the presence of SDS under reducing conditions on a 15% acrylamide gel and electrotransferred from the gel onto a nitrocellulose membrane, followed by chromogenic detection (Immun-Blot Assay, Bio-Rad, Richmond, CA). A goat anti-human IL-1ra antibody (R & D Systems, Minneapolis, MN) was used as the primary antibody. The secondary antibody was rabbit anti-goat IgG alkaline phosphatase conjugate (Sigma, St. Louis, MO). Protein size was estimated by comparison to the 17-kD recombinant human IL-1ra (R & D Systems) and to molecular weight standards.

IL-1ra and IL-1 β assays. IL-1ra was quantitated using a commercially available ELISA kit (R & D Systems). The sensitivity was set at 650 ng/L (38 pmol/L) and the interassay and intraassay coefficients of variation were 8.4% and 5.9%, respectively. IL-1 β measurements were performed by a commercially available kit (Advanced Magnetics, Cambridge, MA). The sensitivity was 4.5 ng/L (265 fmol/L) and the interassay and intraassay coefficients of variation were 9.4% and 9.2%, respectively.

Determination of relative birth weight. Relative birth weight was calculated as follows: (birth weight of infant - mean birth weight of infants of same gestational age and gender)/(SD of birth weight of infants of same gestational age and gender).

Statistical methods. The data are expressed as mean $\pm$ SEM unless otherwise indicated. The Kruskal-Wallis test or regression analysis was used to study association of different variables and IL-1ra levels. The Mann-Whitney test, with Bonferroni correction, was used for post hoc comparisons of diagnostic groups. To detect differences of means between genders, the unpaired two-sided *t* test was used, with Bonferroni correction, where appropriate.

RESULTS

Western blot analysis of amniotic fluid IL-1ra. Amniotic fluid was analyzed by Western blot as described in Subjects and Methods. Amniotic fluid IL-1ra immunoreactivity migrated as one main band around 20 to 22 kD, slightly higher than unglycosylated recombinant human IL-1ra, which migrates around 17 kD (Fig. 1).

IL-1ra concentration in amniotic fluid: dependence on fetal gender. The mean concentration of IL-1ra in amniotic fluid was 100.0 $\pm$ 3.9 μ g/L (*n* = 202) with a range from 5.4 to 330 μ g/L. In samples taken from women carrying a male fetus, the mean concentration of IL-1ra was 74.7 $\pm$ 3.7 (*n* = 119). IL-1ra concentration was significantly higher [136.4 $\pm$ 6.1 (*n* = 83)] in amniotic fluid samples from women carrying a female fetus (Fig.

Fig. 1. Western blot of amniotic fluid IL-1ra. IL-1ra immunoreactivity in unconcentrated amniotic fluid and in amniotic fluid concentrated 10-fold is shown. The 17-kD recombinant human IL-1ra is run in parallel for comparison. On the right-hand side are molecular markers.

2, *p* < 0.0001, two-sided unpaired *t* test). This gender difference was seen at all gestational ages studied (Fig. 2).

Amniotic fluid IL-1ra was not dependent on gestational age (Fig. 2).

In laboring patients, the concentration of IL-1ra was 111.8 $\pm$ 11.8 μ g/L (*n* = 29). In patients not in labor, the concentration was 98.0 $\pm$ 4.2 μ g/L (*n* = 173). This difference was not significant.

Using multiple regression analysis (23), we studied the following two factors for their possible association with amniotic fluid IL-1ra concentration: fetal gender and relative birth weight (Table 2). Of these factors, fetal gender emerged as highly significant (*p* < 0.0001). In addition, relative birth weight was also significantly associated with IL-1ra (*p* = 0.03).

In laboring patients at term, there was no significant difference in the concentration of IL-1ra in amniotic fluid between samples taken from the vaginal pool (130.0 $\pm$ 13.1 μ g/L, *n* = 16) and samples taken transabdominally (100 $\pm$ 21.5 μ g/L, *n* = 8).

Amniotic fluid IL-1ra in different diagnostic categories. The concentration of IL-1ra in amniotic fluid in different patient groups is shown in Figure 3. When both genders were combined, the Kruskal-Wallis test showed a difference between groups (*p* = 0.04). In a comparison of pairwise differences, amniotic fluid IL-1ra in the various groups of pathologic pregnancies did not differ significantly from IL-1ra in normal pregnancies (Fig. 3, upper panel). However, IL-1ra tended to be high in patients with twin pregnancies, as well as in mothers of small for gestational age infants.

