Effects of interleukin-4 on the expression and activity of prostaglandin endoperoxide H synthase-2 in amnion-derived WISH cells

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ABSTRACT

Increased prostaglandin biosynthesis during intra-uterine infection may be a possible mechanism by which preterm labour is initiated. Inflammatory cytokines and growth factors are known to stimulate prostaglandin production through an increase in prostaglandin endoperoxide H synthase (PGHS)-2 synthesis and activity. Interleukin-4 (IL-4), an anti-inflammatory cytokine, can downregulate PGHS-2 expression and inhibit prostaglandin production. Therefore, the aims of the current study were to determine the effects of IL-4 on PGHS-1 and PGHS-2 expression in amnion-derived WISH cells treated with inflammatory cytokines and growth factors. In WISH cells, near-maximal production of the PGHS-2 mRNA occurred using 5 ng/ml EGF, 1 ng/ml IL-1 β or 50 ng/ml TNF- α . Time-course experiments determined that the PGHS-2 mRNA was induced maximally by these stimuli by 1 h. Pretreatment of WISH cells with IL-4 reduced PGHS-2 mRNA levels at 1 h by 67% in cells treated with EGF, 62% in cells treated with

IL-1 β and 54% in cells treated with TNF- α . Pretreatment with IL-4 more effectively inhibited PGHS-2 expression than simultaneous addition with EGF or IL-1 β but not TNF- α . Immunoblot analysis showed a correlation between inhibition of mRNA levels and levels of PGHS-2 protein, although stimulation of PGHS-2 protein production by EGF was undetectable. Levels of PGHS-1 protein and mRNA remained unchanged in all experiments. Increased production of prostaglandin E₂ (PGE₂) in response to TNF- α and IL-1 β treatment was attenuated by IL-4 pretreatment, by 52% and 72%, respectively. No attenuation of EGF-stimulated PGE₂ levels was seen. We conclude that IL-4 inhibits PGHS-2 mRNA and protein production in cytokine-stimulated WISH cells, but does not affect EGF-stimulated PGE₂ production, suggesting that EGF can induce prostaglandin biosynthesis by a mechanism other than through increased PGHS-2 expression.

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INTRODUCTION

Inflammatory processes are evident in approximately 30% of cases of preterm labour (Romero *et al.* 1988), the leading cause of preventable perinatal mortality (van den Berg & Oechsli 1984). The critical event in human parturition is believed to be increased prostaglandin production within intrauterine tissues (Thorburn 1992, Mitchell 1994). Prostaglandin synthesis within the amnion, the inner fetal membrane of the placental sac, has been suggested to be key in the human labour process (Mitchell 1984, Challis & Olson 1988, Olson *et al.* 1993).

Associations between preterm labour and intra-uterine infection (Romero *et al.* 1988) suggest that cytokines, through the induction of prostaglandins, facilitate the infectious process and subsequent untimely parturition (Romero *et al.* 1989a,b). Increased levels of pro-inflammatory cytokines, such as tumour necrosis factor (TNF)- α and interleukin (IL)-1 β , are produced by cells of the host's immune system within the decidual layer of the placental membranes in response to bacterial metabolites (Casey *et al.* 1989, Mitchell *et al.* 1990). The effects of cytokine release include induction of prostaglandin synthesis in the amnion (Romero *et al.* 1989a, Olson *et al.* 1991), activation of the

myometrium, and cervical ripening potentially leading to infection-induced preterm labour (Perkins *et al.* 1996).

Prostaglandin biosynthesis in amnion cells at term is influenced by other stimulatory factors. Epidermal growth factor (EGF) stimulates prostaglandin synthesis by human amnion (Mitchell 1987) and may have an important role in the parturient process as EGF and EGF receptor increase in the amniotic fluid in late pregnancy and during labour, respectively (Romero *et al.* 1989c, Varner *et al.* 1996, Gargiulo *et al.* 1997). As EGF upregulates prostaglandin production in the amnion derived cell-line, WISH (Hayflick 1961, Perkins & Kniss 1997), it is believed that EGF may have important roles in the initiation of labour at term. Roles for EGF in the maturation of the foetal lung (Sundell *et al.* 1980) and adrenal cortisol biosynthesis (Singh-Asa & Waters 1983) have been described in animal models, suggesting links between foetal development and parturition at term.

