

The regulation of prostaglandin output from term intact fetal membranes by anti-inflammatory cytokines

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SUMMARY

Prostaglandins are some of the main mediators which control parturition, and their production by intrauterine tissues can be up-regulated by pro-inflammatory cytokines. Anti-inflammatory cytokines may oppose these effects, and in this study we have investigated how two such cytokines affected fetal membrane function. Interleukin-10 (IL-10) inhibited the output of prostaglandin E₂ (PGE₂) from intact fetal membranes under basal and lipopolysaccharide (LPS)-stimulated conditions, and there was a parallel decrease in the expression of mRNA for COX-2. IL-10 also inhibited the production of interleukin-1 β (IL-1 β) and the expression of mRNA for IL-1 β , indicating that this cytokine has a broad anti-inflammatory effect. Transforming growth factor- β 1 (TGF- β 1), which is generally considered to be anti-inflammatory had opposite effects on PGE₂ production, in that it increased the output of PGE₂ for up to 8 hr. TGF- β 1 increased levels of type-2 cyclo-oxygenase (COX-2) and cytosolic phospholipase A₂ (cPLA₂) protein, and also activated the cPLA₂ enzyme present; the profile of effects is similar to that of the pro-inflammatory cytokine IL-1 β , and was not expected. Combinations of TGF- β 1 with IL-1 β also increased PGE₂ output and caused appropriate changes in prostaglandin pathway enzymes, whereas TGF- β 1 and IL-1 α had more limited effects. Further studies are needed to establish the physiological significance of these findings, but TGF- β 1 does not seem to act as an inhibitory cytokine in intact fetal membranes at term.

INTRODUCTION

Prostaglandin E₂ (PGE₂) and prostaglandin F_{2 α} (PGF_{2 α}) are produced by fetal membranes and other intrauterine tissues,^{1,2} and their levels are elevated in the amniotic fluid at term,³ as well as in labour.^{4,5} This increase in prostaglandin levels is thought to be a critical step in human parturition,^{6,7} and fetal membranes obtained from term human pregnancies may show marked increases in PGE₂ output, and in the expression of the type-2 cyclo-oxygenase (COX-2) before labour.⁸ A number of factors, including the pro-inflammatory cytokines interleukin (IL)-1, IL-6 and tumour necrosis factor- α (TNF- α),^{9–11} cause similar changes in COX-2 expression and prostaglandin output when added to fetal membranes *in vitro*. Human labour at all gestational ages thus has strong similarities with a general inflammatory response; from this it has been suggested that

anti-inflammatory factors will oppose these changes and thus sustain pregnancy. A number of cytokines which possess anti-inflammatory activity have been identified, but in this study we concentrate on two of them, namely IL-10 and transforming growth factor- β 1 (TGF- β 1), for the reasons outlined below.

IL-10 is present in amniotic fluid throughout pregnancy,^{12,13} and can be expressed by a number of tissues.^{14,15} In general, increased levels of IL-10 are linked to the continuation of pregnancy,^{16,17} which is consistent with the anti-inflammatory T helper 2 (Th2) profile of this cytokine.^{18,19} Higher levels have been found during labour;¹³ this has been attributed to increased production in response to the pro-inflammatory changes of labour,²⁰ rather than evidence of a requirement of IL-10 for labour. It is clear that inflammatory factors can induce IL-10 production from such tissues *in vitro*.²⁰ IL-10 generally down-regulates the production of prostaglandins and cytokines in other systems,^{21–23} although this is not a universal finding.²⁴ There seem to be no reports on the effects of IL-10 on fetal membrane prostaglandin output, but a series of studies has shown that IL-10 can inhibit the production of other inflammatory cytokines (IL-6, IL-8 and TNF- α) from human fetal membranes.^{25–27} We therefore studied the effects of IL-10 on the release of IL-1 β and PGE₂ from intact human fetal membranes incubated with bacterial endotoxin.

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Likewise, TGF- β 1 is present in intrauterine tissues; it has been localized immunocytochemically to the decidua,²⁸ and expression of its mRNA has been detected in the decidua, placenta and fetal membranes.²⁹ It is produced by a number of cell types including activated macrophages,³⁰ and can antagonize the biological activities of IL-1.³¹ TGF- β 1 generally inhibits the production of prostaglandins from intrauterine tissues, including amnion^{32,33} and decidual³⁴ cells stimulated with inflammatory factors, but it potentiates epidermal growth factor (EGF)-stimulated PGE₂ production from amnion cells.³⁵ As the functions of cells in intact decidua or placenta differs from those of isolated cells,³⁶⁻³⁸ we have assessed the effects of TGF- β 1 on prostaglandin output from intact fetal membranes. The main regulatory point controlling prosta-

glandin production is considered to be the expression of the inducible enzyme COX-2.^{9,39-43} In particular, it is clear that high levels of mRNA for COX-2 are linked to high levels of PGE₂ output from fetal membranes,⁸ and this is independent of whether the activation of the membranes is spontaneous (*in vivo*) or caused by experimental stimulation (*in vitro*).^{8,44} We will therefore investigate the role of COX-2 in our findings. The substrate for COX-2 (arachidonic acid) is liberated from cell membrane stores by the action of phospholipase A₂ enzymes, and the cytosolic form (cPLA₂) has been implicated in parturition.⁴⁵ In most, but not all, tissues pre-existing cPLA₂ is activated (by phosphorylation),^{46,47} and Ca²⁺-dependent translocation to the endoplasmic reticulum (ER) and nuclear membranes^{48,49} rather than there being increased enzyme

