Secretory leukocyte protease inhibitor concentration increases in amniotic fluid with the onset of labour in women: characterization of sites of release within the uterus

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Abstract

Secretory leukocyte protease inhibitor is a potent inhibitor of neutrophil function, a mediator of mucosal immunity and an inhibitor of NF κ B regulated inflammatory responses. However, its source, function and regulation within the uterus during pregnancy and at parturition are not well defined. In amniotic fluid, the concentration of secretory leukocyte protease inhibitor increased significantly from 2nd trimester (24 ± 3 ng/ml; mean $\pm$ s.e.m.; $n=20$) to term (751 ± 53 ng/ml; $P<0.05$; $n=15$) with a further profound increase ($P<0.005$) with the onset of labour (3929 ± 1076 ng/ml; $n=15$). To establish the intra-uterine sites of secretion, explants ($n=6$ different patients per tissue) were collected at term after elective caesarean section. High levels of secretory leukocyte protease inhibitor were released by decidua (135.2 ± 12.4 pg/mg;

mean $\pm$ s.e.m.) and chorio-decidua (325.1 ± 26.4 pg/mg) with less by amnion (55.6 ± 6.0 pg/mg) and placenta (9.2 ± 1.9 pg/mg). Intense immunoreactivity for secretory leukocyte protease inhibitor was detected predominantly in decidua parietalis cells adherent to the chorion laeve and myometrium, and also in decidua basalis. We propose that, within the pregnant uterus, secretory leukocyte protease inhibitor is released by decidua, fetal membranes and potentially the fetal lung. The increase in secretory leukocyte protease inhibitor may act to modulate pro-inflammatory paracrine interactions for the maintenance of pregnancy and limit those occurring at parturition within the uterus.

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Introduction

Secretory leukocyte protease inhibitor, also known as anti-leukoprotease inhibitor and human mucus proteinase inhibitor, is a 12 kDa member of the chelonianin class of serine protease inhibitors (Francart *et al.* 1997). The protease inhibitory sequence has been identified in the carboxy domain (Meckelein *et al.* 1991). Although initially described as a potent inhibitor of neutrophil elastase and cathepsin-G (Thompson & Ohlsson 1986), secretory leukocyte protease inhibitor has many other functions, including inhibition of mast cell kinases (Pemberton *et al.* 1998), as an anti-bacterial (Hiemstra *et al.* 1996), anti-viral (Shine *et al.* 1997) and anti-fungal (Tomee *et al.* 1997) agent and anti-coagulant (Masuda *et al.* 1995). In addition, it has recently been shown to inhibit the release of matrix metalloproteinases by monocytes via inhibition of cyclo-oxygenase-2 (Zhang *et al.* 1997) and the lipopolysaccharide-induced activation of the pro-inflammatory transcription factor, NF κ B (Jin *et al.* 1997). Secretory leukocyte protease inhibitor is found in mono-

cytes and polymorphonuclear leukocytes (Ohlsson *et al.* 1996) and is secreted in large amounts from bronchial epithelium (Marchand *et al.* 1995) and mucus-secreting glands (Bergensfeldt *et al.* 1996) where it acts as an important mediator of innate mucosal immunity.

In the uterus, secretory leukocyte protease inhibitor has been found in the endometrium and myometrium of pigs (Reed *et al.* 1998), cows and horses (Badinga *et al.* 1994), with concentrations of the protein and mRNA tending to increase during pregnancy. In women, secretory leukocyte protease inhibitor protein has not been demonstrated within non-pregnant endometrium or fetal membranes but has been immunolocalised to the crypts of endocervical glands in both the non-pregnant and pregnant cervix (Helmig *et al.* 1995). In addition, its concentration increases significantly within amniotic fluid during pregnancy to term (Helmig *et al.* 1995). However, any change in concentration with the onset of labour, its site of secretion from uterine tissues and the potential roles of secretory leukocyte protease inhibitor during parturition have not been examined.

The aims of this study were to measure the levels of secretory leukocyte protease inhibitor in amniotic fluid during pregnancy and labour. In addition, the sites of production within the uterus at term have been characterised to determine the putative roles that secretory leukocyte protease inhibitor might play in the paracrine interactions within the uterus for the maintenance of pregnancy and the control of parturition.

