Anti-inflammatory steroid signalling in the human peritoneum

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Abstract

Peritoneal surface epithelial (PSE) cells participate in adhesion formation following inflammatory injury yet adjacent ovarian SE (OSE) cells regenerate without scarification after ovulation. OSE cells show inflammation-associated expression of 11β hydroxysteroid dehydrogenase type 1 (11βHSD1) enzyme, enabling intracrine generation of anti-inflammatory cortisol to minimise tissue damage. We asked if human PSE cells show an 11βHSD1 response to pro-/anti-inflammatory stimulation and if so, how the 11-oxoreductase activity generated compares with OSE. PSE collected from premenopausal women undergoing surgery for benign gynaecological conditions were used to establish primary PSE cell cultures that were treated for 48 h with interleukin-1α (IL-1α) with/without anti-inflammatory steroid (cortisol or progesterone). mRNA levels corresponding to the genes of interest (11βHSD1, 11βHSD2, cyclooxygenase-2, COX-2) were measured by quantitative RT-PCR. IL-1α (0·5 ng/ml) stimulated 11βHSD1 and COX-2 mRNA levels in PSE cells but 11βHSD2 was unaffected. Cortisol (1 μM), not progesterone (1 μM), increased 11βHSD1 mRNA and synergistically enhanced IL-1α action. Cortisol suppressed IL-1α-stimulated COX-2 more effectively than progesterone. PSE cells had a significantly lower basal 11-oxoreductase enzyme activity than OSE cells; IL-1α did not significantly increase the 11-oxoreductase activity in PSE cells but did so in OSE cells. We conclude that PSE cells respond to IL-1α and anti-inflammatory steroids in qualitatively similar ways as OSE. However, the enzymatic activity of 11βHSD1 is lower in PSE and less responsive to IL-1α. This could help explain why peritoneal healing often leads to adhesion formation, whereas postovulatory ovarian healing is scar-free.

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Introduction

An emerging theme in inflammation research is that inflammatory stimuli regulate genes that localise and limit potentially damaging cellular responses. When this machinery fails, pathological sequelae ensue. One such 'anti-inflammatory' gene is *HSD11B1*, encoding 11βhydroxysteroid dehydrogenase type 1 (11βHSD1), a steroidogenic enzyme that metabolises cortisone to cortisol through its 11-oxoreductase enzymatic activity. The activity of 11βHSD1 is offset by 11βHSD2, the encoded product of *HSD11B2* that back converts cortisol to cortisone. Since cortisol – but not cortisone – binds glucocorticoid receptor (GR) and activates downstream anti-inflammatory signalling, relative levels of 11βHSD1 and 11βHSD2 potentially set local inflammatory tone (Draper & Stewart 2005). Differentially expressed 11βHSD1 and 11βHSD2 in a naturally inflamed tissue was first observed in human ovary approaching ovulation. Ovulation bears vascular, haemodynamic and biochemical hallmarks of inflammation, and granulosa cells from periovulatory follicles selectively express 11βHSD1 mRNA over 11βHSD2 mRNA (Tetsuka *et al.* 1997). From work on renal mesangeal cells it emerged that inflammatory cytokines such as interleukin-1α (IL-1α) and tumour necrosis factor α directly stimulate 11βHSD1 and suppress 11βHSD2 activities *in vitro* (Escher *et al.* 1997). This is now known to hold for several other cell types that naturally respond to inflammatory signals, including ovarian granulosa (Tetsuka *et al.* 1999) and surface epithelial cells (Yong *et al.* 2002), bronchial epithelial cells (Feinstein & Schleimer 1999), preadipocytes (Tomlinson *et al.* 2001), osteoblasts (Cooper *et al.* 2001), aortic smooth muscle cells (Cai *et al.* 2001), peritoneal macrophages (Gilmour *et al.* 2006), trophoblast (Li *et al.* 2006) and fetal membranes (Sun & Myatt 2003).