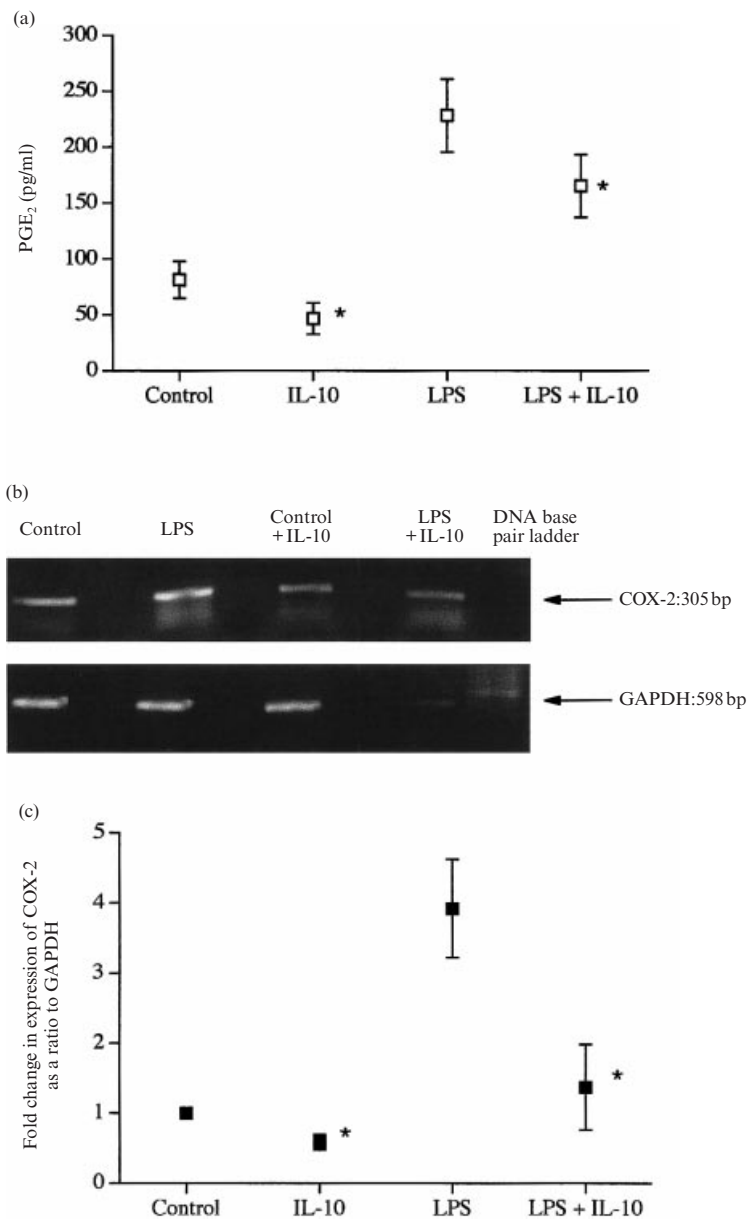


Figure 1. (a) The effects of IL-10 and LPS on the production of PGE₂ from human fetal membranes. All data are means \pm SEM ($n = 5$). (b) The presence of mRNA for COX-2 in typical samples. (c) Quantification of the changes in levels of mRNA for COX-2 in samples after 8 hr of culture. All data are means \pm SEM ($n = 3$). * $P < 0.05$ versus corresponding samples not containing IL-10.

synthesis. We intended initially to study only COX enzyme expression, but it became apparent that the effects of TGF- β 1 on PGE₂ output so resembled those of IL-1 β ⁴⁴ that further studies on cPLA₂ were necessary.

MATERIALS AND METHODS

The methods used in this study are essentially those described in our recent papers.^{8,44} The main features are given here.

Tissue collection and culture

Fetal membranes were collected from uncomplicated pregnancies at term (38–40 weeks gestation) after elective Caesarean

section in the absence of labour or infection. Patients were not suffering from pre-eclampsia and had not taken anti-inflammatory drugs for 2 weeks prior to delivery. Ethics committee approval was obtained to use tissues which would normally be discarded. Tissue was washed with phosphate-buffered saline containing 10% penicillin, streptomycin and L-glutamine (Sigma, Poole, UK). 1.5 cm discs of intact tissue were cut using a sharpened punch and cultured in Medium 199 supplemented with ITS (insulin, transferrin, selenium and linoleic acid all at 0.63 mg/ml, and bovine serum albumin at 0.13 mg/ml (Sigma)) in multiwell tissue culture plates for 24 hr at 37° in an atmosphere of 5% CO₂ : 95% air. The medium was also supplemented with 1 mM aspirin, as we have shown that