Materials and Methods

Tissue collection

Samples of amniotic fluid from the second trimester ($n=20$ women; 15–18 weeks) were obtained from women undergoing an amniocentesis as part of the karyotype screening programme for Down's Syndrome. All pregnancies were karyotypically normal. At term, samples of amniotic fluid were obtained from women undergoing elective caesarean section ($n=15$ women; >37 weeks) and after spontaneous vaginal delivery ($n=15$ women; >37 weeks, uncomplicated labour, not induced). Fetal urine samples, which were clear and not contaminated with blood, were collected at first micturition immediately after delivery by elective caesarean section from uncomplicated pregnancies ($n=12$ neonates; >37 weeks). Fetal serum samples were collected at term (>37 weeks) from the placental venous cord vein after spontaneous vaginal delivery ($n=5$ neonates) and emergency caesarean section (spontaneous active labour, $n=5$ neonates). All samples were centrifuged at 1000 *g* for 5 min and stored at -20°C prior to immunoassay. Tissues used for explants were obtained from women undergoing an elective caesarean section at term ($n=6$ women; >37 weeks; not associated with labour). For placental perfusion, placentae were collected after spontaneous vaginal delivery at term ($n=6$; >37 weeks, active labour, not induced). For immunohistochemistry, tissues ($n=4$; >37 weeks, elective caesarean; $n=4$; >37 weeks, emergency caesarean section, active labour, not induced) were fixed in 10% neutral buffered formalin for 24 h before washing twice in 70% ethanol prior to mounting in paraffin wax. Ethical approval for the collection of all samples was obtained from the Lothian Trust Ethical Committee with the informed and written consent of patients.

Culture of explants of amnion, chorio-decidua, decidua and placenta

Discs of amnion (12 mm diameter; wet weight 15–20 mg) and chorio-decidua (9 mm diameter; wet weight 15–25 mg) were prepared using a cork borer. Decidua (wet weight 15–25 mg) was dissected off the myometrial aspect of the posterior uterine wall from a site away from the placental bed and pieces of villous placental tissue

dissected from the middle of a central cotyledon (wet weight 15–30 mg). Tissues explants (four explants of each tissue from each woman) were maintained in culture, as described previously by this laboratory (Brennand *et al.*, 1995). All reagents were obtained from Sigma, Poole, UK unless otherwise stated. Explants were placed on absorbent capillary matting (The Fyba Pot Company Ltd, Knottingley, UK) in a 24-well plate (Costar, High Wycombe, UK) and maintained in complete culture medium (RPMI 1640 with 2 mM L-glutamine, Gibco, Paisley, UK supplemented with 10% fetal calf serum, Gibco; 50 IU/ml penicillin, Gibco; 50 µg/ml streptomycin, and insulin/transferrin/selenium, Gibco) for 24 h at 37°C in 95% air and 5% CO_2 under humid conditions in 24 well culture plates (Costar). The harvested media was then frozen at -20°C until analysis.

Preparation and culture of amnion and chorion cells

Amnion and chorion were collected immediately after delivery from patients undergoing elective caesarean section at term (>37 weeks, not in labour), transported to the laboratory in Dulbecco's phosphate buffered saline (DPBS; Gibco) containing heparin (10 U/ml; LeoLabs Ltd, Risborough, UK), then incubated in DPBS containing gentamicin (80 µg/ml) and amphotericin B (5 µg/ml) for 60 min at 23°C . Amnion and chorion cells were prepared for and maintained in culture adapting methods described previously (Jones *et al.* 1989). Excess decidua was scraped from the chorion laeve, and amnion and chorion were minced, washed and incubated in digestion medium (RPMI 1640 containing 5 mg/ml trypsin and 20 µg/ml DNase) with mechanical agitation for 40 min at 37°C . Dispersed cells were collected by passing through a 0.16 mm nylon mesh (Lockertex, Warrington, UK) three times. Cells were washed in complete culture medium and viabilities for all preparations were assessed as $>90\%$ by trypan blue exclusion. Cells were cultured in complete culture medium for 2–4 days. Chorion fibroblasts were obtained by dilution of chorion trophoblast cell preparations with subsequent overgrowth of fibroblasts to confluence within 28 days. Chorion fibroblasts were used up to passage five. Amnion cell preparations were $>95\%$ positive for the epithelial cell marker cytokeratin and $<5\%$ positive to the fibroblast/mesenchyme marker vimentin; chorion trophoblast cells preparations were $>90\%$ cytokeratin positive and $<5\%$ vimentin positive and chorion fibroblasts were $>95\%$ vimentin positive.