At the ovarian level, cortisol generated through intracrine 11βHSD signalling potentially contributes to the natural injury-repair process associated with ovulation. In particular, the ovarian surface epithelium (OSE), which is contiguous with the peritoneal SE (PSE), undergoes serial inflammation-associated injury and repair with each follicular rupture (Rae & Hillier 2005). We have previously shown that OSE cells express 11βHSD1 mRNA and 11-oxoreductase enzyme activity catalysed by the encoded 11βHSD1 protein (Yong *et al.* 2002). We have also shown that IL-1α-induced experimental 'inflammation' of OSE cells measured as an increased expression of cyclooxygenase-2 (COX-2) is suppressed by cortisol and to a lesser extent progesterone (Rae *et al.* 2004). Both cortisol and

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progesterone are anti-inflammatory steroids that act via nuclear GR and progesterone (PR) receptors in target cells to repress pro-inflammatory transcription factors such as nuclear factor κ B and activating protein-1 (van der Burg & van der Saag 1996, Rhen & Cidlowski 2005). Since micromolar concentrations of cortisol and progesterone accumulate in follicular fluid at ovulation (Andersen 1991, Andersen & Hornnes 1994), either or both might participate in postovulatory ovarian healing and act on adjacent PSE.

The mesothelial cells lining the peritoneal surface are also potential glucocorticoid and progesterone targets and are inevitably exposed to high concentrations of follicular steroids following follicular rupture. Interestingly, when postovulatory OSE repairs it does so rapidly without scarification or involving i.p. adhesions. On the other hand, inflammatory injury to the PSE frequently leads to adhesion formation with adverse clinical sequelae including pain, bowel obstruction and, in the female, infertility. Thus, despite their common embryological origin, the anti-inflammatory machineries of PSE and OSE cells likely differ. Here, we describe a simple method to collect and culture human PSE cells, based on the one we have previously used to study human OSE cells (Hillier *et al*. 1998, Auersperg *et al*. 2001). Using this culture system, we define interactions between an inflammatory cytokine (IL-1α) and two anti-inflammatory steroids (cortisol and progesterone) on 11βHSD1, 11βHSD2 and COX-2 mRNA expression in PSE cells *in vitro*. We also compare basal and cytokine-responsive 11-oxoreductase activities of PSE and OSE cells. Our results define properties of PSE cells that differ from OSE, which could bear on the particular way in which the peritoneum responds to inflammation and becomes involved in gynaecological disease states *in vivo*.

Materials and Methods

Patients

Samples of PSE were obtained at the time of surgery from premenopausal women undergoing surgery for benign gynaecological conditions. OSE cells for comparative purposes were also obtained from a subset of patients. All participants gave informed consent and the Local Research Ethics Committee approved the study. Relevant clinical details of the patients who donated cells are shown in Table 1. Patients with endometriosis or overt signs of peritoneal pathology were excluded from study.

Collection and culture of PSE cells

The method for PSE collection and culture was similar to that previously described for OSE (Hillier *et al*. 1998). The culture medium was Medium 199:MCDB 105 (1:1, v/v) supplemented with fetal calf serum (15% v/v), penicillin (50 IU/ml), streptomycin (50 μg/ml) and L-glutamine (2 mmol/l), all from Sigma–Aldrich Company Ltd. Briefly, the peritoneum was gently brushed using a sterile Aylesbury spatula or cytobrush (Cook Ireland Ltd, Limerick, Ireland) and then rinsed into prewarmed culture medium. Two sites were chosen for sampling PSE cells: the anterior abdominal wall, which has no contact with the OSE or pelvic organs, and the uterine fundus. The purpose of using these two sites was to determine whether the Müllerian source of PSE behaves in a manner similar to cells sourced from the non-Müllerian abdominal wall. The medium used to collect cells was transferred to 75 cm^2 plastic tissue culture flasks (Corning BV Life Sciences, Schiphol-Rijk,

Table 1 Relevant clinical details of patients donating peritoneal surface epithelial (PSE) cells from which cultures were established and measurements obtained

Age (year)	Parity	Contraception	Day of cycle	Surgery	Indication for surgery	Measurement
38	1+2	DMPA	NA	Lap Ster	Unwanted fertility	mRNA
40	3+0	Fem Ster	2	TAH	HMB	mRNA
42	0+1	None	?	TAH	HMB, dysmen	mRNA
50	2+0	None	11	TAH&BSO	HMB, fibroids	mRNA
33	0+0	None	19	Diag Lap	Pelvic pain, dyspareunia	mRNA
25	0+1	None	?	Diag Lap	Pelvic pain	mRNA
31	0+0	COCP	NA	Diag Lap	Pelvic pain, dyspareunia	mRNA
39	1+0	None	5	TAH	HMB, dysmen	mRNA
34	2+0	Fem Ster	27	LAVH	HMB, dysmen	mRNA
47	2+0	LNG-IUS	NA	TAH&BSO	Irreg bleeding, fibroids	mRNA
48	3+0	Fem Ster	2	TAH	HMB, fibroids	mRNA
43	2+0	Barrier	?	Lap Ster	Unwanted fertility	11-oxo-reductase
29	3+0	None	13	Diag Lap	Dyspareunia	11-oxo-reductase
22	1+0	DMPA	NA	Lap ovarian cystectomy	Benign ovarian cyst	11-oxo-reductase
28	0+0	COCP	NA	Diag Lap	Pelvic pain, dyspareunia	11-oxo-reductase