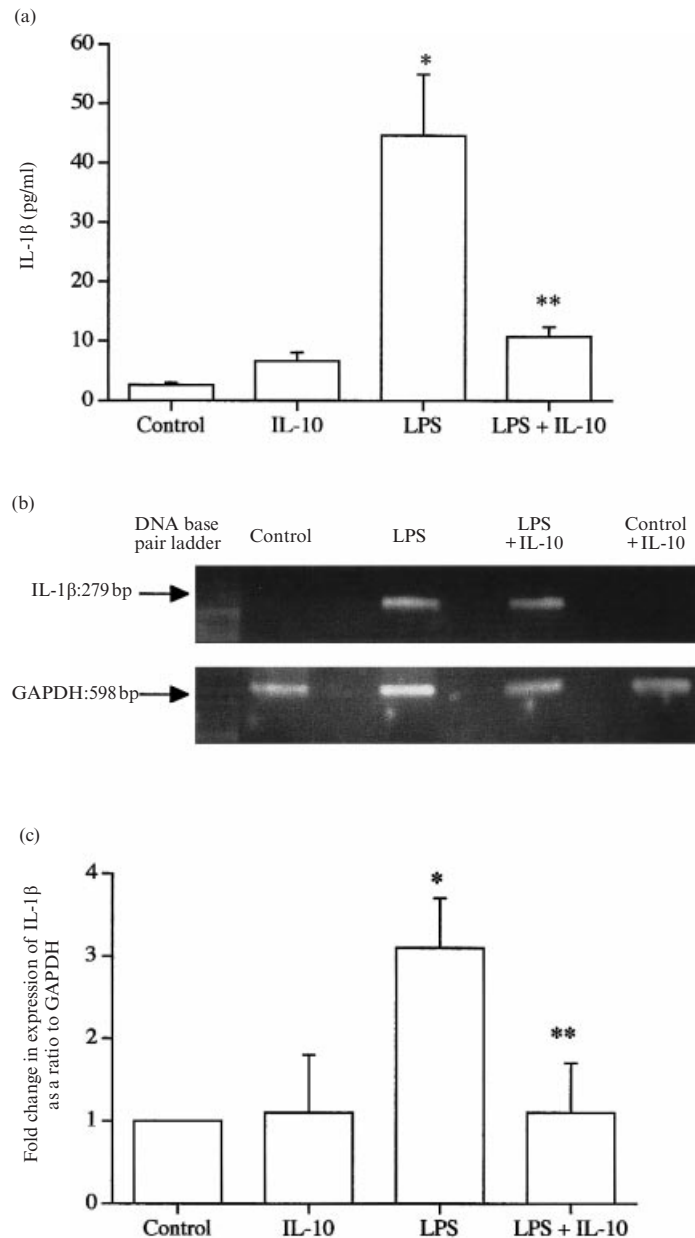


Figure 2. (a) The effects of IL-10 and LPS on the production of IL-1 β from human fetal membranes. All data are means \pm SEM ($n = 6$). (b) The presence of mRNA for COX-2 in typical samples. (c) Quantification of the changes in levels of mRNA for COX-2 in samples after 8 hr of culture. All data are means \pm SEM ($n = 3$). * $P < 0.05$ versus control; ** $P < 0.05$ versus LPS alone.

fetal membranes may be preactivated and spontaneously release high levels of PGE₂.⁸ After this time the medium was changed, the tissue washed with medium containing no aspirin, and incubated with IL-10 (alone or with lipopolysaccharide; LPS), or TGF- β 1 (alone or with IL-1 α or IL-1 β) for various time periods. All cytokines were added at 1 ng/ml, and LPS at 10 ng/ml ~ these concentrations were chosen on the basis of previous experience or pilot studies. Samples from different membranes were used for the IL-10 and TGF- β 1 studies. The tissues were snap frozen in liquid nitrogen and stored at -80°. The supernatants were then removed and frozen at -20° until analysis for PGE₂ levels by enzyme-linked immunosorbent assay (ELISA) (Amersham Pharmacia Biotech, Little Chalfont, Bucks, UK). The coefficients of variation were 7.5–8.1% (within assay) and 10.6% (between assay). Levels of PGE₂ and IL-1 β were determined in at least five separate replicate experiments of each culture condition.

LPS was used as a general inflammatory stimulus which stimulated the production of both IL-1 β and PGE₂, making it possible to assess the effects of IL-10 on two different aspects of

fetal membrane function which may be linked to labour. The surprising effects of TGF- β 1 on PGE₂ output resembled those of IL-1 α and IL-1 β reported previously,⁴⁴ so we used co-incubations to assess further the similarities and difference in the effects of these cytokines.