Dual perfusion of placental cotyledons

A peripheral cotyledon was selected which was macroscopically intact with parallel chorionic artery and vein, cannulated and mounted in a perfusion chamber as described previously (Schneider *et al.* 1972, Schneider & Huch 1985), with minor modifications (Benediktsson *et al.*

1997). The maternal and fetal compartments were perfused with Krebs's solution at flow rates of 10 and 6 ml/min, and gassed with 95% O₂/5% CO₂ and 95% N₂/5% CO₂, respectively. The perfusate on the fetal side was supplemented with dextran (20 g/l; average molecular weight 74 kDa; Sigma Chemical Co., Poole, UK). Perfusions were started within 10 min of delivery. After a 40 min equilibration period, which permitted the residual blood to be eluted and circulatory perfusion pressures to stabilise, consecutive 10 min samples were collected from both maternal and fetal circuits. Samples were stored at -20 °C before being assayed. The integrity and viability of each preparation were established throughout the experimental period by ensuring that (a) the rate of perfusate input in both the fetal and maternal circuits equalled the rate of output, (b) there was adequate exchange of O₂ from the maternal to fetal circuits (step up pressures of >40 mm Hg in fetal perfusate), (c) lactate concentrations remained within the normal low range (<2 mM; Benediktsson *et al.* 1997), (d) at the end of the experiment a bolus of noradrenaline induced an increase in fetal vascular resistance of >2 mm Hg and (e) subsequent microscopic histological investigations revealed no significant morphological changes.

Secretory leukocyte protease inhibitor assay

Secretory leukocyte protease inhibitor was measured by a competitive enzyme linked immunosorbent assay. Plates (96 well; Nunc Maxi-Sorp, Gibco) were coated with recombinant human secretory leukocyte protease inhibitor (R&D Systems, Oxford, UK; 0.025 µg/ml in PBS/1% carbonate buffer; 400 mM NaHCO₃; 40 mM Na₂CO₃; pH 9.6; 300 µl/well) for 60 min at 23 °C then blocked with milk powder (0.1 mg/ml in H₂O; 400 µl/well) for 30 min at 23 °C. Plates were then washed (150 mM NaCl; 100 mM Tris; 0.05% Tween-20; pH 7-7.5) and ELISA assay buffer (150 mM NaCl; 100 mM Tris; 50 mM Phenol Red solution; 2 mM EDTA; 1 mM 2-ethylisothiazolone, Boehringer Mannheim, Lewes, UK; 1 mM bromonitrodioxane, Boehringer Mannheim; 2 mg/ml BSA; 0.05% Tween-20 to final pH of 7.2; 250 µl/well) added for determination of non-specific binding (NSB). Recombinant standards (R&D Systems; highest concentration 50 ng/ml; 200 µl/well) and samples (200 µl/well) were added, followed by the polyclonal antibody raised in goat against recombinant human secretory leukocyte protease inhibitor (R&D Systems; 2 µg/ml in ELISA buffer; 50 µl/well) and incubated for 120 min at 23 °C. Plates were washed and anti sheep/goat IgG Fab fragments raised in donkey conjugated to peroxidase (Boehringer Mannheim; diluted 1:500 in ELISA buffer; 100 µl/well) added and incubated for 120 min at 23 °C. Finally, plates were washed again and substrate (0.3 g/l urea-hydrogen peroxide; 0.1 g/l tetramethyl benzidine in 100 mM sodium acetate, pH 6.0; 200 µl/well) was added prior to

quenching with 2N H₂SO₄ (50 µl/well). Absorption was read at 450 nm within 30 min of quenching. The intra- and inter-assay precisions were 9.2% relative standard deviations (r.s.d.) and 10.1% r.s.d., respectively and the detection limit of the assay was 9.8 pg/ml. To validate the assay, serial dilutions of amniotic fluids were compared with that of the recombinant standard and assay results were compared with those obtained using a commercial kit which uses the same antibodies (R&D Systems).