Prostaglandin endoperoxide H synthase (PGHS) catalyses the committed step in prostaglandin biosynthesis. In this reaction, the oxygenation of arachidonic acid, followed by the reduction of prostaglandin G₂, can be catalysed by either of two PGHS isoforms, PGHS-1 or PGHS-2 (reviewed by Smith & DeWitt 1995). Each isoform is encoded by separate genes (Funk *et al.* 1991, Jones *et al.* 1993) and they are believed to metabolise arachidonic acid from differing cellular stores, because of their distinct cellular location. PGHS-1 has been shown to be located in the nuclear envelope and the endoplasmic reticulum, whereas PGHS-2 is found mainly in the nuclear envelope (Morita *et al.* 1995). In general, PGHS-1 is constitutively expressed and non-responsive to inflammatory stimuli, whereas PGHS-2 is expressed in response to growth factors and inflammatory mediators such as EGF, TNF- α , and IL-1 β (Han *et al.* 1990, Smith *et al.* 1991, Pueringer & Hunninghake 1992, DeWitt *et al.* 1993, Albert *et al.* 1994, Herschman 1996). The PGHS-2 gene is believed to be an early response gene, because of the rapid upregulation of PGHS-2 mRNA in the presence of protein synthesis inhibitors after its induction (Herschman 1991). This mRNA is also subject to post-transcriptional regulation, suggested by its rapid degradation in the cytoplasm (Evelt *et al.* 1993, Ristimaki *et al.* 1996, Hansen *et al.* 1998).

Interleukin-4 (IL-4) is a T helper 2 (T_h2)-derived cytokine produced by T-cells, mast cells and placental tissues (Yokata *et al.* 1988, Brown & Hural 1997, De Moraes-Pinto *et al.* 1997). It downregulates release of many proinflammatory mediators, including TNF- α (Lehn *et al.* 1989) and

IL-1 β (Paul & Ohara 1987). IL-4 may play a part in human labour by suppressing responses to factors that stimulate prostaglandin production. Interestingly, however, it has been shown to increase PGE₂ synthesis in amnion cells in primary culture (Adamson *et al.* 1994).

In the current investigation, we examined the regulation of PGHS-2 expression and PGE₂ biosynthesis by EGF, IL-1 β , and TNF- α , and the ability of IL-4 to antagonise their actions. The human amnion-derived cell line, WISH, was chosen as a model for amnion epithelial cell regulation of PGHS-2 expression in response to cytokines and growth factors. EGF, IL-1 β , and TNF- α have been shown previously to enhance PGE₂ biosynthesis in WISH cells (Harris *et al.* 1988, Albert *et al.* 1994, Perkins & Kniss 1997) in addition to regulating PGHS-2 expression. Furthermore, pretreatment with IL-4 before stimulation has been shown to attenuate PGHS-2 increases in monocytes and WISH cells to a greater extent than the attenuation seen with concurrent additions of IL-4 and stimulant (Harding *et al.* 1996, Mertz *et al.* 1996). As intrauterine infection-driven prostaglandin release is a feature in many cases of preterm labour, it is important to understand the balance that exists between those molecules that induce and those that inhibit prostaglandin biosynthesis. Therefore, we sought to investigate the effects of the anti-inflammatory cytokine, IL-4, on PGHS-1 and -2 expression and PGE₂ production in the presence of stimulants known to be present in the amniotic fluid during normal term labour and preterm labour with infection.