RNA extraction and reverse transcription-polymerase chain reaction (RT-PCR)

Fetal membrane discs were selected for further analysis on the basis of their output of PGE₂ or IL-1 β in response to the stimuli and other cytokines used. Discs were taken from three separate replicate experiments. Total RNA was extracted and stored at -80° until use.⁵⁰ RNA samples (1 μ g) were used as the template for the production of cDNAs and stored at -80° until PCR amplification.⁴⁴ The primers used have been reported previously: COX-1 and COX-2;⁵¹ cPLA₂ and sPLA₂;⁴⁴ glyceraldehyde phosphate dehydrogenase (GAPDH).⁵² PCR was performed according to the manufacturer's method (Bioline). Primer annealing temperatures were 58° for GAPDH and COX-2; 55° for sPLA₂ and cPLA₂. Cycle profiles were performed as described previously to ensure that the exponential phase was used to amplify the products.⁵¹ The cycle numbers chosen are indicated in the figure legends. Aliquots of the PCR products were separated by agarose gel electrophoresis and visualized under UV light.

To quantitate the PCR, 5 μ l of each PCR reaction was dotted onto Hybond nylon filters. The filters denatured, neutralized and washed and the DNA was fixed to the filters by UV crosslinking. Filters were first prehybridized for 1–2 hr at 65° followed by hybridization overnight with the appropriate ³²P dicytosine triphosphate (dCTP)-labelled cDNA probe at 65°. Excess probe was removed by washing in buffers of increasing stringency to 0.1 \times sodium saline citrate (SSC). The levels of cDNA were then determined by β -counting of the filter sections. The expression of each product was then expressed as a ratio relative to the expression of GAPDH. The ratios were then represented as a fold increase or decrease in mRNA expression.

Immunoblot analysis for protein phosphorylation of cPLA₂

Fetal membrane discs were homogenized in buffer (containing 1 mM sodium orthovanadate and the protease inhibitors pepstatin A (1 μ M), phenylmethylsulphonyl fluoride (PMSF) (0.1 mM), and E-64 (trans-epoxysuccinyl L-leucylamido-(4-guanidino)butane) (10 μ M)). Total lysate was centrifuged for 10 min at 13000 g to pellet the cell debris. Protein levels in the supernatant were determined by the method of Bradford⁵³ using bovine serum albumin (BSA) as the standard and 200 μ g of each sample was loaded on a 12% sodium dodecyl sulphate (SDS)-polyacrylamide gel. The stacking gel used an acrylamide:bis-acrylamide ratio of 30:0.5 and 67 mM imidazole to improve protein band resolution,⁵⁴ and hence demonstrate a phosphorylation-stimulated shift in cPLA₂ mobility.^{47,55} Purified cPLA₂ was also included on each gel as a positive control (Genetics Institute, Cambridge, MA). The proteins were electrophoretically transferred to a nitrocellulose filter, which was blocked in phosphate-buffered saline (PBS) containing 0.1% Tween and 5% non-fat milk. The filter was incubated with a rabbit polyclonal immunoglobulin G (IgG) antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA), followed by an anti-rabbit IgG antibody labelled with peroxidase (Sigma). Immunolocalized proteins were detected

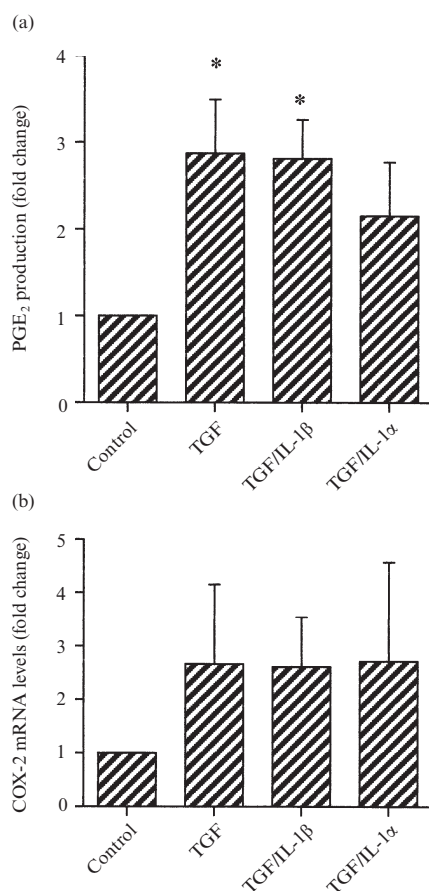


Figure 3. The effects of TGF- β 1 alone, or in combination with IL-1 β or IL-1 α on (a) the output of PGE₂ from intact fetal membranes during 4 hr of culture or (b) the expression of mRNA for COX-2. All cytokines were present at 1 ng/ml. PGE₂ output was compared to control from triplicate determinations from five separate replicate experiments (means \pm SEM). Basal PGE₂ output was 20–80 pg PGE₂/ml/4 hr, with a maximum stimulated output of 50–200 pg/ml/4 hr. COX-2 mRNA levels were assessed in fetal membranes from four separate experiments (means \pm SEM). * $P < 0.05$ versus control.