Localisation of secretory leukocyte protease inhibitor in tissues by immunohistochemistry

Sections (5 µm) were dewaxed, rehydrated and endogenous peroxidase quenched with H₂O₂ (1% v/v in methanol) for 30 min at 23 °C. Non-specific binding was eliminated by pre-blocking with 10% normal donkey serum (Scottish Antibody Production Unit, Carlisle, UK) for 30 min at 37 °C. The polyclonal secretory leukocyte protease inhibitor primary antibody, previously described (R&D Systems), was then applied (25 µg/ml in 10% normal donkey serum) for 17 h at 4 °C. Sections were washed and specific binding detected using an anti-sheep/goat Fab fragment raised in donkey conjugated to peroxidase (R&D Systems) applied for 90 min at 23 °C. This peroxidase activity was localised using 3,3'-diaminobenzidine (Vector Labs Ltd, Peterborough, UK) as chromagen. Sections were counter-stained with Harris' haematoxylin, dehydrated, mounted and visualised by light microscopy.

Statistical analysis

Statistical analysis of the data was performed using ANOVA (Statview 4.1, Abacus Inc., Berkeley, CA, USA). The data were normally distributed and are expressed as pg/ml, ng/ml or pg/mg/wet weight tissue (mean ± s.e.m.) with a statistically significant difference defined as $P < 0.05$.

Results

Validation of secretory leukocyte protease inhibitor assay

Serial dilutions of amniotic fluids gave dose-response curves parallel to that of the recombinant standard (Fig. 1). The results from this assay and those obtained by a commercial assay (R&D Systems), which uses the same antibodies, were very highly correlated ($r = 0.99$, total degrees of freedom, $n = 5$).

Presence of secretory leukocyte protease inhibitor in amniotic fluid

Secretory leukocyte protease inhibitor was present within amniotic fluid in increasing concentrations from second

Figure 1 Dose-response relationships (B/BO) for recombinant secretory leukocyte protease inhibitor standards and two serially diluted amniotic fluid samples. Serial dilutions of amniotic fluid gave dose responses parallel to that of recombinant standard.

trimester (24 ± 3 ng/ml; mean $\pm$ S.E.M.) to term not in labour (751 ± 53 ng/ml) to term in labour (3929 ± 1076 ng/ml), with this rise being significant from second trimester to term ($P < 0.05$) to term in labour ($P < 0.005$; Fig. 2).

Secretory leukocyte protease inhibitor release from amnion, chorion-decidua, decidua and placenta and its concentration in cord serum and fetal urine

Amnion, chorion-decidua, decidua and placenta explants demonstrated a distinct pattern of secretory leukocyte protease inhibitor release with low levels being secreted by placenta (9.2 ± 1.9 pg/mg), more by amnion (55.6 ± 6.0 pg/mg; $P < 0.05$) and significantly higher levels from decidua (135.2 ± 12.4 pg/mg; $P < 0.01$) and chorion-decidua (325.1 ± 26.4 pg/mg; $P < 0.005$; Fig. 3). Amnion epithelial cells, chorion trophoblast cells and chorion fibroblasts released 613 ± 88 pg/ml/ 10^5 cells, 389 ± 62 pg/ml/ 10^5 cells and 1658 ± 250 pg/ml/ 10^5 cells secretory leukocyte protease inhibitor, respectively.

Secretory leukocyte protease inhibitor was secreted into both the maternal (7080 ± 840 pg/min) and fetal (1790 ± 230 pg/min) circulations in the perfused placental cotyledon system (Fig. 4), with significantly higher ($P < 0.005$)

Figure 2 Increase in concentration of secretory leukocyte protease inhibitor in amniotic fluid during pregnancy. The concentration increased significantly during pregnancy from the second trimester ($n=20$) to term ($n=15$) to term in labour ($n=15$). Values are expressed as ng/ml $\pm$ S.E.M. Significance: * $P < 0.05$, term not in labour to second trimester; ** $P < 0.005$, term not in labour to term in labour.

amounts being released into the maternal than fetal circulation. However, it was undetectable in all cord serum samples ($n=10$) and below the detection limit of the assay in nine out of the twelve fetal urine samples assayed, with levels being 358 pg/ml, 320 pg/ml and 188 pg/ml, respectively in the remaining three samples. There was no evidence of infection or neonatal sepsis in the three neonates in which secretory leukocyte protease inhibitor was detectable.