Parity is defined in $x+y$ format indicating number of pregnancies over 24 weeks (x)+number of pregnancies failing to reach 24 weeks (y).
NA, not applicable; ?, uncertain; DMPA, depo-medroxyprogesterone acetate; Fem Ster, female sterilisation; COCP, combined oral contraceptive pill; LNG-IUS, levonorgestrel intra-uterine system; Lap Ster, laparoscopic sterilisation; TAH, total abdominal hysterectomy; BSO, bilateral salpingo-oophorectomy; Diag Lap, diagnostic laparoscopy; HMB, heavy menstrual bleeding; dysmen, dysmenorrhoea.

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The Netherlands) precoated with donor calf serum. Each flask was examined by phase-contrast microscopy to verify that sufficient cellular material had been obtained. Cultures were then established by incubating the flasks at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air for up to 42 days. The flasks were inspected regularly, with medium renewal every 7 days.

Experimental treatment of PSE cultures

Confluent cell monolayers were washed twice with PBS prior to incubation in 1×Trypsin/EDTA solution (Invitrogen) for 5 min at 37 °C. Dissociated cells were then aspirated and sedimented by centrifugation for 5 min at 800 ***g***. The resulting cell pellet was resuspended in 2 ml culture medium. Cell number and viability were estimated using a haemocytometer and vital staining with Trypan Blue (Sigma–Aldrich). To provide cell monolayers for immunohistochemical assessment (see below), PSE cells were seeded into eight-well chamber slides (VWR, Batavia, IL, USA) at a density of 5–10×10^4 cells/0·5 ml medium and incubated at 37 °C overnight. For experiments involving hormone treatment and subsequent analysis of effect, cells were distributed into six-well plates (Corning) at a density of 4–5×10^5 cells/0·5 ml medium (for mRNA analysis) or 12-well plates at a density of 2×10^5 cells/0·5 ml medium (for 11-oxoreductase analysis). The medium was then aspirated and replaced with serum-free culture medium, containing 0·01% BSA (Sigma–Aldrich). IL-1α (R&D Systems Europe Ltd, Abingdon, Oxon, UK) was diluted in serum-free medium to a final concentration of 0·5 ng/ml, this concentration having been previously shown to be maximally effective in inducing 11βHSD1 mRNA expression and activity in OSE cells cultured in the same way (Yong *et al*. 2002). Cortisol and progesterone (stored as 100 μM stock solutions in ethanol) were added to culture medium at a final concentration of 1 μM. Control culture medium received a similar dilution (1% v/v) of ethanol alone. The incubation was done for 48 h at 37 °C, whereupon media were aspirated and the cell monolayers processed as described below.

Immunohistochemistry

Following methanol fixation and triplicate 3-min washes in PBS, cell monolayers were sequentially blocked with avidin (Vector, Peterborough, UK), biotin (Vector) and non-immune serum (horse, Vector). Primary monoclonal antibodies for human GR (Santa Cruz Biotechnology Inc., Heidelberg, Germany), cytokeratin or PR (Dako Corp., Glostrup, Denmark), diluted 1/100, 1/1000 and 1/50 respectively were applied for 1 h at 37 °C. After three washes with PBS+0·01% Tween 20 (Sigma–Aldrich; 3 min each), secondary antibody (horse-antimouse) diluted in non-immune horse serum was applied for 1 h at room temperature. Sequential washes with PBS+0·01% Tween 20 (3×3 min) were then performed before incubating with Vector-ABC Elite reagents and visualisation via chromagen (diaminobenzidine) staining. Negative controls consisted of non-immune mouse IgG2 substituted for primary antiserum.

RNA extraction and quality analysis

Total RNA was extracted using RNEasy Minispin columns (Qiagen) as per the manufacturer's instructions. RNA was quantified and quality assessed by microfluidic analysis (Agilent 2100 bioanalyser, Agilent technologies, Cheshire, UK).