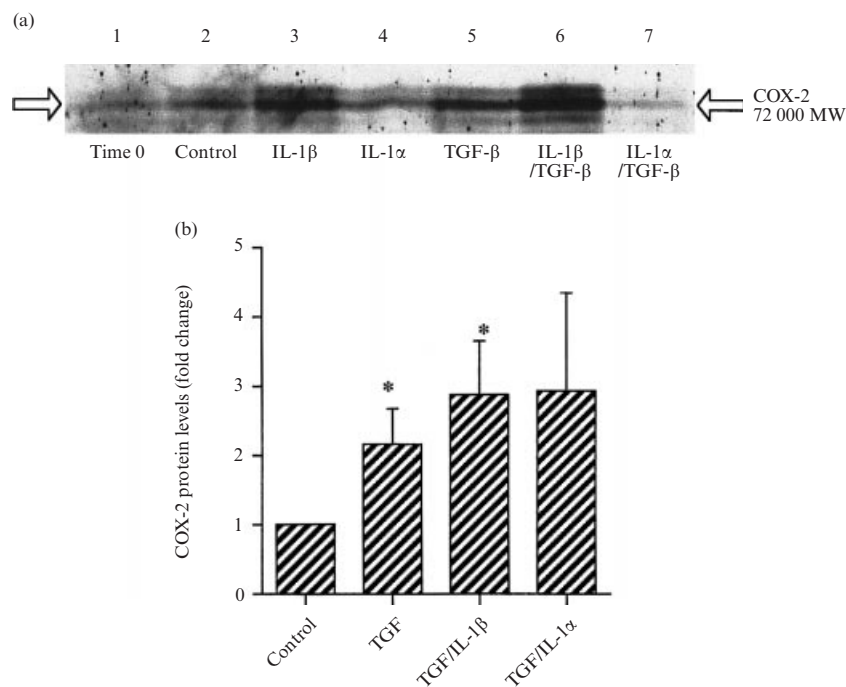


Figure 4. Studies on the presence of COX-2 protein in intact fetal membranes under the culture conditions shown. (a) Immunoblot for COX-2 protein from different membranes. (b) Quantification of changes in COX-2 protein normalized to β -actin after 4 hr stimulation with the cytokines shown (means \pm SEM $n = 3$). * $P < 0.05$ versus control.

using a chemiluminescent detection system (ECL, Amersham, Pharmacia Biotech). The filters were also incubated with a β -actin goat polyclonal antibody to verify equal loading of the samples. The presence of COX-1 and COX-2 protein was assessed on the same filters after they were stripped, washed, blocked as before and incubated with either a COX-1 or COX-2 goat polyclonal antibody (Santa Cruz). Human platelets were used as a positive control for COX-1, and for COX-2 lymphocytes stimulated with LPS were used.

Immunoblot detection of cPLA₂ translocation from the cytosol to the membrane

Fetal membrane discs were homogenized in 0.5 ml translocation buffer and the total lysate was centrifuged to remove cell debris. Cytosol (supernatant) and membrane (pellet) fractions were generated by ultracentrifugation at 100 000 g for 1 hr at 4°. 100 μ g of protein from the cytosol fractions and the whole membrane fraction were loaded on a 12% SDS-polyacrylamide gel. The proteins were electroblotted and cPLA₂ and β -actin were immunodetected as described above.

Analysis of immunoblots

The autoradiographs of each blot were scanned by a UMAX Mirage D-16L scanner (UMAX Technologies Inc., Fremont, CA) and the integrated density of each band determined using the software package Whole Band Analyser (Bioimage Services Ann Arbor, MI). The integrated density for cPLA₂ or COX-2 was then expressed relative to the density of β -actin for each sample.

Statistical analysis

The results are shown as mean \pm SEM. For statistical analysis, the results were log-transformed and investigated by ANOVA

with *post hoc* analysis by Fisher's exact test. Differences of $P < 0.05$ were considered to be significant.

RESULTS

LPS increased the output of PGE₂ from human fetal membranes three- to fourfold (Fig. 1a). Co-incubation with IL-10 diminished the effect of LPS, and IL-10 also decreased basal PGE₂ output (Fig. 1a). All fetal membrane samples expressed mRNA for COX-2 (Fig. 1b). Quantification of mRNA levels showed that IL-10 decreased both LPS-stimulated and basal COX-2 levels (Fig. 1c).

LPS also increased the output of IL-1 β from the intact fetal membranes (Fig. 2a). IL-10 substantially inhibited this increased IL-1 β output (Fig. 2a), whereas alone it had little effect on basal IL-1 β production (Fig. 2a). Fetal membranes expressed the mRNA for IL-1b (Fig. 2b), and quantification showed that IL-10 completely inhibited the LPS-stimulated increase in mRNA for IL-1 β (Fig. 2c).

