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Increased Plasma Concentrations of Interleukin-1 Receptor Antagonist in Neonatal Sepsis

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ABSTRACT

Newborns are prone to severe infections and sepsis. Cytokines such as tumor necrosis factor-α and IL-1β play a major role in the initiation of the host response to infections. IL-1 receptor antagonist (IL-1ra) is a naturally occurring antagonist of IL-1β. We hypothesized that low IL-1ra plasma concentrations might contribute to the high morbidity and mortality of neonatal sepsis. We studied IL-1ra plasma concentrations during neonatal sepsis. Eleven newborns with severe infection or sepsis, 28 newborns suspected as having sepsis, and eight healthy newborns were enrolled in the study. IL-1ra plasma concentrations proved to be increased in the newborns with severe infections or sepsis (5635 ± 411 ng/L) *versus* the concentrations in the suspected group (2597 ± 433 ng/L) and the control group (273 ± 88 ng/L) ($p < 0.001$). After the start of antibiotic therapy, the IL-1ra plasma concentrations remained high during the first 16 h. The IL-1β plasma concentrations were increased in the group with a proven infection (78 ± 27 ng/L) *versus* the suspected group (37 ± 7 ng/L) ($p < 0.05$). Interestingly, the mean Il-1RA plasma concentration is a factor 50–100 higher than the IL-1β plasma concentrations. We conclude that IL-1ra in newborns is produced in an amount equal to that in adults. An inadequate IL-1ra response does not seem to contribute to the increased morbidity and mortality of neonatal sepsis. (***Pediatr Res* 37: 626–629, 1995**)

Abbreviations

TNF, tumor necrosis factor
IL-1R, IL-1 receptor
IL-1ra, IL-1 receptor antagonist

IL-1 is an important pro-inflammatory mediator and initiates in concert with tumor necrosis factor-α (TNF-α) the systemic host response cascade after infection (1–3). IL-1 potentiates the effect of TNF-α severalfold (4, 5). IL-1 is produced by monocytes in response to various stimuli (3, 6). It induces fever and hypotension when given i.v. (1, 7, 8). Moreover, IL-1 stimulates its own production and that of TNF-α and IL-6 in the monocyte (4, 9). This positive feedback mechanism deteriorates the patients' hemodynamic condition. On the other hand IL-6 suppresses the IL-1 and TNF production *in vitro* (10). Human IL-1 exists in two active forms: IL-1α and IL-1β, which are structurally related and are produced as precursors (11). Proteases cleave IL-1α and IL-1β precursors to give rise to the active forms of IL-1α and IL-1β (11–13). IL-1α has a function in the monocyte as an autocrine messenger and a function on the cell membrane as a paracrine messenger. IL-1α is rarely detected in the circulation (11). IL-1β is released in the blood and can be detected in the circulation (2, 3, 11). Both IL-1α and IL-1β bind to the IL-1 receptor (IL-1R), leading to several intracellulair processes and clinical phenomena as described elsewhere (11).

IL-1 receptor antagonist (IL-1ra) is a naturally occurring antagonist of IL-1 (14–16). IL-1ra binds competitively to the IL-1R (17). The IL-1ra/IL-1R complex on the cell membrane does not give rise to activation of the cell, as opposed to the IL-1/IL-1R complex. *In vitro* and in animals IL-1ra blocks IL-1-induced phenomena as TNF-α and IL-6 production, hypotension, and shock (8, 18–20). *In vitro* studies demonstrated that IL-1ra and IL-1β are often produced in the same monocyte, but are regulated differently (21, 22).

Thus far no data are available with regard to the role of IL-1ra in the pathogenesis of neonatal sepsis. The aim of the present study was to investigate whether a low IL-1ra plasma concentration is a reason for the high morbidity and mortality during neonatal sepsis (23, 24).

METHODS

Cytokine measurements. EDTA blood specimens were obtained by venipuncture or from an arterial catheter and immediately transported on ice to the laboratory. Plasma was separated from the blood within 30 min. Aliquots were stored at −80°C until assayed. IL-1β was measured using an EASIA assay (Medgenix) for IL-1β. IL-1ra was measured with an

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ELISA method (Amersham Corp.). Because of the small blood sample we used an adapted method (100 μL for IL-1β measurements and 50 μL for the IL-1ra measurement). The control, interassay, and intraassay values obtained with this procedure were within the same range as the original method. The detection limits were 4 ng/L for IL-1β and 100 ng/L for IL-1ra. In the septic group the first blood sample was taken before the start of antibiotic treatment.

Informed consent was obtained from the parents of each newborn before the start of the study. The study was approved by the hospital ethics committee.