Within each patient group, amniotic fluid IL-1ra tended to be higher in female- than in male-bearing gestations (Fig. 3, lower panel). The male-female difference was significant in the follow-

Fig. 2. Amniotic fluid IL-1ra at different gestational ages: dependence on fetal gender. The amniotic fluid concentrations of IL-1ra in women bearing a male or a female fetus are shown as a function of gestational age. IL-1ra is expressed in ng/mL or $\mu\text{g/L}$ ($1 \mu\text{g/L} = 59 \text{ pmol/L}$). The regression lines and 90% confidence bands for means are shown for each gender separately.

ing four patient groups: normal, preterm PROM, maternal diabetes, and miscellaneous (Fig. 3).

Concentration of IL-1 β in amniotic fluid. Amniotic fluid IL-1 β was not associated with fetal gender. As expected (20), it was not associated with amniotic fluid IL-1ra, either. In patients with PROM at gestational age of less than 34 weeks, amniotic fluid IL-1 β concentration was $84.9 \pm 48.7 \text{ ng/L}$ (14), and amniotic fluid IL-1ra concentration was $133.3 \pm 19.9 \mu\text{g/L}$ ($n = 15$). In patients without PROM of similar gestational age, IL-1 β was above the detection limit only in two (concentrations 9.8 and 4.5 ng/L) of a total of 31 specimens ($p = 0.015$ vs. PROM), but the concentration of IL-1ra was similar ($108.2 \pm 9.3 \mu\text{g/L}$, $n = 31$, $p = 0.20$) to that in patients with PROM.

Concentration of IL-1ra in urine at birth: gender dependence. All urine samples collected before 48 h of age from preterm and term neonates contained IL-1ra. The mean IL-1ra in newborn urine was 91.1 ± 17.5 ($n = 21$), with a range from 5.0 to 297.1 $\mu\text{g/L}$.

The concentration of IL-1ra in neonatal urine was higher in female newborns than in male newborns ($p = 0.04$) (Table 3). The gestational ages and birth weights of the female and male neonates were similar.

IL-1ra in neonatal urine was not dependent on gestational age. The urinary concentration of IL-1ra was $71.7 \pm 25.6 \mu\text{g/L}$ (range 5.0–203 $\mu\text{g/L}$) in preterm neonates (gestational age less than 37 wk, $n = 10$) and $108.7 \pm 23.7 \mu\text{g/L}$ (range 19.7–297 $\mu\text{g/L}$) in term neonates ($n = 11$).

DISCUSSION

The present results show that women carrying a female fetus have a higher concentration of IL-1ra in amniotic fluid than women carrying a male fetus. To our knowledge, sex-specific differences in the concentration of IL-1ra in any body fluids have not previously been reported.

IL-1ra was present in the urine of newborn infants in very high concentrations, comparable to those in amniotic fluid. An important source of amniotic fluid IL-1ra is therefore likely to be fetal urine, the major contributor to amniotic fluid volume. Because keratinocytes contain considerable amounts of IL-1ra (24) and cells from fetal skin are desquamated to amniotic fluid, fetal skin is another possible source of amniotic fluid IL-1ra. In addition, fetal membrane and decidual cells release IL-1ra *in vitro* and may thus also contribute to amniotic fluid IL-1ra (21, 25).

As a preliminary finding, we report here that IL-1ra concentration in the urine of female newborns tends to be higher than in male newborns. Most of the infants studied were suffering from severe diseases. A study involving a larger number of patients including normal newborns is needed before any definite conclusion can be drawn regarding the gender dependence of IL-1ra concentration in the urine of newborn infants.

There are limited data available on the concentration of IL-1ra in body fluids, and no reports have been published on the concentration of IL-1ra in urine or sera of newborns or children. IL-1ra is present in urine of patients with fever or myelocytic leukemia (26–28). After experimental endotoxemia in human volunteers, IL-1ra concentrations in plasma increased from 0.46 to 15 $\mu\text{g/L}$ (29). In critically ill patients, plasma IL-1ra has been reported to range from 0.32 to 55 $\mu\text{g/L}$ (29). IL-1ra concentration in serum of patients with Hodgkin's disease ranges from 0.63 to 16 $\mu\text{g/L}$ (30). The concentrations found in amniotic fluid or in urine of newborn infants thus greatly exceed those reported previously in the sera or plasma of adults.

IL-1 is a mediator in both local and systemic infections. Furthermore, it has functions in host defense mechanisms, particularly immunologic and hematologic responses (1). It induces fever, decreases blood pressure, lowers systemic vascular resistance, induces neutrophilia, and increases the synthesis of collagenases (1). IL-1 up-regulates the production of other mediators such as platelet-activating factor, prostaglandins, and nitric oxide (5). IL-1 can also induce its own production and that of other cytokines that participate in inflammatory processes (2–4, 31, 32). In addition, the growth of virulent strains of *Escherichia coli* is enhanced by IL-1 (33).