TGF- β 1 increased the production of PGE₂ from fetal membranes after both 4 hr of culture (Fig. 3a) and after 8 hr; control, 41.0 \pm 16.6 pg PGE₂/ml, TGF- β 1, 110.6 \pm 19.5 pg PGE₂/ml (triplicate results from a single experiment, typical of three separate experiments). The combination of TGF- β 1 with IL-1 β had similar effects (Fig. 3a), but TGF- β 1 with IL-1 α did not significantly increase PGE₂ output. The expression of mRNA for COX-2 in these samples was variable, so that there were no statistically significant changes (Fig. 3b). We therefore studied levels of COX-2 protein by Western blotting, and found that the protein was present in all samples (Fig. 4a). TGF- β 1 alone, or in combination with IL-1 β , increased COX-2 protein levels (Fig. 4b). The combination of TGF- β 1 and IL-1 α

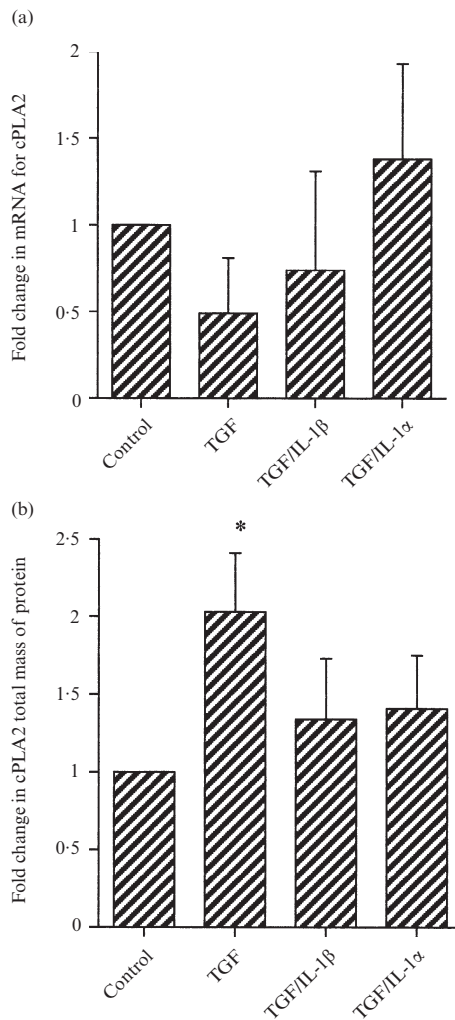


Figure 5. The effects of TGF- β 1 alone, or in combination with IL-1 β or IL-1 α on (a) levels of mRNA for cPLA₂ or (b) levels of cPLA₂ protein. In (a) the expression of mRNA was normalized to GAPDH prior to expression relative to control tissue samples. In (b) cPLA₂ levels were normalized to β -actin levels and expressed as a fold change (means \pm SEM $n = 4$). * $P < 0.05$ versus control.

was without significant effect, although the mean level of mRNA was similar to the other data (Fig. 4b).

To further explore the mechanism of action of TGF- β 1 on human fetal membranes, we investigated the effects of this cytokine on cPLA₂. Levels of mRNA were unaffected (Fig. 5a), but protein concentration was increased twofold by TGF- β 1 (Fig. 5b). This increase was abrogated by IL-1 α or IL-1 β (Fig. 5b).

Post-translational changes involved in the activation of cPLA₂, namely phosphorylation and translocation from the cytosol to the membrane, were also investigated. The phosphorylated and dephosphorylated forms of cPLA₂ could be resolved with polyacrylamide gel electrophoresis (PAGE) (Fig. 6a). Treatment with TGF- β 1 increased the level of phosphorylated cPLA₂ compared to control (Fig. 6b). Co-incubation with IL-1 β had no additional effect, and IL-1 α inhibited the changes in P-cPLA₂ caused by TGF- β 1 (Fig. 6b).

Cytosolic and membrane fractions of intact fetal membranes were prepared as described in the Methods section. Phosphorylated and de-phosphorylated forms of cPLA₂ could be visualized in both fractions (Fig. 7a). Quantification of the data showed that TGF- β 1 increased the translocation of cPLA₂ to the membrane fraction (Fig. 7b). The inclusion of IL-1 β or IL-1 α did not alter the effect of TGF- β 1.

DISCUSSION

The data obtained with IL-10 were consistent with the broadly anti-inflammatory profile of this cytokine established in previous studies on fetal membranes.^{32–34} The down-regulation of IL-1 β protein and mRNA in LPS-stimulated tissues was complete and clear-cut (Fig. 2), which was consistent with a direct effect of IL-10 on the induction process as reported in other cell-types.^{21,22} IL-10 had no effect on IL-1 β when added alone, but inhibited both basal and LPS-stimulated PGE₂ production and COX-2 expression (Fig. 1). In other tissues, IL-10 has variable effects on prostaglandin production; in human neutrophils and monocytes IL-10 decreased the production of PGE₂, which is linked to decreased expression of COX-2 protein and mRNA,^{21,22} whereas in human alveolar cells IL-10 had little effect on PGE₂ production,²³ and in mouse mast cells IL-10 potentiated the effects of other stimuli.²⁴ Our data do not discriminate between a direct effect of IL-10 on PGE₂ production from fetal membranes, and one mediated through changes in IL-1 β expression, although the difference in effect of IL-10 on basal PGE₂ and on basal IL-1 β output would indicate that separate mechanisms are involved. Further studies are needed to resolve this question, but from a physiological viewpoint it is clear that IL-10 is potentially a pro-pregnancy factor via the inhibition of inflammatory pathways. This is consistent with data from other studies,^{21,22,25–27} which show that Th2 cytokines (including IL-10) are linked to normal pregnancy.^{56,57}