Immunolocalisation of secretory leukocyte protease inhibitor within fetal membranes, decidua and placenta

Intense specific immunoreactivity for secretory leukocyte protease inhibitor was localised to the layer of decidua parietalis adherent to chorion trophoblast (Fig. 5A). Similarly, intense immunostaining was present in decidua parietalis collected from the myometrial aspect (Fig. 5B). Only a few isolated cells, which may be fibroblasts, stained immunopositive within the amnion and chorion trophoblast, with the majority of the cells constituting these layers

Figure 3 Release of secretory leukocyte protease inhibitor by explants of third trimester amnion, chorio-decidia, decidua and placenta maintained in culture for 24 h. Significantly more secretory leukocyte protease inhibitor was released by chorio-decidia and decidua than by placenta and amnion. Values are expressed as pg/mg/wet weight tissue $\pm$ S.E.M.; tissues collected from $n=6$ different women. Significance: * $P<0.05$, amnion compared with placenta; ** $P<0.01$, decidua compared with amnion and placenta; *** $P<0.005$, chorio-decidia compared with decidua, amnion and placenta.

being immunonegative (Fig. 5A). In placenta, the decidua basalis which underlies the placenta also demonstrated intense specific immunoreactivity (Fig. 5C). The extra-villous trophoblast was immunonegative, as was the majority of the placenta apart from a small amount of staining around the margins and within fetal blood vessels of the villi. Substitution of the primary antibody with normal donkey (Fig. 5D) and normal goat serum (data not shown) resulted in absence of staining in decidua, fetal membranes and placenta.

Discussion

We demonstrate that secretory leukocyte protease inhibitor is present in increasing concentrations within amniotic fluid during pregnancy and further increases with the onset of labour. Secretory leukocyte protease inhibitor was secreted predominantly by explants of decidua and

Figure 4 Release of secretory leukocyte protease inhibitor from the dually perfused placental cotyledon. Significantly more secretory leukocyte protease inhibitor was released into the maternal than the fetal circulation. Values are expressed as pg/min $\pm$ S.E.M.; $n=6$ placentae. Significance: * $P<0.005$, maternal compared with fetal effluent.

chorio-decidia, with lower levels released by amnion and placenta. Immunohistochemistry demonstrated cell-specific localisation of secretory leukocyte protease inhibitor to both decidua parietalis and basalis.

The profound increase in the concentration of secretory leukocyte protease inhibitor within amniotic fluid after the onset of labour is a novel finding. The presence of secretory leukocyte protease inhibitor within amniotic fluid during the second trimester and at term has been previously demonstrated (Helmig *et al.* 1995) with the reported values being comparable to our data. Term amniotic fluid comprises mainly of secretions from the surrounding fetal membranes, decidua, fetal lung and from fetal urine (Gilbert & Brace 1993). Release of secretory leukocyte protease inhibitor from amnion explants *in vitro* was comparatively low. However, the potential release of secretory leukocyte protease inhibitor by amnion *in vivo* may be considerable, due to the large surface area of the amniotic membrane. In addition, it has been previously demonstrated that secretory leukocyte protease inhibitor is present in high concentrations within tracheal aspirates from neonates (Ohlsson *et al.* 1992) with levels correlating positively with gestational age (Sluis *et al.* 1994). Although we have demonstrated that fetal urine contains secretory leukocyte protease inhibitor, it was only detectable in one quarter of all samples tested and therefore unlikely to account for the consistently high levels of secretory

Figure 5 Localisation of secretory leukocyte protease inhibitor by immunohistochemistry in: A, Fetal membranes and adherent decidua collected at emergency caesarean section in active labour. Intense immunostaining for secretory leukocyte protease inhibitor is localised predominantly in decidual cells and in some cells of fibroblast morphology in chorion and amnion. Key: a=amnion; c=chorion laeve; d=decidua parietalis adherent to the chorion; t=trophoblast cell layer of chorion. B, Decidua parietalis collected from the myometrial aspect after emergency caesarean section in active labour with specific positive immunoreactivity in decidual stromal cells. C, Placenta collected at emergency caesarean section in active labour with little or no immunostaining in syncytiotrophoblast cells or extravillous trophoblast in the placental bed. Some positive immunostaining is present in cells of the villous core in the stroma and associated with the vasculature. There is intense immunostaining in cells of the decidua basalis. Key: s=syncytiotrophoblast cells; ev=extravillous trophoblast; db=decidua basalis. D, Parallel negative control section of fetal membranes and adherent decidua with no non-specific staining. Scale bars: A and D, 200 µm; B and C, 100 µm.

leukocyte protease inhibitor demonstrated in all labouring amniotic fluid samples. It is likely therefore that release and transfer of secretory leukocyte protease inhibitor from decidua, as has been suggested for prolactin (Rosenberg *et al.* 1980), amnion epithelial and fetal lung secretions may all contribute to secretory leukocyte protease inhibitor in amniotic fluid.