MATERIALS AND METHODS

Cell culture dishes and plastic ware were obtained from Nunc, Naperville, IL, USA and Becton Dickinson, Lincoln Park, NJ, USA. Cell culture medium, fetal calf serum (FCS) and 0.24-9.5-kb RNA ladder were purchased from GIBCO Life Technologies, New Zealand. [α -³²P]dCTP (3000 Ci/mmol) and [5,6,8,11,12,14,15(n)-³H]PGE₂ tracer were obtained from Amersham Life Science, Buckinghamshire, UK. The random prime labelling kit was a product of Pharmacia Biotechnology, Uppsala, Sweden. GeneScreen Plus and gel-blotting paper were purchased from DuPontNEN, Boston, MA, USA and Schleicher and Schuell, Dussel, Germany, respectively. Guanidine thiocyanate was obtained from Fluka, Buchs, Switzerland. Salmon sperm DNA was purchased from Boehringer, Mannheim, Germany. Trypsin 1:250 was from Difco, MN, USA. PGHS-1

polyclonal antibody was a product of Oxford Biomedical Research, Oxford, MI, USA. Polyacrylamide and protein molecular weight markers were from BioRad, Hercules, CA, USA. Prostaglandin E₂ standard and prostaglandin E₂ antibody were acquired from Cayman Chemicals, MI, USA and PerSeptive Biosystems, MA, USA. All other biochemicals were from Sigma, St Louis MO, USA and Reidel de Haehn, Seelze, Germany.

Cell culture

WISH cells were maintained in a Dulbecco's modified Eagle's medium/F12 plus 10% heat-inactivated FCS at 37 °C in a 95% air/5% CO₂ atmosphere. Medium was supplemented with 60 µg/ml penicillin and 100 µg/ml streptomycin. WISH cells were grown to confluence in 6-cm tissue culture dishes for RNA and protein studies and 24-well plates for PGE₂ production experiments. At confluence, medium was removed and replaced with the test substances in fresh medium.

PGE₂ radioimmunoassay

Prostaglandin E₂ was measured using sensitive and specific radioimmunoassay on unextracted media samples as described previously (Lundin-Schiller & Mitchell 1991). The bicinchoninic acid method (Redinbaugh & Turley 1986) calibrated against bovine serum albumin was used to determine cellular protein concentration. PGE₂ production was normalised to the untreated control and expressed as fold induction over control ($n=4$ wells per treatment). The assay had a sensitivity of approximately 7 pg/ml and an intra-assay precision of 7%.

RNA extraction

After treatment, WISH cells were lysed with 0.8 ml GTC (4 M guanidine thiocyanate, 25 mM sodium citrate pH 7, 0.5% Na-*N*-lauryl sarcosine, 0.1 M β-mercaptoethanol) and stored in 2-ml Eppendorf tubes at -70 °C until required for processing. To each sample, 80 µl 2 M sodium acetate pH 4, 0.8 ml H₂O-saturated phenol, and 160 µl chloroform : isoamyl alcohol (v/v 49 : 1) were added, with vortexing between each addition. Samples were placed on ice until phase separation became apparent, upon which they were centrifuged for 12 min at 10 000 *g*, at 4 °C. The aqueous layer was removed and combined with 0.8 ml isopropanol, vortexed and left overnight at -20 °C. RNA was pelleted by centrifugation for 12 mins at 10 000 *g*, washed in 80% ice-cold ethanol, and resuspended in

110 µl formamide with heating at 55 °C. RNA concentration within each sample was determined by measuring absorbance at 260 nm.