Statistical analysis. Differences between groups were analyzed with Wilcoxon-Mann-Whitney test. Differences were considered significant at p values equal or less than 0.05. All data are expressed as mean ± SEM.

Patients. During a 12-mo period 47 consecutive newborns with clinical suspicion of sepsis were included in the study. Patients with bronchopulmonary dysplasia, ventilator dependency for more than 14 d, or indomethacin use were excluded. Signs suggesting sepsis were lethargy, apneic spells, a poor peripheral circulation, and poor feeding (24–26).

Sepsis was defined on clinical grounds and a positive blood culture. These patients and patients with a well-defined severe localized bacterial infection were included in group 1. Patients suspected for sepsis but without a positive blood culture or a localized bacterial infection were included in group 2. Group 3 were healthy newborns admitted for reasons other than infectious or immunologic diseases.

Two IL-1β plasma concentrations are lacking in the septic group due to inadequate sample volume. The antibiotic regime was ampicillin (50 mg/kg/d, every 8 h) and ceftazidime (100 mg/kg/day, every 8 h), when newborns became septic in the first 5 d of life. In newborns aged 7 d or more and suspected as having sepsis, the initial antibiotic therapy was cefuroxime (100 mg/kg/day every 12 h) and amikacin (according to gestational age and birth weight every 12 h), because of local resistance patterns of nosocomial isolates.

RESULTS

Eleven out of 47 patients fulfilled the definition for sepsis or had a severe bacterial infection. Nine patients had a positive blood culture, and two patients had a urinary tract infection (seven male and four female newborns, mean gestational age 34.1 ± 1.4 wk, mean birth weight 2.3 ± 0.5 kg, mean age 11.8 ± 4.1 d) (group 1) (Table 1). In patient 10, many leukocytes and bacteria were seen in the urine sediment, but the culture was lost. Congenital urinary tract abnormalities were not found. There were no deaths during the study period.

In 28 newborns sepsis was suspected on clinical grounds but not confirmed by culture (group 2) (16 male and 12 female infants, mean gestational age 29.4 ± 1.9 wk, mean birth weight 1.9 ± 0.1 kg, mean age 7.6 ± 1.6 d). Empirical antibiotic therapy was discontinued after 3 d. All patients but two recovered within a few days. One patient died after 3 d due to congenital hypoplasia of the lungs, which was confirmed at autopsy. The second patient died after 5 d due to a therapy-reistant coagulation disorder, intracranial hemorrhage, and respiratory insufficiency.

The control group included eight healthy control subjects (three male and five female infants, mean gestational age 35.6 ± 1.2 wk, mean birth weight 2.1 ± 0.2 kg, mean age 10.9 ± 4.9 d). Blood for cytokine measurements was obtained once when blood samples were taken for glucose, bilirubin, or hemoglobin measurements.

In Figure 1 the individual IL-1ra plasma concentrations are shown. The mean IL-1ra plasma concentration in the septic group (5635 ± 411 ng/L) was significantly higher than the concentrations on the suspected group (2597 ± 433 ng/L) ($p < 0.001$) and the control group (273 ± 88 ng/L) ($p < 0.001$). The mean IL-1ra plasma concentration in the suspected group was significantly increased *versus* the control group ($p < 0.01$). After the start of the antibiotic treatment the IL-1ra plasma concentrations remained high during the first 16 h (data not shown). In comparison with IL-1 plasma concentrations the IL-1ra plasma concentrations was 50–100-fold higher. No correlation is found between IL-1ra and gender or gestational age.

Also the Il-1β plasma concentrations were increased in the septic group (78 ± 27 ng/L) *versus* the suspected group (37 ± 7 ng/L) ($p < 0.05$) (data not shown), confirming the data of our previous study (27).

Table 1. *Clinical data of patient group, group 1*

Patient	Gestational age (wk)	Birth weight (g)	Sex	Age at diagnosis (d)	Culture	Site	IL-1β (pg/mL)	IL-1ra (pg/mL)
1	33	1510	M	6	Coagulase-negative *Staphylococcus*	Blood	20	5636
2	36	3680	F	47	*Enterobacter* sp. (peritonitis)	Blood	<4	5024
3	32	1295	F	0	*E. coli*	Blood	232	5636
4	40	4035	M	1	*Listeria monocytogenes*	Blood	29	8934
5	31	1740	M	20	Coagulase-negative *Staphylococcus*	Blood	22	6038
6	27	1280	M	0	*L. monocytogenes*	Blood	196	6038
7	30	1490	M	7	*Enterobacter* sp.	Blood	126	5636
8	31	1120	M	10	Coagulase-negative *Staphylococcus*	Blood	ND	5902
9	33	1740	M	15	*Staphylococcus aureus*	Blood	ND	4809
10	40	4040	F	2	UWI	Urine	47	5508
11	42	3790	F	2	Group B hemolytic *Streptococcus*	Urine	29	2826