The predominant source of secretory leukocyte protease inhibitor secretion in the uterus is likely to be the decidua, with explants of decidua parietalis and chorio-decidua secreting high levels. In decidua parietalis, intense immunoreactivity for secretory leukocyte protease inhibitor was localised in the maternal aspect of the decidua and in that adjacent to chorion. This adherent decidua is likely to be the principal site of production in chorio-decidual explants. Decidua collected from the myometrial aspect by curettage is usually contaminated

with some myometrium, which is immunonegative for the protein. This may explain why decidua explants secrete slightly less secretory leukocyte protease inhibitor compared with chorio-decidua, unless there is a gradient of secretion with highest outputs from decidua adjacent to the chorion. Term decidua is heterogeneous, consisting of stromal cells (53%), macrophages (19%), granulocytes (16%) and T-cells (8%) (Adeleye *et al.* 1996). Hence the stromal cells, macrophages and granulocytes, which are capable of secretory leukocyte protease inhibitor secretion in other sites (Ohlsson *et al.* 1996), are potential sources. In the fetal membranes, amnion explants, which consist of an epithelium and sub-epithelial layers containing fibroblasts, secrete secretory leukocyte protease inhibitor. The potential for paracrine interactions between the different cell types present in the term amnion, chorion, composed of reticular fibroblast and the trophoblast cell layers and

decidua, to regulate the release of secretory leukocyte protease inhibitor at the materno–fetal interface requires further investigation.

Placental explants of villous core tissue released low levels of secretory leukocyte protease inhibitor, which is in agreement with only slight immunostaining within the fetal vasculature. This immunostaining could represent either secretory leukocyte protease inhibitor bound to elastin (Rudolphus *et al.* 1994) within the blood vessel wall or specific staining of residual fetal blood cells in the villi. Within the isolated perfused placental cotyledon, secretory leukocyte protease inhibitor was released mainly into the maternal and not the fetal circuit and was undetectable in cord serum. Decidua basalis adherent to the perfused placental cotyledon stains intensely for secretory leukocyte protease inhibitor reactivity and may be the principal source of the secretory leukocyte protease inhibitor secretion into the maternal circulation effluent.

Parturition has been likened to an inflammatory reaction (Liggins *et al.* 1977). Secretory leukocyte protease inhibitor may be involved in the regulation of this response at the materno–fetal interface. At the onset of labour, there is increased expression and release of pro-inflammatory cytokines such as IL-1 and IL-6 within amniotic fluid (Cox *et al.* 1997) and decidua (Dudley *et al.* 1992), which stimulate pro-inflammatory cascades involved in the initiation and maintenance of parturition. However, IL-1 and IL-6 can also stimulate release of secretory leukocyte protease inhibitor (Sallenave *et al.* 1994, Jin *et al.* 1998). The significant rise in IL-1 and IL-6 within amniotic fluid and decidua at parturition could therefore enhance release of secretory leukocyte protease inhibitor (Sallenave *et al.* 1994, Jin *et al.* 1998) from amnion epithelium, fetal respiratory tract mucosa and decidua. Secretory leukocyte protease inhibitor could then exert a negative feedback on release of inflammatory cytokines via inhibition of NFκB (Jin *et al.* 1997) and neutrophil function by inhibition of neutrophil elastase. Secretory leukocyte protease inhibitor could also inhibit release of PGE₂ and matrix metalloproteinases, which are also involved in membrane remodelling and rupture, by inhibiting cyclo-oxygenase-2 (Zhang *et al.* 1997). During parturition, the uterine cavity is at high risk of invasion and infection by microbial organisms. Secretory leukocyte protease inhibitor, by virtue of its anti-bacterial, anti-fungal and anti-viral properties, could therefore in addition act as an endogenous block to infection within the decidua and the amniotic cavity.