The data obtained with TGF- β 1 were in marked contrast to most of the previous studies on intrauterine tissues which showed that this cytokine was anti-inflammatory.^{32–34} All those studies were done on isolated cells, and the difference in the data obtained may be linked to this. It has been shown that tissue dissociation increases the production of TGF- β 2 (and many other cytokines) from first trimester human decidua,³⁷ and it seems reasonable that the responses to cytokines may be altered. Comparison with other studies on intact fetal membrane shows that TGF- β 1 had effects very similar to those of IL-1 α and IL-1 β .⁴⁴ PGE₂ output was increased two- to threefold by all three cytokines, linked to moderate changes in mRNA and protein for COX-2 – again two- to threefold (Figs 3 and 4), with varying degrees of statistical significance. The phosphorylation of cPLA₂ was increased 1.5-fold (Fig. 6 and 44), and cPLA₂ was redistributed into the membrane fraction (Fig. 7). One major difference was that TGF- β 1 increased the levels of cPLA₂ protein (Fig. 5), whereas IL-1 α or IL-1 β were without effect.⁴⁴

The combination of TGF- β 1 and IL-1 β generally had similar effects to the individual cytokines (PGE₂, COX-2 mRNA and protein, cPLA₂ phosphorylation and redistribution) – the only exception was that IL-1 β diminished the effect of TGF- β 1 on cPLA₂ total protein. There was no evidence that TGF- β 1 inhibited the effects of IL-1 β on intact fetal

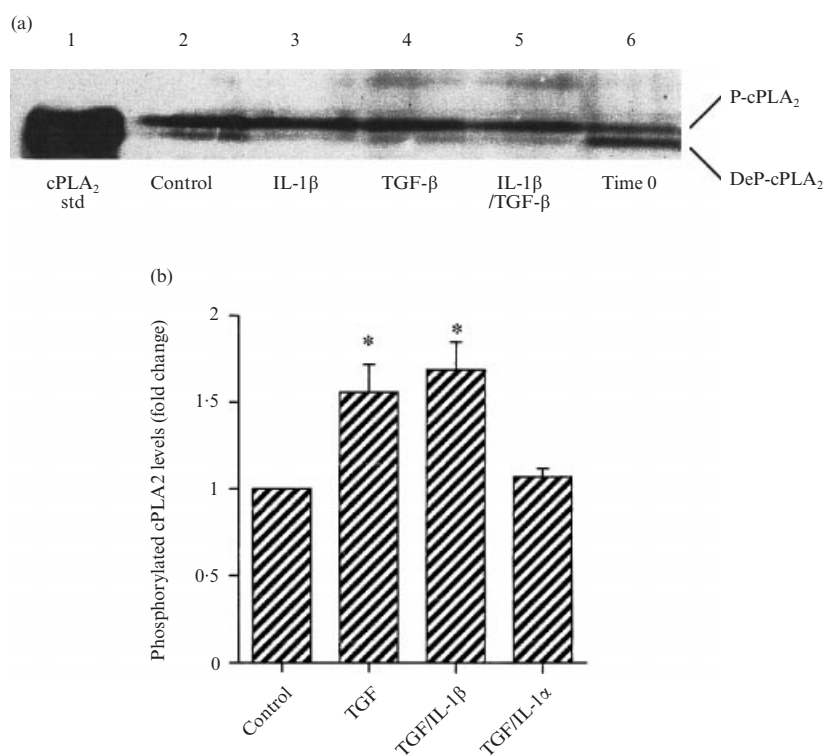


Figure 6. (a) The separation of phosphorylated (P-cPLA₂) and dephosphorylated (DeP-cPLA₂) forms of cPLA₂ by PAGE, followed by immunoblotting. Time 0 samples were cut from the tissues and frozen immediately at -70° . (b) Quantification of the levels of P-cPLA₂ from replicate experiments, after 2 hr incubation with the cytokines shown. Data were corrected for β -actin levels and expressed as a fold change (means \pm SEM $n=4$). * $P < 0.05$ versus control.

membranes, and it was therefore not exhibiting anti-inflammatory activity.

IL-1 α generally had more variable effects on fetal membranes than did IL-1 β ,⁴⁴ and this also applied to the combination of TGF- β 1 with IL-1 α . The only significant change was in the distribution of cPLA₂ (Fig. 7b). The combination of cytokines had limited effects on PGE₂ production and COX-2 mRNA and protein (Figs 3 and 4), but the quantity and phosphorylation of cPLA₂ were unaffected (Figs 5 and 6). TGF- β 1 did diminish the effects of IL-1 α on PGE₂ output (Fig. 3), and on the levels of phosphorylated cPLA₂ (Fig. 6), but otherwise it could not be stated that TGF- β 1 inhibited the effects of IL-1 α .

We have shown in this study that TGF- β 1 increases PGE₂ production from intact fetal membranes after 4 hr or 8 hr of treatment. Other studies performed on amnion or decidual cells found TGF- β 1 to inhibit PGE₂ production in the presence of IL-1,^{32–34} whereas TGF- β 1 potentiated the effects of EGF.³⁵ However, our study was performed using intact tissue and may therefore be a better representative model of the events that take place *in vivo* compared to those in isolated cells.^{36–38} In particular, enzymatic digestion of decidual tissue activates production of TGF- β 2 and so is likely to also increase the production of TGF- β 1.³⁷ This may contribute to the different findings obtained from digested and intact tissues.

