Journal of Reproduction and Fertility (1994) **100**, 81–84

Transfer of cytokines through human fetal membranes

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Intact human fetal membranes (amnion, chorion and decidua) were incubated with ^{125}I-labelled cytokines added to the fetal or maternal sides of the membrane. The transfer of ^{125}I-labelled interleukin-6 (IL-6), ^{125}I-labelled tumour necrosis factor α (TNF-α), ^{125}I-labelled interleukin-1α (IL-1α) and ^{125}I-labelled interleukin-1β (IL-1β) was determined by measurement of radioactivity in a gamma counter and the integrity of the cytokines was assessed by acid precipitation and by radioimmunoassay. IL-1α and IL-1β were transferred through human fetal membranes in both feto–maternal and materno–fetal directions at similar rates. Only 2–4% of the cytokine originally added appeared to be intact on the opposing side of the membrane after 24 h of culture. Transfer of intact TNF-α (5–7%) and IL-6 (8–17%) was greater than that of the IL-1 isomers. Low but variable amounts of the four cytokines tested may be transferred through the human fetal membrane. This finding suggested that concentrations of cytokines in amniotic fluid would not reflect those produced by decidua if the fetal membranes are intact.

Introduction

The biochemical changes accompanying human labour have been investigated and it is apparent that prostaglandin E_2 (PGE_2) is involved in cervical ripening (Lorenz *et al.*, 1984), whereas $PGF_{2\alpha}$ co-ordinates myometrial contractility (Green *et al.*, 1974). Beyond this, the control of human labour remains obscure, such that the factors controlling prostaglandin synthesis at the end of pregnancy have not been identified. However, in the case of intrauterine infection and pre-term labour, substantial progress has been made, showing that increased prostaglandin production may be accompanied by increased concentrations of the cytokine interleukin-1β in the amniotic fluid (Romero *et al.*, 1987, 1989a). A series of studies have shown that the interleukins-1α, -1β, -6 and -8 together with tumour necrosis factor α (TNF-α) are present at high concentrations in amniotic fluid from pregnant women with obvious evidence of intrauterine infection (Romero *et al.*, 1989a, b, 1990), suggesting that these cytokines are involved in the activation of prostaglandin production from fetal membranes. This contention is supported by a number of studies that have shown that these cytokines stimulate the production of PGE_2 from cultured amnion cells (Romero *et al.*, 1989c; Mitchell *et al.*, 1991) and from cultured decidual cells (Casey *et al.*, 1989; Mitchell *et al.*, 1990; Ishihara *et al.*, 1992). A major source of cytokines in amniotic fluid is inflammatory cells present within the infected uterus (Romero *et al.*, 1988), but it is not known whether the decidua is a source of cytokines detected in amniotic fluid in the absence of intrauterine infection and ruptured fetal membranes. In addition, it is thought that the decidua may be the major source of prostaglandins involved in labour (Khan *et al.*, 1991, 1992) and we have found that IL-1α and IL-1β increase the production of prostaglandins by fetal membranes (Kent *et al.*, 1993), the accessibility of amniotic fluid cytokines to the decidua therefore needs to be assessed.

The aim of this study was to examine the transfer of cytokines in both the feto–maternal and materno–fetal directions through cultured human fetal membranes. It is clear from previous studies that fetal membranes actively metabolize prostaglandins (Keirse and Turnbull, 1976; Sullivan *et al.*, 1992); the metabolism of cytokines during transfer through the membranes was therefore also assessed.

Methods

The culture of intact human fetal membranes has been described in detail by Roseblade *et al.* (1990). In brief, fetal membranes were obtained from women with term normal pregnancies after delivery by elective Caesarean section. The use of this tissue has been approved (by the Ethical Committee of the Royal Postgraduate Medical School, Hammersmith Hospital and Queen Charlotte's Hospital Special Health Authority) and permission to use the tissue was obtained from the patients. Fetal membrane discs were held over the end of glass cylinders (14 mm diameter) with silicone rubber rings, and incubated with 1.5 ml medium (Medium 199 with 10% horse serum, 2 mmol glutamine l^{-1} and 1% penicillin–streptomycin: Gibco, Paisley) on each side. The cells in the membrane are viable by trypan blue (Sigma, Poole) exclusion and diaphorase histochemistry (Roseblade *et al.*, 1990) for at least 120 h of culture. All experiments described in this paper were terminated at no more than 96 h; the results should therefore not be affected by changes in the cultured membrane. Preliminary studies showed that preincubation of the

Received 22 February 1993.

membranes for 24, 48 or 72 h did not affect transfer of the cytokines through the membranes.

