Interleukin 8 concentrations in amniotic fluid and peripheral venous plasma during human pregnancy and parturition

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To establish the gestational and labour-associated changes in interleukin 8 (IL-8) release, we have determined the concentration of this cytokine in maternal peripheral plasma and amniotic fluid from 15 weeks of gestation to term and in association with spontaneous-onset labour at term and preterm. No statistically significant changes in peripheral plasma IL-8 concentration were observed during pregnancy or in association with labour onset (mean concentration 56.5 ± 14.5 ng/l. N = 64). The IL-8 concentrations in amniotic fluid were up to 50-fold greater than those observed in peripheral plasma (p<0.05) and increased significantly (p<0.05) during pregnancy. At term, but before the onset of labour, amniotic fluid concentrations of IL-8 averaged 969.2 ± 55.3 ng/l (N = 12). In association with labour at term, IL-8 concentrations increased to 3895.8 ± 1414.4 ng/l (N = 6, p<0.03). The concentration of IL-8 in amniotic fluid obtained from women in preterm labour averaged 1854.7 ± 1352.6 ng/l (N = 6) but was not statistically different from the concentration of IL-8 in amniotic fluid obtained from gestational aged-matched non-labouring controls. Although the precise role of intrauterine IL-8 at the time of parturition awaits elucidation, these data support the concept that this cytokine may be involved in the biochemical events associated with the onset and/or propagation of normal labour in the human.

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The mechanisms that initiate increased uterine contractions and cervical ripening at the time of human labour remain to be elucidated clearly. Evidence obtained to date, however, implicates the prostaglandin group of hormones in the regulation of these processes (for reviews, see Refs. 1 and 2). Furthermore, prostaglandin concentration, in association with labour, increases in amniotic fluid (3, 4), plasma (5, 6) and urine (7). The concentration of free arachidonic acid, the substrate for prostaglandin synthesis, also increases in association with labour (8), as does the synthesis of prostaglandins by gestational tissues (9).

Previously, it has been proposed that the biochemical and cellular events that occur at the time of labour are similar to those that occur in association with inflammatory responses (10). The similarities between these two processes include vascular changes, leukocyte infiltration and increased prostaglandin synthesis. Further experimental support for this hypothesis has been obtained recently with the identification of the inflammatory-mediating cytokines interleukin 1 (IL-1), IL-6 and tumour necrosis factor-α (TNF-α) within the intra-uterine compartment (11–13), the demonstration that gestational tissues release these cytokines in vitro (12, 14–16) and that they stimulate prostaglandin formation by human gestational tissues (17–19).

The cytokine group of hormones includes various polypeptide autacoids, ranging in molecular weight from 8 to 30 kD. One of the smallest of these is the recently characterized cytokine IL-8. Interleukin 8 has inflammatory and growth-regulating properties, but is notable for its selective chemotaxis, degranulation and activation of neutrophils (20). The role of IL-8 in human pregnancy and labour has yet to be established; IL-8-mediated neutrophil degranulation and the release of degradative enzymes such as elastase may contribute to cervical ripening and/or the rupture of fetal membranes owing to its ability to degrade type III collagen. This mechanism has been suggested as a possible cause of premature rupture of the membranes (PROM) (21, 22). Furthermore, IL-8 activation of neutrophils may stimulate eicosanoid synthesis, both lipoxygenase and cyclooxygenase products of arachidonic acid metabolism, by these cells (23).

To investigate further the role of IL-8 in human parturition, the aim of this study was to quantify maternal peripheral plasma and amniotic fluid concentrations of IL-8 during pregnancy and labour, both at term and preterm.
Materials and methods

Patients

Plasma and amniotic fluid samples were collected from women attending the Monash Medical Centre (Clayton, Victoria). The project was approved by the Monash Medical Centre Research and Ethics Committee, and informed consent was obtained from all participating subjects.