In summary, we demonstrate that secretory leukocyte protease inhibitor is present in increasing concentrations within amniotic fluid during pregnancy and labour. Secretory leukocyte protease inhibitor is also released by and immunolocalised to term decidua. This tissue, together with fetal membranes and possibly the fetal lung, are the likely sources of secretory leukocyte protease inhibitor within the uterus. We suggest that secretory

leukocyte protease inhibitor might act both to limit the pro-inflammatory cascades ongoing during parturition and to protect against microbial invasion and the response to infection. Further studies are required to investigate the regulation of secretory leukocyte protease inhibitor release by intra-uterine tissues and to examine its potential roles in the maintenance of pregnancy and the initiation and progression of parturition, both preterm and at term.

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References

- Adeleye TA, Elder MG & Sullivan MHF 1996 Preparation of a population of macrophages from human 3rd trimester decidua. *Human Reproduction* **11** 451–456.
- Badinga L, Michel FJ, Fields MJ, Sharp DC & Simmen RCM 1994 Pregnancy-associated endometrial expression of antileukoprotease gene is correlated with epitheliochorial placentation. *Molecular Reproduction and Development* **38** 357–363.
- Benediktsson R, Calder AA, Edwards CRW & Seckl JR 1997 Placental 11β-hydroxysteroid dehydrogenase: a key regulator of fetal glucocorticoid exposure. *Clinical Endocrinology* **46** 161–166.
- Bergenfelt M, Nystrom M, Bohe M, Lindstrom C, Polling A & Ohlsson K 1996 Localization of immunoreactive secretory leukocyte protease inhibitor (SLPI) in intestinal-mucosa. *Journal of Gastroenterology* **31** 18–23.
- Brennan JE, Leask R, Kelly RW, Greer IA & Calder AA 1995 Changes in prostaglandin synthesis and metabolism associated with labour, and the influence of dexamethasone, RU486 and progesterone. *European Journal of Endocrinology* **133** 527–533.
- Cox SM, Casey ML & MacDonald PC 1997 Accumulation of interleukin-1 beta and interleukin-6 in amniotic fluid: a sequela of labour at term and preterm. *Human Reproduction Update* **3** 517–527.
- Dudley DJ, Trautman MS, Araneo BA, Edwin SS & Mitchell MD 1992 Decidual cell biosynthesis of interleukin-6: regulation by inflammatory cytokines. *Journal of Clinical Endocrinology and Metabolism* **74** 884–889.
- Francart C, Dauchez M, Alix AJP & Lippens G 1997 Solution structure of r-elafin, a specific inhibitor of elastase. *Journal of Molecular Biology* **268** 666–677.
- Gilbert WM & Brace RA 1993 Amniotic-fluid volume and normal flows to and from the amniotic cavity. *Seminars in Perinatology* **17** 150–157.
- Helmig R, Uldbjerg N & Ohlsson K 1995 Secretory leukocyte protease inhibitor in the cervical-mucus and in the fetal membranes. *European Journal of Obstetrics Gynecology and Reproductive Biology* **59** 95–101.
- Hiemstra PS, Maassen RJ, Stolk J, Heinzelwieland R, Steffens GJ & Dijkman JH 1996 Antibacterial activity of antileukoprotease. *Infection and Immunity* **64** 4520–4524.
- Jin FY, Nathan C, Radzioch D & Ding A 1997 Secretory leukocyte protease inhibitor: a macrophage product induced by and antagonistic to bacterial lipopolysaccharide. *Cell* **88** 417–426.
- Jin FY, Nathan CF, Radzioch D & Ding AH 1998 Lipopolysaccharide-related stimuli induce expression of the secretory leukocyte protease inhibitor, a macrophage-derived lipopolysaccharide inhibitor. *Infection and Immunity* **66** 2447–2452.