The mechanisms by which TGF- β 1 increased PGE₂ output were compared with those of IL-1 α and IL-1 β .⁴⁴ None of the factors used in this study affected the expression of mRNA for

cPLA₂, although TGF- β 1 increased cPLA₂ protein levels, and was the only factor to do so (Fig. 2). There was no shift from non-phosphorylated cPLA₂ to the phosphorylated (active) form in the presence of TGF- β 1, but the increase in protein led to an overall increase in phosphorylated cPLA₂ (Fig. 3). This may be contrasted with the interleukins, which caused a shift in phosphorylation without affecting cPLA₂ protein levels.¹⁰ All the cytokines caused translocation of cPLA₂ from cytosol to cell membranes, which is consistent with increased release of arachidonic acid from membrane phospholipids. This analysis may not reflect total cPLA₂ levels, as some membranes will be lost in the initial sample preparation, but should reflect the relative distribution between cytosol and membrane fractions.

The important point is that TGF- β 1 had similar effects on COX-2 protein and mRNA as did the pro-inflammatory interleukins, indicating the TGF- β 1 is not functioning as the anti-inflammatory factor we anticipated on the basis of previous data.^{32–34} A TGF- β response element is present in the human COX-2 promoter,⁵⁸ and a similar response element may mediate the stimulatory effects of TGF- β 1 on COX-2 expression and PGE₂ production in murine osteoblastic cells.⁵⁹ These findings provide a precedent for our data. Other studies have indicated that TGF- β can interact in complex ways with many other cytokines, including IL-1 β , IL-1 receptor antagonist,^{34,60} the IL-2–IL-2 receptor pathway³⁰ and IL-6.⁶¹ Human fetal membranes are very heterogeneous at the cellular level, and further studies are needed to investigate the possible cross-talk between different cells and cytokines.

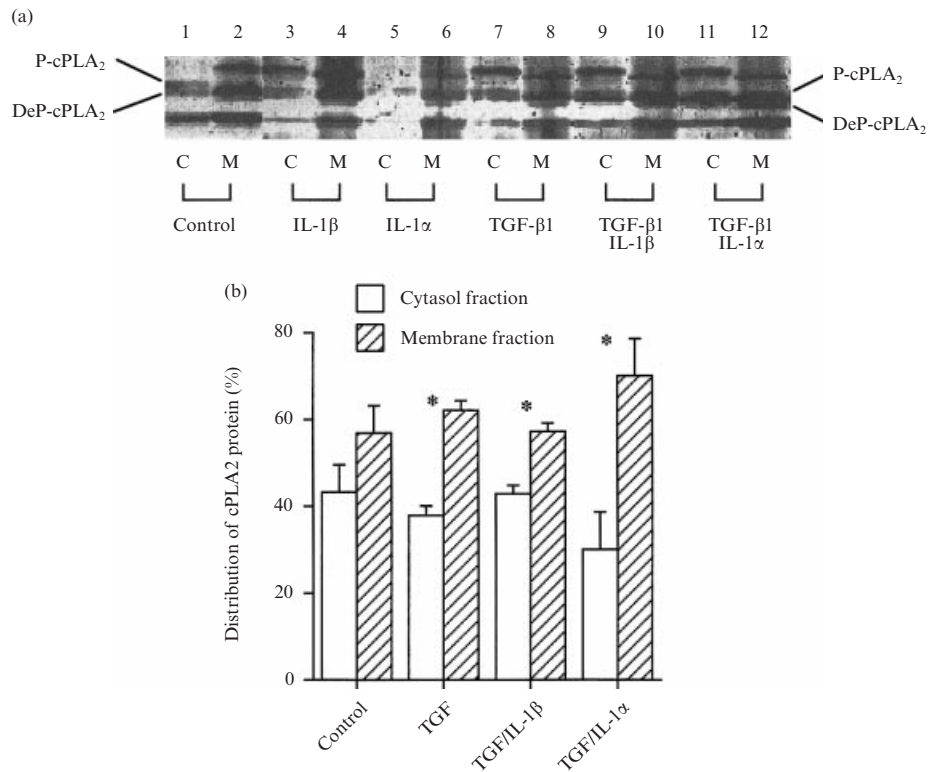


Figure 7. (a) The separation of P-cPLA₂ and DeP-cPLA₂ from cytosolic (C) and membrane (M) fractions of intact fetal membranes, followed by immunoblotting. (b) Distribution of total cPLA₂ after correction for β-actin levels (means ± SEM n = 3). * P < 0.05 for difference between cytosol and membrane fractions for each treatment group.

We conclude that cytokines whose functions have been identified in other tissues may not exert similar effects in human fetal membranes, and that detailed studies are required for each cytokine. IL-10 had the anti-inflammatory effects expected from previous studies, whereas TGF-β1 more closely resembled a pro-inflammatory cytokine.

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