Whole blood was collected from: non-pregnant women (NP); non-labouring women during pregnancy; and women in spontaneous-onset labour both at term and preterm. Plasma samples obtained from women during pregnancy were assigned to three groups: less than 20 weeks (early pregnancy, EP): 20–36 weeks inclusive (preterm not in labour, PNIL); and greater than 37 weeks of gestation (term not in labour, TNIL). Plasma samples collected from women in spontaneous-onset labour were designated as preterm in labour (PIL), i.e. less than 37 weeks of gestation, and term in labour (TIL), i.e. greater than or equal to 37 weeks of gestation.

Whole blood was collected into ethylenediaminetetraacetic acid (EDTA)-coated tubes (Beckton Dickinson Vacutainer Systems, Rutherford, NJ, USA). Plasma was separated by centrifugation (GLC-2B; Sorvall/Du Pont Instruments, Wilmington, DE, USA) at 4000 rpm for 5 min. The plasma was then stored in 2-ml tubes at −80°C until assayed.

Amniotic fluid samples were collected from five groups of women with gestational ages as defined above for the collection of plasma samples. The groups were as follows: women undergoing transabdominal amniocentesis (EP) at 16–20 weeks of gestation for genetic studies; women requiring caesarean section preterm prior to labour (PNIL) because of maternal and/or fetal conditions, and those undergoing elective caesarean section at term prior to labour (TNIL). The fourth and fifth groups of women were those undergoing emergency (i.e. in labour) caesarean section as a result of clinical complications, after the onset of preterm labour (PIL) or term labour (TIL), respectively.

Amniotic fluid samples were initially collected into plain polypropylene tubes (Hardy Health Care Products, Oakleigh, Victoria, Australia) and processed as described above for plasma samples.

Interleukin 8 assay

Plasma and amniotic fluid concentrations of IL-8 were quantified by radioimmunoassay. Standard curves (0, 98, 195, 781, 3125, 12500 and 25000 ng/l) were prepared by diluting human recombinant IL-8 (Advanced Magnetics, Cambridge, MA, USA) in charcoal-stripped human serum. Radio-iodinated human recombinant IL-8 (254 kBq/ml) was purchased from Advanced Magnetics and diluted in bovine serum albumin and phosphate-buffered saline (BSA-PBS; 0.01 mol/l Na₂HPO₄, 1.8 mmol/l KH₂PO₄, 3 mmol/l KCl, 140 mmol/l NaCl, 0.1% BSA and 0.1% sodium azide, pH 7.0) to an activity of 2.27 kBq/ml. Lyophilized rabbit anti-human recombinant IL-8 serum was reconstituted with BSA-PBS and utilized at a dilution of 1:3000. The assays were performed in polycarbonate test tubes (12 × 75 mm; Disposable Products, Victoria, Australia) by incubating 200 µl of standard or sample with 100 µl of IL-8 antibody for 16 h at room temperature. Total counts and non-specific binding tubes (i.e. containing no primary antibody) were prepared and incubated in parallel. Following the initial incubation, iodinated IL-8 (100 µl; 0.227 kBq) was added and the tubes were vortexed for 30 s and then incubated for 4 h at room temperature. Magnetic goat anti-rabbit immunoglobulin G diluted in 0.01 mol/l PBS containing <0.1% EDTA (pH 7.4) (500 µl; 1 mg protein/ml; Advanced Magnetics) was then added, vortexed (30 s) and incubated for 20 min at room temperature. Antibody-bound radio-iodinated IL-8 was then separated from free radiolabel by centrifugation at 1000 g for 20 min at 4°C. The supernatant was decanted and discarded. The pellets were resuspended in 0.01 mol/l TRIS buffer (pH 7.4) containing 1.0 mol/l NaCl, 2 mmol/l EDTA, 1.0% BSA and 0.1% sodium azide and then recentrifuged at 1000 g for 20 min at 4°C. The supernatant was discarded and the radioactivity remaining in the pellets was quantified by gamma scintillation spectrometry.