- Jones SA, Brooks AN & Challis JRG 1989 Steroid modification of corticotropin releasing hormone production in human fetal membranes and placenta. *Journal of Clinical Endocrinology and Metabolism* **68** 825–830.
- Liggins GC, Forster CS, Grieves SA & Schwartz, AL 1977 Control of parturition in man. *Biology of Reproduction* **16** 39–56.
- Marchand V, Tournier JM, Chevillard M, Polette M, Beorchia A & Klossek JM 1995 Identification of antileucoprotease in remodelled human adult nasal surface epithelium. *European Respiratory Journal* **8** 15–21.
- Masuda K, Kamimura T, Watanabe K, Suga T, Kanesaki M, Takeuchi A, Imaizumi A & Suzuki Y 1995 Pharmacological activity of the C-terminal and N-terminal domains of secretory leukoprotease inhibitor *in vivo*. *British Journal of Pharmacology* **115** 883–888.
- Meckelein B, Kemme M, Nikiforov T, Appelhans H & Gassen HG 1991 Expression and characterization of recombinant 2nd domain variants of human mucus proteinase-inhibitor (MPI). *Biomedica et Biochimica Acta* **50** 673–676.
- Ohlsson K, Sveger T & Svenningsen N 1992 Protease inhibitors in bronchoalveolar lavage fluid from neonates with special reference to secretory leukocyte protease inhibitor. *Acta Paediatrica* **81** 757–759.
- Ohlsson K, Linder C, Lundberg E & Axelsson L 1996 Release of cytokines and proteases from human peripheral-blood mononuclear and polymorphonuclear cells following phagocytosis and LPS stimulation. *Scandinavian Journal of Clinical and Laboratory Investigation* **56** 461–470.
- Pemberton AD, Huntley JF & Miller HRP 1998 Differential inhibition of mast cell chymases by secretory leukocyte protease inhibitor. *Biochimica et Biophysica Acta—General Subjects* **1379** 29–34.
- Reed KL, Blaaser LL, Dantzer V, Green ML & Simmen RCM 1998 Control of secretory leukocyte protease inhibitor gene expression in the porcine peri-implantation endometrium: a case of maternal-embryo communication. *Biology of Reproduction* **58** 448–457.
- Rosenberg SM, Maslar IA & Riddick DH 1980 Decidual production of prolactin in late gestation: further evidence for a decidual source of amniotic fluid. *American Journal of Obstetrics and Gynaecology* **138** 681–685.
- Rudolphus A, Kramps JA, Mauve I & Dijkman JH 1994 Intratracheally-instilled antileukoprotease and α_1 -proteinase inhibitor – effect on human neutrophil elastase-induced experimental emphysema and pulmonary localization. *Histochemical Journal* **26** 817–824.
- Sallenave JM, Shulmann J, Crossley J, Jordana M & Gauldie J 1994 Regulation of secretory leukocyte proteinase-inhibitor (SLPI) and elastase-specific inhibitor (ESI/Elafin) in human airway epithelial-cells by cytokines and neutrophilic enzymes. *American Journal of Respiratory Cell and Molecular Biology* **11** 733–741.
- Schneider H & Huch A 1985 Dual *in vitro* perfusion of an isolated lobe of human placenta: method and instrumentation. *Controversies in Gynaecology and Obstetrics* **13** 40–47.
- Schneider H, Panigel M & Dancis J 1972 Transfer across the perfused human placenta of antipyrine, sodium and leucine. *American Journal of Obstetrics and Gynecology* **114** 822–828.
- Shine N, Konopka K & Duzgunes N 1997 The anti-HIV-1 activity associated with saliva. *Journal of Dental Research* **76** 634–640.
- Sluis KB, Darlow BA, Vissers MCM & Winterbourn CC 1994 Proteinase–antiproteinase balance in tracheal aspirates from neonates. *European Respiratory Journal* **7** 251–259.
- Thompson RC & Ohlsson K 1986 Isolation, properties and complete amino-acid sequence of human secretory leukocyte protease inhibitor, a potent inhibitor of leukocyte elastase. *Proceedings of the National Academy of Sciences of the USA* **83** 6692–6696.
- Tomee JFC, Hiemstra PS, HeinzlWieland R & Kauffman HF 1997 Antileukoprotease: an endogenous protein in the innate mucosal defence against fungi. *Journal of Infectious Diseases* **176** 740–747.
- Zhang YH, DeWitt DL, McNeely TB, Wahl SM & Wahl LM 1997 Secretory leukocyte protease inhibitor suppresses the production of monocyte prostaglandin H synthase-2, prostaglandin E(2), and matrix metalloproteinases. *Journal of Clinical Investigation* **99** 894–900.

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