^{125}I-labelled IL-1α, ^{125}I-labelled IL-1β, ^{125}I-labelled IL-6 and ^{125}I-labelled TNF-α (Amersham International, Amersham) at 100 000 ± 15 000 d.p.m. ml^{-1} were added to either the fetal side or the maternal side of the cultured fetal membranes for 4–24 h. At the end of the experiments, the medium was taken from both sides of the membrane. The membranes were also removed and split into amnion and chorio–decidual components. Concentrations of ^{125}I in 150 μl aliquots of medium and in the split membranes were assessed immediately, and the remainder of the medium was frozen at − 20°C (for less than 7 days) until further analysis for integrity of the cytokines. The degradation of the cytokines was assessed by acidifying 150 μl aliquots of medium with 10% trichloroacetic acid and centrifuging at 1500 *g* for 10 min (Habberfield *et al.*, 1986). Small molecular weight fragments remain in solution under these conditions, whereas large polypeptides are precipitated. The extent of cytokine degradation on both sides of the membrane was calculated. Storage of ^{125}I-labelled cytokines at − 20°C for less than 7 days did not affect the extent of protein precipitation (about 90%) in control samples. The data are expressed as percentages to allow comparison between different experiments. Concentrations of immunoreactive cytokines were assessed by radioimmunoassays (Amersham International, Amersham). Data were compared using Student's *t* test.

Results

^{125}I-labelled IL-1β was added to the fetal side of cultured membranes after 48 h preincubation, and a detectable transfer of ^{125}I from the fetal to the maternal side of the membranes occurred within 4 h (Fig. 1a). Radioactivity in the medium on the maternal side of the membrane and in the chorio–decidual tissue increased for up to 24 h of incubation (Fig. 1a), whereas levels in the amnion remained low. The transfer of radioactivity through the membrane was similar when the ^{125}I-labelled IL-1β was added to the maternal side of the membrane (Fig. 1b), which suggested that the transfer of ^{125}I-labelled IL-1β was equivalent in both directions through the membrane. Similar results were obtained if the preincubation period was 24 h or 72 h (results not shown), indicating that the handling of IL-1β did not change during this period of culture.

Analysis of the ^{125}I-labelled IL-1β incubated at 37°C for 24 h in culture medium alone (with no tissue present) revealed that 80–90% of the parent compound was precipitated by the acid treatment, indicating that it was not being broken down. A similar percentage of intact protein was present in the medium from the side of the membranes to which the ^{125}I-labelled IL-1β was added (78.6 and 75%, from Table 1). Most of the radioactive compounds detected on the opposing side of the membrane were not acid-precipitable (Table 1), which suggests that most of the cytokine was degraded during transfer through the membrane. A small percentage (2% of the original radioactivity) appeared to be intact, indicating that limited transfer of cytokines through the membranes may occur. The integrity of the IL-1β was also assessed by radioimmunoassay of medium from both sides of the membrane. 20 ng IL-1β ml^{-1} was added to the fetal side of the membrane. After 24 h of