IL-1ra alleviates inflammatory effects of IL-1 (7). It inhibits the production of cytokines induced by IL-1 (34, 35). In animal models, treatment with IL-1ra improves survival in *Escherichia coli* (9), *Staphylococcus epidermidis* (12), and polymicrobial Gram-negative sepsis (10), as well as in endotoxemia (8, 11). IL-1ra is endogenously produced in response to endotoxemia and septic shock (29). Production of IL-1ra can be considered a host defense mechanism to block deleterious effects of IL-1 (5). The balance between the amount of IL-1 and the amount of IL-1ra may be a critical factor in determining whether the host is able to block the symptoms mediated by IL-1.

Male newborns have increased risks of infection-mediated morbidity and mortality (36–41). Thus, male newborns are two to six times more likely to develop perinatal sepsis or meningitis than female newborns (36–38). The risk of perinatal bacterial infection after PROM is also higher in male than in female

Table 2. Multiple regression analysis of factors potentially affecting concentration of IL-1ra in amniotic fluid

Factor	Numerical variable	Regression coefficient	Standard error	p
Fetal sex	Male = 1, female = 2	60.9	7.2	<0.0001
Relative birth weight	Birth weight in SD of mean	−4.8	2.1	0.026

Table 3. *IL-1ra in newborn urine samples obtained before 48 h of age*

	Both genders (n = 21)	Male newborns (n = 10)	Female newborns (n = 11)	Male vs female (p)*
Birth weight (g)	2649 ± 272	2598 ± 517	2690 ± 284	0.87
Gestational age (d)	251 ± 7	243 ± 11	259 ± 8	0.26
IL-1ra (μg/L) in urine	91.1 ± 17.5	54.0 ± 19.2	124.0 ± 25.2	0.04

* The two-sided unpaired *t* test was used to compare males with females.

Fig. 3. Amniotic fluid IL-1ra in different patient categories. The upper panel shows the concentration of IL-1ra in the indicated patient groups with both genders combined. Horizontal lines show means. Significant differences between pairs of groups are indicated by a horizontal line and star above the data ($p < 0.05$, Mann-Whitney test with Bonferroni correction). The lower panel shows the concentrations of IL-1ra (mean ± SEM) separately for male- and female-bearing gestations. IL-1ra is expressed in ng/mL or μg/L. Significant differences (unpaired *t* test with Bonferroni correction) are indicated as follows: *, $p < 0.05$; **, $p < 0.01$; and ***, $p < 0.001$.

neonates (40). Amniotic fluid invaded by microorganisms is an important source of fetal and perinatal infections (42–44). Infection leads to activation of the cytokine network and to increased production of cytokines such as IL-1 (17). IL-1 mediates symptoms and signs of infection. IL-1ra has been shown to reduce the severity of diseases such as hemodynamic shock and lethal sepsis (8–12). Endogenously produced IL-1ra may similarly prevent the onset and development of inflammatory lesions. Amniotic fluid IL-1ra concentration is constitutively high, as shown by present and previous (20) results. Fetal skin is exposed to IL-1ra present in amniotic fluid. Through swallowing of amniotic fluid and fetal breathing, the gastrointestinal tract and the airways of the fetus are also exposed to IL-1ra. The increased concentration of IL-1ra in the amniotic fluid surrounding female fetuses and the increased production of IL-1ra by female fetuses and newborns may contribute to the better resistance to infection of female compared with male fetuses and newborns. However, at present there is no direct evidence indicating that high endogenous IL-1ra protects against severe infections.

A higher incidence of prematurity in male births than in female births has been documented in several studies (45–48). According to one study, males are more likely than females to be born at

33 to 36 wk of gestation (48). Other studies have found an excess of male births at gestational ages between 24 and 37 wk (45–47). Due to its presence in amniotic fluid in preterm labor occurring in the setting of infection and its stimulatory effects on fetal membrane prostaglandin synthesis, IL-1 has been proposed to act as a mediator of preterm labor (19). IL-1ra inhibits the IL-1-induced prostaglandin production by human amnion cells (20, 21). In mice, preterm delivery induced by IL-1 can be blocked by IL-1ra (22). A high IL-1ra in fetal urine and amniotic fluid may inhibit IL-1-induced inflammatory changes in the fetal membranes and in the fetus. The higher IL-1ra concentration in amniotic fluid surrounding the female fetus and bathing her fetal membranes may be one factor contributing to the lower incidence of infections and preterm deliveries among female fetuses.

The sources of IL-1ra in the fetus and newborn infant and the regulation of its production remain to be elucidated. The possibility that, in addition to IL-1ra, other cytokines are gender dependent merits investigation.

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