Northern blot analysis

Fifteen micrograms total RNA were added to an equal volume of RNA loading buffer (40 mM 3-(*N*-morpholino)propanesulphonic acid (MOPS), 2 mM sodium acetate, 12% formaldehyde, 2 mM EDTA, 10% glycerol, 0.1% bromophenol blue, 0.1% xylene cyanol). The samples were heated at 70 °C for 10 min and maintained on ice before being loaded onto a 1% agarose gel containing 20 mM MOPS, 6% formaldehyde, 1 mM EDTA, and 1 mM sodium acetate pH 7.0. Electrophoresis was carried out overnight at 1.0 V/cm. The gel was rinsed in de-ionised water and equivalence of loading assessed under u.v. light. The gel was shaken in a 50 mM NaOH for 20 min, followed by a 1-h neutralisation in 0.25 M NaH₂PO₄ pH 6.5. RNA was transferred to GeneScreen Plus by standard capillary transfer procedures in a 25-mM NaH₂PO₄ buffer pH 6.5, overnight. Transfer was assessed under u.v. light and the blot subsequently irradiated with u.v. 1.2×10^5 µJ in a Stratagene Stratalinker. After prehybridisation at 37 °C in 50% formamide, 25 mM Na₂HPO₄ pH 7.2, 1 mM EDTA, 250 mM NaCl, 7% w/v SDS, and 100 µg/ml salmon testis DNA, the blots were hybridised overnight with a random-prime-labelled cDNA probe [2.0 kb EcoRI/Xba I fragment of PGHS-1 (Funk *et al.* 1991) or the 1.8-kb EcoRI/Xba I fragment of the PGHS-2 (Hla & Neilson 1992) cDNA ($>10^9$ c.p.m./µg)] at a concentration of 5 ng/ml. The blots were rinsed three times and washed twice for 20 min in Wash 1, twice in Wash 2 and 3 at 53 °C for 10 min each. Wash 1: 2 × SSC, 0.5% SDS; Wash 2: 50 mM Na₂HPO₄ pH 7.2, 1 mM EDTA, 0.5% SDS; Wash 3: 50 mM Na₂HPO₄ pH 7.2, 1 mM EDTA, 0.5% SDS. Band intensity was quantitated by electronic autoradiography on a Packard InstantImager. For re-probing, the blots were stripped by agitation in a 2% glycerol solution at 80 °C for 10 min.

mRNA half-life analysis

WISH cells were grown to confluence and incubated in the presence or absence of IL-4 (10 ng/ml) for 1 h. TNF-α was then added to the cells at a final concentration of 50 ng/ml. After 1 h of TNF-α treatment, the RNA polymerase II-specific inhibitor, 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB), was added to the cells at a final concentration of 65 µM. At 0, 0.5, 1, and 2 h

after the addition of DRB, medium was removed and total RNA was isolated. Control cells were treated with ethanol carrier at a final concentration of 0.5%.

Immunoblot analysis

After treatment, WISH cells were lysed with 400 µl hypotonic lysis buffer (100 mM Tris, 6.8, 2% SDS, 0.25 M sucrose, 2 mM EDTA, 1 µg/ml pepstatin A, 100 µg/ml phenylmethylsulfonylfluoride, 15 mM sodium azide) and boiled for 5 min. Protein concentration was determined as previously described and 2-mercaptoethanol was then added to a final concentration of 5% (v/v). Twenty micrograms total cellular protein were separated on a 10% SDS-PAGE gel and transferred to nitrocellulose using the Bio-Rad Mini Trans-Blot electrophoretic transfer system. The blots were blocked overnight with 5% Blotto (PBS, 0.1% thiomersol, 5% non-fat dry milk powder) at 4 °C, then probed with a 1:2000 dilution of rabbit anti-human PGHS-2 antiserum in PBS containing 1% Blotto and 2% horse serum. The antiserum has been shown previously to be specific for the PGHS-2 isoform (Trautman 1996). Goat anti-rabbit IgG conjugated to horseradish peroxidase was used as the secondary antibody. The Amersham Enhanced Chemiluminescence system was used to visualise the immunoreactive protein. Identical conditions were used for detection of the PGHS-1 protein. Band intensity was quantitated using the MD30 Image analysis System (Leading Edge, Australia) and video camera mounted on a Leitz Diaplan microscope.

Statistical analysis and data expression

Measurements of PGHS-2 mRNA levels and PGE₂ production are depicted as the mean ± S.E.M. Statistical significance was determined by ANOVA followed by a post-hoc Dunnett's test. Significance was set at a *P* value of <0.05.

RESULTS

Determination of cytokine and growth factor concentration

Confluent WISH cells were treated with increasing concentrations (0.1–100 ng/ml) of EGF (Fig. 1A), IL-1β (Fig. 1B), and TNF-α (Fig. 1C) and for 1 h to determine the concentration that resulted in near maximal expression of PGHS-2 mRNA. Northern blot analysis of WISH cell total RNA revealed that all factors induced PGHS-2 mRNA expression in a concentration-dependent fashion at 1 h: 5 ng/ml EGF (Fig. 1A) induced a 2.76 ± 0.43 -fold increase,

FIGURE 1. Effect of increasing concentrations of (A) EGF (0–100 ng/ml), (B) IL-1β (0–100 ng/ml), and (C) TNF-α (0–100 ng/ml) on PGHS-1 and PGHS-2 mRNA expression. Total RNA was isolated 1 h after treatment and 15 µg were blotted and probed with ³²P-labelled PGHS-2- and PGHS-1-specific cDNA probes. Signal intensity was quantitated on a Packard InstantImager. Mean increases ± S.E.M. in PGHS-2 mRNA levels at optimal cytokine/growth factor concentrations are given in the text.