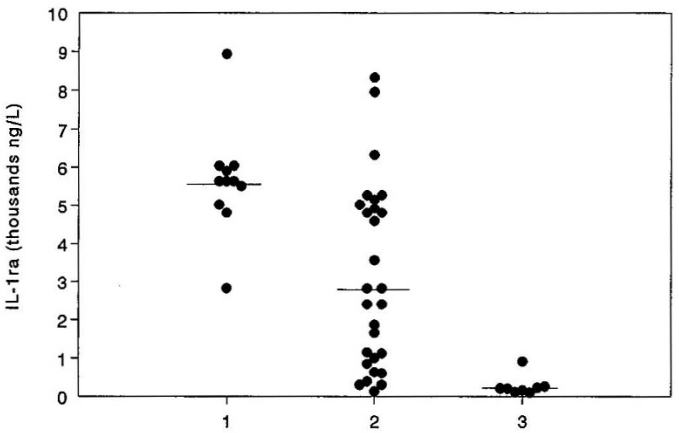

Figure 1. Individual plasma concentrations of IL-1ra for the three groups of newborns: *1*, newborns with confirmed sepsis; *2*, newborns suspected of having sepsis; and *3*, control group. *Bars* indicate median.

DISCUSSION

This study shows that newborns with infections, bacteremia, and/or sepsis have elevated IL-1ra plasma concentrations, as high as adults during sepsis (28). IL-1ra is a natural occurring inhibitor that blocks the action of IL-1 by competitive binding to its receptor. IL-1ra can block *E. coli*-induced shock in rabbits and baboons when given 15 min before bacterial infusion (29, 30). Experimental evidence suggests that IL-1ra blocks the action of IL-1 at the level of the IL-1R, because the IL-1 concentrations are not influenced by the presence of IL-1ra (29, 30). In addition the IL-1ra infused in baboons attenuated the sustained IL-1β response (30). During *in vitro* studies with human mononuclear cells, IL-1ra given 30 min before stimulation inhibits IL-1-induced IL-1, TNF-α, and IL-6 production in a dose-dependent manner as well as endotoxin-induced cytokine synthesis (31, 32). Anderson *et al.* (22) demonstrated that the peak of IL-1ra production by peripheral blood mononuclear cells was at 4 h after stimulation. Monocytes can produce IL-1 and IL-1ra at the same time (22). This corresponds with studies in volunteers after experimental endotoxemia and in critically ill patients (28, 33).

To our knowledge no study on the role of IL-1ra during neonatal sepsis has been published. An increased morbidity to infection and sepsis in newborns could be due to a low IL-1ra plasma concentration. This was not demonstrated in the present study. The IL-1ra plasma concentrations during infections were as high as in adults. The moderately increased mean IL-1ra plasma concentration in the suspected group of newborns is interesting. It supports the idea that monocytes and macrophages are also activated by other diseases but to a lesser degree than in infectious diseases.

In the septic group the IL-1ra plasma concentrations were 50–100-fold higher than concurrent IL-1 plasma concentrations, confirming data from endotoxemia experiments (28). No correlation between IL-1β and IL-1ra was found. It is possible that IL-1β has a high tissue concentration, and relatively small amounts can be measured in the circulation, whereas IL-1ra has a more systemic distribution.

IL-1 has been proposed as a mediator in preterm labor (34, 35). High IL-1ra concentrations in the amniotic fluid are found (36). The origin of the high IL-1ra concentrations (range, 10–100 μg/L) in amniotic fluid is not clear. Recently a gender difference was described; female newborns have higher IL-1ra concentrations in the amniotic fluid and in the urine than do male newborns (37). This gender difference was not found during the present study. Probably the high IL-1ra concentrations in the amniotic fluid are from maternal origin because plasma concentrations in healthy newborns are very low (0.1–1 μg/L). Interestingly, recently a gender difference in adults was studied; in adult women with normal ovarian function, the produced and urinary excreted IL-1ra is higher than in men (38).

In conclusion, IL-1ra plasma concentrations are increased during neonatal sepsis in comparison with IL-1ra plasma concentrations of newborns suspected for sepsis or a control group. IL-1ra probably plays a role in reducing the actions of IL-1 during neonatal sepsis. IL-1ra plasma concentrations do not contribute to the increased morbidity and mortality of newborns with sepsis.

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