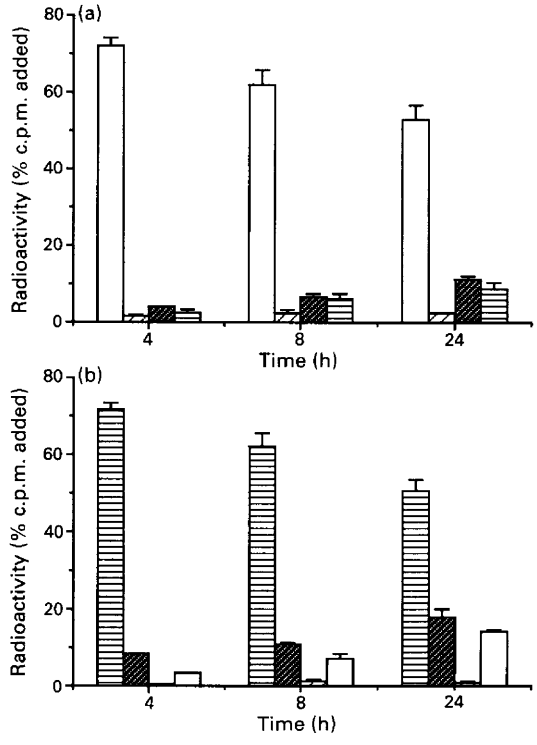

Fig. 1. ^{125}I-labelled IL-1β (113 000 d.p.m. ml^{-1}) was added to the fetal side (a) of cultured intact fetal membranes after 48 h of preincubation in medium alone. After the incubation periods shown, the radioactivity (expressed as the percentage of radioactivity added) in the fetal medium (□) and maternal medium (▤) was determined. The membrane disks were split into amnion (▨) and chorio–decidua (▩), and radioactivity measured. In (b) ^{125}I-labelled IL-1β (102 000 d.p.m. ml^{-1}) was added to the maternal side of the membrane and levels of radioactivity determined in the maternal medium (▤), chorio–decidua (▩), amnion (▨) and fetal medium (□). Data are means ± SEM (n = 3).

culture 7.7 ± 1.1 ng IL-1β ml^{-1} remained on the fetal side and only 0.4 ± 0.1 ng ml^{-1} was detected on the maternal side (2.0 ± 0.5% transfer). Comparable figures were obtained after the addition of 20 ng IL-1β ml^{-1} to the maternal side (8.3 ± 0.3 ng ml^{-1} on the maternal side and 0.6 ± 0.2 ng ml^{-1} on the fetal side), indicating 3 ± 1% transfer. Comparison of intact IL-1β concentrations after addition to the fetal side showed 50.1 ± 4.6% (acid precipitation) and 38.5 ± 5.5% (radioimmunoassay), which are not statistically different ($P > 0.05$). Corresponding data after addition to the maternal side were 57.9 ± 14.3% and 41.5 ± 1.5% ($P > 0.05$), which overall show that about 50% of the original cytokine remains intact on the side to which it was added after 24 h of culture.

Essentially similar data were obtained with the other cytokines tested (IL-1α, IL-6 and TNF-α), in that limited time-dependent transfer of intact cytokine occurred (results not shown), and that transfer of cytokines through the membrane was similar in the feto–maternal and materno–fetal directions. The extent of the degradation of the transferred cytokines varied considerably (Table 1). Very little of the IL-1α was intact, but higher levels of acid-precipitable radioactivity were found when the transfer of TNF-α and IL-6 were assessed. A minority of the radioactive material that passed though the

Table 1. Metabolism of cytokines on both sides of human fetal membranes

Cytokine added	Radioactivity not transferred (% added)		Radioactivity transferred (% added)	
	Total	Acid-precipitable	Total	Acid-precipitable
To fetal side				
IL-1β	63.7 ± 3.4	50.1 ± 4.6	7.6 ± 2.5	**2.0 ± 0.7**
IL-1α	89.0 ± 1.1	70.1 ± 12.0	6.8 ± 2.9	**2.4 ± 1.2**
IL-6	80.4 ± 1.9	73.4 ± 2.2	19.1 ± 2.3	**16.3 ± 2.5**
TNF-α	73.1 ± 4.1	55.8 ± 3.4	20.4 ± 3.1	**6.8 ± 2.1**
To maternal side				
IL-1β	77.2 ± 2.9	57.9 ± 14.3	11.1 ± 3.1	**3.7 ± 1.2**
IL-1α	82.1 ± 4.6	58.7 ± 8.2	8.8 ± 3.6	**2.7 ± 1.5**
IL-6	86.6 ± 2.6	79.4 ± 5.3	16.2 ± 1.9	**8.6 ± 1.6**
TNF-α	73.1 ± 3.3	55.4 ± 4.8	20.8 ± 2.0	**5.9 ± 0.1**

^{125}I-labelled cytokines (87 000–125 000 d.p.m. ml^{-1}) were added to the fetal or maternal side of cultured fetal membranes. Media were removed from both sides of the membrane after incubation for 24 h, and total and acid precipitable radioactivity determined. The percentage of the added radioactivity remaining on the side to which the cytokine was added (counts not transferred) and on the opposing side of the membrane (counts transferred) were calculated. All data are means ± SD from three experiments. Data in **bold** show transfer to intact cytokine through the membranes. IL-1: interleukin 1; TNF-α: tumour necrosis factor α.

membrane was acid-precipitable when IL-1α, IL-1β and TNF-α were used (25–35%), whereas metabolism of IL-6 seemed to be much less (50–85% acid precipitable). The lower metabolism of IL-6 was also apparent on the side to which it was added (more than 90% intact, from Table 1), whereas the other cytokines were 71.5–78.8% intact (from Table 1).