1 ng/ml IL-1β (Fig. 1B) induced an 8.12 ± 0.99 -fold increase, and TNF-α (50 ng/ml) (Fig. 1C) resulted in a 10.29 ± 3.21 -fold increase in PGHS-2 mRNA expression over that in control cells. These concentrations were chosen for all subsequent experiments. No factor-induced changes in expression were observed for the PGHS-1 mRNA (Fig. 1).

Pretreatment with IL-4 inhibits PGHS-2 mRNA expression

To determine the concentration of IL-4 that most effectively attenuated cytokine and growth factor-stimulated PGHS-2 mRNA levels by 1 h, increasing concentrations of IL-4 (0.1–100 ng/ml) were added to cells either 1 h before or concurrently with treatment with EGF, IL-1β, or TNF-α (Fig. 2).

FIGURE 2. Pretreatment with IL-4 more effectively inhibits induction of PGHS-2 mRNA expression in WISH cells. Increasing concentrations of IL-4 (0.1–100 ng/ml) were added to WISH cells either 1 h before EGF, IL-1 β , or TNF- α (Pre) or simultaneously with EGF, IL-1 β , or TNF- α (Con). Total RNA was isolated 1 h after treatment with EGF, IL-1 β , or TNF- α and probed with 32 P-labelled PGHS-2- and PGHS-1-specific cDNA probes. Signal intensity was quantitated on a Packard InstantImager and is representative of three separate experiments. Mean inhibition $\pm$ S.E.M. of PGHS-2 mRNA levels at optimal IL-4 concentration (10 ng/ml) is given in the text. U, untreated cells; C, cells treated with cytokine or growth factor only.

One hour after the addition of cytokine/growth factor, RNA was isolated and probed for PGHS-1 and -2 mRNA. In untreated cells, the PGHS-2 mRNA was expressed at very low levels. In cells with the addition of only cytokine or growth factor, PGHS-2 mRNA levels increased relative to those in untreated cells. IL-4 (10 ng/ml) was more effective at inhibiting PGHS-2 expression when added before IL-1 β than when added concurrently with IL-1 β ($62 \pm 4\%$ compared with $33 \pm 2\%$) or EGF ($67 \pm 3\%$ compared with $20 \pm 11\%$). In cells treated with TNF- α , the time of addition of IL-4 (10 ng/ml) had no effect on the inhibition of PGHS-2 mRNA expression ($49 \pm 1\%$ compared with $54 \pm 7\%$).

Induction of the PGHS-2 mRNA is inhibited by IL-4

We compared the induction of the PGHS-2 mRNA in response to EGF, IL-1 β , and TNF- α (Fig. 3). In cells treated with these factors alone, an increase in PGHS-2 mRNA was evident within 30 min after their addition and was greatest at 1 h. All factors elicited an initial induction of PGHS-2 mRNA with similar temporal characteristics, whereas only the inflammatory cytokines, TNF- α and IL-1 β , induced a second upregulation of PGHS-2 mRNA at 8 and 16 h. The addition of IL-4 to the cells inhibited the accumulation of the PGHS-2 mRNA at 1 h by $59 \pm 9\%$ in cells treated with EGF, $52 \pm 11\%$ in cells treated with IL-1 β , and $54 \pm 8\%$ in cells treated with TNF- α . Equivalent degrees of

inhibition were observed at all times at which stimulation was evident, including the later induction of PGHS-2 expression by IL-1 β and TNF- α . The levels of PGHS-1 mRNA were unaffected by the addition of IL-4 (data not shown).