Under the assay conditions utilized, maximal and non-specific binding averaged 50.0% and 0.8% (N = 3), respectively. The sensitivity of the assay (defined as two standard deviations from maximum binding) (24) was 12.8 ng/tube (64 ng/l). The intra- and interassay coefficients of variation (determined in three assays) were 7.3% and 7.6%, respectively. The antisera displays less than 0.001% cross-reactivity with other human cytokines, including IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-6, IL-7, interferon (IFN)-γ, TNF-α, granulocyte macrophage colony stimulating factor (GM-CSF) and macrophage colony stimulating factor (M-CSF). Neither amniotic fluid nor maternal pregnant plasma at a final dilution of 1 in 4.5 interfered in the assay, as indicated by the parallel dilution of recombinant IL-8 dissolved in amniotic fluid or pregnant plasma with the standard curve. There were no statistically significant differences (p > 0.05) between linear regression lines for standard curves prepared in serum diluent (r = 0.994) versus amniotic fluid (r = 0.998) or serum diluent (r = 0.999) as compared to maternal plasma (r = 0.999). The recovery of known amounts of exogenously added IL-8 to amniotic fluid and plasma averaged 99.4 ± 2.5% (N = 3) and 106.2 ± 16.9% (N = 6), respectively. All samples were therefore assayed at a final dilution of 1–4.5.

Analysis of data

The data were initially assessed for homogeneity of variance using a Bartlett’s test. The variance was determined to be non-homogeneous (p < 0.01), thus
non-parametric statistical analysis (Wilcoxon signed rank test) was utilized. Data were subsequently analysed using Wilcoxon signed rank tests. The gestational variation in amniotic fluid concentrations of IL-8 was assessed statistically by Spearman’s rank correlation analysis. The data are presented as means ± SEM (N). The IL-8 concentrations that were below the limit of sensitivity of the assay were numerically equated to zero for the determination of the mean and standard error terms.

Results

Interleukin 8 concentrations were determined in peripheral plasma samples collected from non-pregnant women (NP) and women during pregnancy (EP, PNIL, TNLIL) or labour (PIL, TIL) (Fig. 1). Immunoreactive IL-8 was detected in 24% (18/74) of plasma samples assayed. The IL-8 concentration in maternal plasma did not change significantly in association with labour or during pregnancy. The data were therefore pooled and the concentration of IL-8 in maternal peripheral plasma during pregnancy averaged 52.8 ± 17.8 ng/l (N = 31); this was not statistically significantly different from the mean IL-8 concentration measured in NP women (10.1 ± 10.1 ng/l, N = 10). Similarly, no statistically significant difference between the mean plasma concentrations of IL-8 obtained from women in spontaneous-onset labour at term or preterm (75.8 ± 28.4 ng/l, N = 19; 46.1 ± 25.0 ng/l, N = 14; p > 0.6, respectively) was detected. The data were therefore pooled and averaged 48.8 ± 14.1 ng/l (N = 74).

The concentration of IL-8 in amniotic fluid during pregnancy and in association with term and preterm onset of labour is presented in Fig. 2. Immunoreactive IL-8 was detected in 60% (24/40) of amniotic fluid samples assayed. The IL-8 concentrations in amniotic fluid were 2–50-fold higher than the IL-8 concentrations in plasma. The difference between plasma and amniotic fluid IL-8 concentrations increased with gestational age.

During pregnancy, IL-8 concentrations in amniotic fluid increased from 236.7 ± 92.0 ng/l at less than 20 weeks to 969.2 ± 553.5 ng/l at term (assessed by Spearman’s rank correlation analysis, p < 0.05, N = 31). In association with spontaneous labour at term, the concentration of IL-8 in amniotic fluid increased four-fold compared with a gestational-age-matched control group (TNIL = 969.2 ± 553.5 ng/l, N = 12; TIL = 3895.8 ± 1414.4 ng/l, N = 6; p < 0.03). With respect to spontaneous-onset preterm labour, no statistically significant change in IL-8 concentration in amniotic fluid was detected.