Discussion

The intact IL-1α, IL-1β and TNF-α remaining in contact with the amnion or the decidua decreased by about 25% during 24 h of culture (see above). Spontaneous degradation was about 15%, which suggests that about 10% of these cytokines was metabolized by amnion and decidua. In contrast, 65–75% of the IL-1α, IL-1β or TNF-α passing through the membrane was metabolized, indicating that the major breakdown of the cytokines occurred during transfer through the membranes. To cross the membrane, the cytokines must pass through the chorion; we therefore suggest that most of the metabolism occurs in this tissue. Similar data have been reported for PGE_2 (Roseblade *et al.*, 1991).

The transfer and metabolism of IL-6 differed from that of the other three cytokines tested. In particular, relatively high amounts of intact IL-6 passed through the membrane. We attribute this high transfer to low metabolism of IL-6 within the membrane on the following basis: metabolism of the other cytokines during transfer was 65–75% (25–35% intact); applying these figures to the total IL-6 transferred (16.2–19.1%) would suggest a maximum transfer of intact IL-6 of 6.7% (19.1% × 0.35) and a minimum transfer of 4.1% (16.2% × 0.25). These results resemble the actual data obtained for the other cytokines. It is clear that fetal membranes may produce large amounts of IL-6 (Romero *et al.*, 1990), and this may act as a block on metabolism of IL-6 added to either side of the membrane, but further studies are needed to assess this. The cytokine metabolites were not identified, although the loss of immunoreactivity and the solubility of the radioactive compounds in the presence of 10% trichloroacetic acid indicates that small peptides were being produced.

The transfer of intact IL-1α and IL-1β was similar to the transfer of intact PGE_2 through fetal membranes (Bennett *et al.*, 1990; McCoshen *et al.*, 1990; Roseblade *et al.*, 1990), in the range of 1–4%. These results suggest that these bioactive compounds are likely to be synthesized within, or very close to, the tissues on which they act. Thus the prostaglandins, which act on the myometrium, and cytokines, which act on the decidua, may be produced by the decidual–myometrial region of the uterus. This contention supports earlier suggestions that decidual activation has a major role in preterm labour associated with infection (Romero *et al.*, 1988), and is consistent with a recent report implicating decidual activation in term labour (Khan *et al.*, 1992). The enzymes responsible for the metabolism of the cytokines observed in the study have not been identified, although it seems likely that nonspecific proteases are most likely to be involved, as three of the four cytokines were degraded to the same extent. The internalization and degradation of IL-1β after binding to the cell-surface receptor depends on the type of receptor present (Horuk, 1991), in that cells with the 80 kDa (type I) receptor internalized, but did not degrade, the cytokine to acid soluble products, whereas cells with the type II receptor internalized 15% of the bound cytokine, of which 40% was released as acid-soluble products (Horuk, 1991). It is not known whether decidual cells possess only one or both receptors; further studies will therefore be needed to determine the contributions of decidua and chorion to overall cytokine metabolism.

Cytokines have been implicated in normal labour (Ishihara *et al.*, 1992; Khan *et al.*, 1992), but concentrations of the interleukins in amniotic fluid do not increase greatly during this

process (Romero *et al.*, 1989a, b, 1990). We suggest that when the fetal membrane is intact, decidual cytokines have only limited access to the amniotic fluid, and the changes cannot be detected by the techniques currently used. Only after membrane rupture would there be ready transfer of cytokines between the amniotic fluid and the decidua. This may also indicate that in women with intrauterine infection either the amnion or fetus produces cytokines, or the fetal membranes are damaged or ruptured so that decidual cytokines may gain access to the amniotic fluid, as it has been clearly demonstrated that in such cases there are very high concentrations of cytokines in the amniotic fluid (Romero *et al.*, 1989a, b, 1990). We have recently found that IL-1α and IL-1β activate prostaglandin production by human fetal membranes (Kent *et al.*, 1993), and that there seems to be some transfer of bioactive cytokines, between 1–4%, which supports the direct measurements in this study and implies that cytokines will be active in the tissues in which they are produced.

The authors thank Action Research for a Perinatal Research Fellowship (A. S. H. Kent).

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