IL-4 does not affect the decay rate of the PGHS-2 mRNA

Although time-course experiments suggested that IL-4 inhibited PGHS-2 mRNA transcription, we

FIGURE 3. The temporal induction of the PGHS-2 mRNA is inhibited by IL-4 pretreatment. Confluent WISH cells were fed and treated with IL-4 (10 ng/ml) 1 h before the addition of EGF, IL-1 β , or TNF- α . Total RNA was isolated at the times indicated and probed with 32 P-labelled PGHS-2- and PGHS-1-specific cDNA probes. Signal intensity was quantitated on a Packard InstantImager and is representative of three separate experiments. The mean inhibition $\pm$ S.E.M. of PGHS-2 mRNA levels at 1 h is given in the text. - IL-4, no pretreatment with IL-4; +IL-4, pretreatment with IL-4.

FIGURE 4. IL-4 does not affect the decay rate of the PGHS-2 mRNA in cells treated with TNF- α . WISH cells were treated as described in Methods. Total RNA was isolated at the times indicated and probed with 32 P-labelled PGHS-2-specific cDNA probes. Signal intensity was quantitated on a Packard InstantImager and is representative of two separate experiments.

FIGURE 5. Expression of PGHS-2 protein is inhibited by IL-4 in TNF- α - and IL-1 β -stimulated WISH cells. Time-course experiments were repeated as previously described. Total protein was isolated and PGHS-2 protein levels were determined by immunoblotting. Signal intensity was quantitated by densitometry and is representative of three separate experiments. std, standard. The mean inhibition $\pm$ S.E.M. of PGHS-2 protein levels at 8 h is given in the text.

assessed the effects of IL-4 on the stability of PGHS-2 mRNA (Fig. 4). In the presence of TNF- α alone, the PGHS-2 mRNA decayed with a half-life of 30 min. The addition of IL-4 1 h before the addition of TNF- α had no effect on the PGHS-2 mRNA decay rate.

Expression of PGHS-2 protein is inhibited by IL-4

The same conditions were used to determine the effects of IL-4 on the production of PGHS-2 and PGHS-1 protein in response to EGF, IL-1 β , and TNF- α (Fig. 5). Immunoreactive PGHS-2 protein was induced rapidly by IL-1 β and TNF- α within

FIGURE 6. IL-4 attenuates PGE₂ production in cytokine- but not EGF-treated WISH cells. WISH cells were pretreated for 1 h with 10 ng/ml IL-4. TNF- α (10 ng/ml), IL-1 β (0.2 ng/ml), and EGF (5 ng/ml) treatment proceeded for 16 h and medium was removed for PGE₂ RIA. Data shown are from seven experiments. - IL-4, no pretreatment with IL-4; +IL-4, pretreatment with IL-4. Significant decreases in PGE₂ production (*) were observed in IL-1 β and TNF- α treated cells ($P < 0.05$).

1 h and in these cytokine-treated cells, the amount of PGHS-2 protein remained increased for at least 16 h. The addition of IL-4 attenuated this induction at 8 h by $65 \pm 3\%$ and $41 \pm 2\%$ in cells treated with IL-1 β and TNF- α , respectively. In contrast, we were unable consistently to detect PGHS-2 protein in EGF-stimulated cells. No changes in PGHS-1 protein levels were observed in response to any of the treatments (data not shown).

IL-4 inhibits PGE₂ production in cytokine but not growth factor-treated cells

EGF, IL-1 β , and TNF- α stimulated PGE₂ production in WISH cells by 18.77 ± 2.68 -fold, 257 ± 43.38 -fold, and 45 ± 2.95 -fold over control, respectively (Fig. 6). Pretreatment with IL-4 resulted in a reduction of the fold production of PGE₂ by 72% in IL-1 β -treated cells (to 71.07 ± 12.92 -fold) and 52% in TNF- α -treated cells (to 21.77 ± 3.35 -fold). However, IL-4 had no significant effect on EGF-stimulated PGE₂ production (21.8 ± 2.6 -fold).

DISCUSSION

In the present study, we have demonstrated that the anti-inflammatory cytokine, IL-4, inhibits the

production of PGHS-2 mRNA in cells treated with IL-1 β , TNF- α , or EGF. Using these stimulatory factors at concentrations that elicit near maximal expression of PGHS-2 mRNA at 1 h, we have shown that they promote similar effects on PGHS-2 mRNA expression but have no effect on the constitutive PGHS-1 isoform. IL-4 exerted its effects on the production of both the PGHS-2 mRNA and the PGHS-2 protein.