Discussion

Inflammatory foci are characterized by increased release of cytokines and eicosanoids, which orchestrate...
homeostatic adjustments to injury or infection. Recent evidence suggests that the biochemical mediation of human labour may be analogous to that of an acute inflammatory response. To further explore this analogy, in this study we quantified IL-8 concentrations in amniotic fluid and peripheral venous plasma during pregnancy and labour both at term and preterm.

The data obtained in this study support a role for IL-8 of intrauterine origin in the processes of spontaneous-onset labour at term. In amniotic fluid obtained from women in active labour at term (i.e., >37 weeks of gestation), the concentration of IL-8 was fourfold greater than that measured in amniotic fluid obtained from women at a similar gestational age but not in labour. The observations that the concentrations of IL-8 in peripheral plasma did not vary in association with labour onset and that they were up to 50-fold lower than amniotic fluid concentrations are consistent with the release of IL-8 by intrauterine tissues and with an intrauterine site of action of this cytokine.

With respect to preterm labour, the concentration of IL-8 in amniotic fluid was not statistically different from that of the gestational-age-matched control group. The observation that 50% (3/6) of amniotic fluid samples collected from women in preterm labour contained no detectable IL-8 may reflect the heterogeneous aetiology of human preterm labour. This is in contrast to the term labour group, in which all amniotic fluid samples assayed (6/6) contained IL-8 at concentrations greater than 800 ng/l.

These data are consistent with the findings of Romero et al. (25), who reported an increased median concentration of IL-8 in amniotic fluid in association with labour at term. In addition, although no significant difference between median IL-8 concentrations was detected, these authors reported that a greater proportion of amniotic fluid samples with IL-8 concentrations greater than 1000 ng/l were identified at term (not in labour) than at midgestation. The identification, in this study, of a significant correlation between amniotic fluid IL-8 concentrations and gestational age confirms and extends these observations.

The cellular origin of amniotic fluid IL-8 during pregnancy and at the time of labour have yet to be established. Recently, however, Kelly et al. (26) reported that human chorioan and decidual cells maintained in culture release substantial amounts of IL-8. These cells may represent intrauterine origins of amniotic fluid IL-8. The release of IL-8 from chorionic and decidual cells obtained before labour onset (elective cesarean section), however, was reported to be similar to that released by tissue obtained after labour and delivery. This latter observation is in contrast to the observed increase in the concentration of IL-8 in amniotic fluid at the time of spontaneous-onset labour.

The role and mechanism(s) of action of IL-8 in human labour, both term and preterm, remain to be defined. Previously, IL-8 has been identified as a potent chemo-tactic and activating factor for neutrophils (20). As neutrophils are capable of releasing elastase (which can act on collagen), it is tempting to speculate that intrauterine activation of neutrophils by IL-8 may cause rupture of fetal membranes. Kanayama et al. (21) investigated the presence of neutrophils and elastase in fetal membranes obtained at the site of rupture, or otherwise, in cases of labour either associated with or without PROM. Their study reported neutrophil infiltration and elastase staining for all cases of PROM. No staining was observed in cases without PROM.

An additional site of action for IL-8 may be the cervix. One of the characteristics of cervical ripening is an inflammatory cell infiltrate (10). Interleukin 8 may therefore participate in the infiltration of inflammatory cells into the cervix and thereby in the process of cervical ripening.

In summary, the aim of this study was to characterize gestational and labour-associated changes in amniotic fluid and peripheral venous plasma IL-8 concentrations. The data are consistent with the local intrauterine release and action of IL-8 in association with term labour. It remains to be established, however, whether or not increased IL-8 amniotic fluid concentration plays a causal role in the initiation and/or maintenance of human labour.

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