IL-4 pretreatment has previously been proposed to enhance the inhibitory effects of IL-4 on PGHS-2 mRNA expression by disruption of early signalling events in the cytokine signal transduction pathway (Mertz *et al.* 1996). In WISH cells, IL-4 was much more effective at inhibiting PGHS-2 expression when added to the cells 1 h before the addition of cytokine or growth factor.

It is most likely that the effects of IL-4 on PGHS-2 mRNA levels were mediated through downregulation of transcription, as the early increases in PGHS-2 mRNA were inhibited by IL-4. IL-4 has been shown to have similar effects on the expression of other genes such as E-selectin. In monocytes, IL-4 promotes signal transducer and activation of transcription factor 6 (STAT6) binding to sequences within the E-selectin promoter. The mechanism of action was shown to be preferential binding of the STAT6 protein to a TTA(N)_{3,4}GCC motif that overlapped an NF- κ B response element in the promoter, which was required for induction of E-selectin transcription by the TNF- α (Bennett *et al.* 1997). The PGHS-2 promoter contains at least two STAT6 sequence motifs at different locations in the PGHS-2 promoter (Tazawa *et al.* 1994).

The effects of IL-4 on PGHS-2 mRNA expression, however, may not have been limited solely to transcription. We determined the effects of IL-4 on the post-transcriptional characteristics of the PGHS-2 mRNA in WISH cells treated with TNF- α . The decay rate of the mRNA was unaffected by the presence of IL-4. However, at the time of addition of DRB (1 h after the addition of TNF- α), PGHS-2 mRNA levels were 70% of those in cells treated with TNF- α alone. Therefore, we conclude that IL-4 most likely regulates the PGHS-2 gene at the transcriptional level in WISH cells.

Of particular interest in this study are the differential effects of IL-4 on the biosynthesis of PGE₂ in cytokine and EGF-stimulated WISH cells. In our studies, IL-4 was a potent inhibitor of WISH cell PGE₂ production in cells that had been stimulated with IL-1 β or TNF- α . However, IL-4 did not inhibit EGF-stimulated PGE₂ biosynthesis. This indicates that the mechanism through which EGF stimulates prostaglandin biosynthesis in

WISH cells may be uncoupled from the pathways that stimulate the expression of both the PGHS-2 mRNA and protein. In support of this, PGHS-2 protein levels remained undetectable by immunoblotting in EGF-treated cells, although the mRNA was induced. It is possible that this PGHS-2-independent prostaglandin biosynthetic mechanism could represent a post-translational effect on the PGHS-1 protein, as no changes in PGHS-1 mRNA or protein expression were evident in this study, although we are unaware of any examples in support of this hypothesis. Alternatively, alterations in arachidonic acid release in response to the combination of IL-4 and EGF may be the cause of the failure to measure changes in prostaglandin production in the face of decreases in PGHS-2 mRNA levels.

The ability of IL-4 to inhibit amnion cell prostaglandin and PGHS-2 mRNA production has interesting implications with regard to intra-uterine prostaglandin production during infection-associated preterm labour. IL-4 is detectable in amniotic fluid and is moderately increased with infection (Dudley *et al.* 1996). The presence of IL-4 during intrauterine infection may have suppressive effects on prostaglandin production; however, its role in the amniotic fluid has not been determined. Although our data suggest that administration of IL-4 might be a possible candidate for therapeutic intervention during infection-associated preterm labour, other clinical factors would have to be considered.

We have presented evidence that IL-4 has potent inhibitory effects on cytokine and EGF-induced PGHS-2 expression in WISH cells. However, there is a divergence in its effects at the level of prostaglandin biosynthesis in EGF-stimulated cells. It will be of great interest to determine both the sequences and factors through which IL-4 exerts its effects on PGHS-2 expression and to uncover the PGHS-2-independent pathway through which EGF induces prostaglandin biosynthesis in WISH cells.

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