Quantitative RT-PCR analysis

DNAseI-treated total RNA (200 ng) was reverse-transcribed to cDNA (random hexamer kit, Applied Biosystems, Warrington, Cheshire, UK) and 2 μl resultant cDNA was analysed per reaction. The reaction mixture (25 μl) consisted of 200 nmol/l probe and 300 nmol/l primers and was analysed using the ABI Prism 7900 real-time PCR system (Applied Biosystems). Primer and probe sequences have been reported previously (Rae *et al*. 2004). Target mRNA was quantified in relation to the abundance of 18S rRNA in each sample. Controls included human liver, RT negative (RNA template but lacking reverse transcriptase), RT no template control (water instead of RNA template) and a Taqman step negative control (water instead of cDNA).

11-Oxoreductase assay

11-Oxoreductase activity was determined by measuring the conversion of [1,2,6,7-^{3}H]cortisone to [1,2,6,7-^{3}H]cortisol, as described previously (5). Briefly, each culture well received 0·5 ml serum-free culture medium containing 0·1 μCi [1,2,6,7-^{3}H]cortisone and 50 pmol carrier cortisone (total substrate concentration 0·1 μM). Incubation was for 8 h at 37 °C. The medium was then aspirated and vortexed with 5 ml dichloromethane to extract steroids. The organic phase was then collected and evaporated to dryness under a stream of nitrogen. Dry steroid extracts were reconstituted in fresh dichloromethane and spotted onto silica gel precoated aluminium sheets (Sigma–Aldrich) for the separation of cortisone and cortisol by thin layer chromatography, using chloroform:ethanol (92:8 v/v) as the solvent system. Radiolabelled steroids on the chromatogram were located and quantified using a Bioscan 200 imaging detector (Lablogic Systems, Sheffield, UK).

Statistical analysis

Quantitative RT-PCR measurements were normalised to the control (no treatment) value for each mRNA measured. Data from multiple patients (see figure legends) were grouped and expressed as mean±S.E.M. Statistical differences due to treatment were determined by repeated measures ANOVA of raw data reflecting the mean C_T difference between 18S rRNA and target mRNA. The mean 11-oxoreductase enzymatic activity expressed as pmol/culture per h was compared between treated and untreated samples of the same cell type using paired Student's *t*-tests, and between OSE and

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PSE samples using unpaired Student's *t*-tests. Treatment effects were considered significant at $P<0{\cdot}05$.

Results

PSE cell morphology

The morphology of PSE cells in culture and their responses to experimental treatment *in vitro* were unaffected by the site within the abdominal cavity from which they had been obtained (data not shown). Sheets of PSE cells collected into culture medium usually attached to the serum-coated flask surface within 48 h. Further incubation allowed multiple epithelial cell colonies to become established, eventually producing confluent monolayers. The 'cobblestone' morphology (Fig. 1a) and the mode of cytokeratin expression (Fig. 1b) shown by cultured PSE cells was similar to that previously described for OSE cells cultured in the same way (Hillier *et al*. 1998). Flasks that became contaminated by cells showing a fibroblastic phenotype were excluded from experimentation. Overall, out of the 29 patient PSE specimens collected, 21 (72·4%) produced cultures suitable for further study.

PSE cell steroid receptor status

PSE cell monolayers contained GR and PR mRNA levels similar to those present in OSE cells cultured under identical conditions (data not shown). Immunostaining with antisera to GR (Fig. 1c) or PR (Fig. 1d) revealed the presence of both the steroid receptor proteins in PSE cells, with a predominantly nuclear location.

PSE cell responses to IL-1α and cortisol

To determine influences of IL-1α and cortisol on inflammation-associated gene expression in PSE cells, we assessed 11βHSD1, 11βHSD2 and COX-2 mRNA levels, reasoning that the 11βHSD isoform expression profile defines the potential for intracrine generation of anti-inflammatory cortisol, while COX-2 provides a referent inflammatory readout.

The treatment of PSE cell cultures from five patients with IL-1α significantly increased the expression of 11βHSD1 mRNA relative to non-treated control values (mean fold induction, 9·75, $P<0{\cdot}01$; Fig. 2a). PSE cells sampled from the anterior abdominal wall and the uterine serosal surface responded similarly to IL-1α (average fold induction, 10·4 and 9·0 respectively). Cortisol alone also up-regulated 11βHSD1 mRNA expression 12·6-fold ($P<0{\cdot}001$). In combination, cortisol and IL-1α synergised to increase 11βHSD1 expression 67·9-fold relative to the control ($P<0{\cdot}001$).

Neither IL-1α nor cortisol, alone or in combination, significantly affected 11βHSD2 mRNA expression (Fig. 2b).

The exposure to IL-1α stimulated COX-2 mRNA expression on average was 10·6-fold ($P<0{\cdot}001$; Fig. 2c).

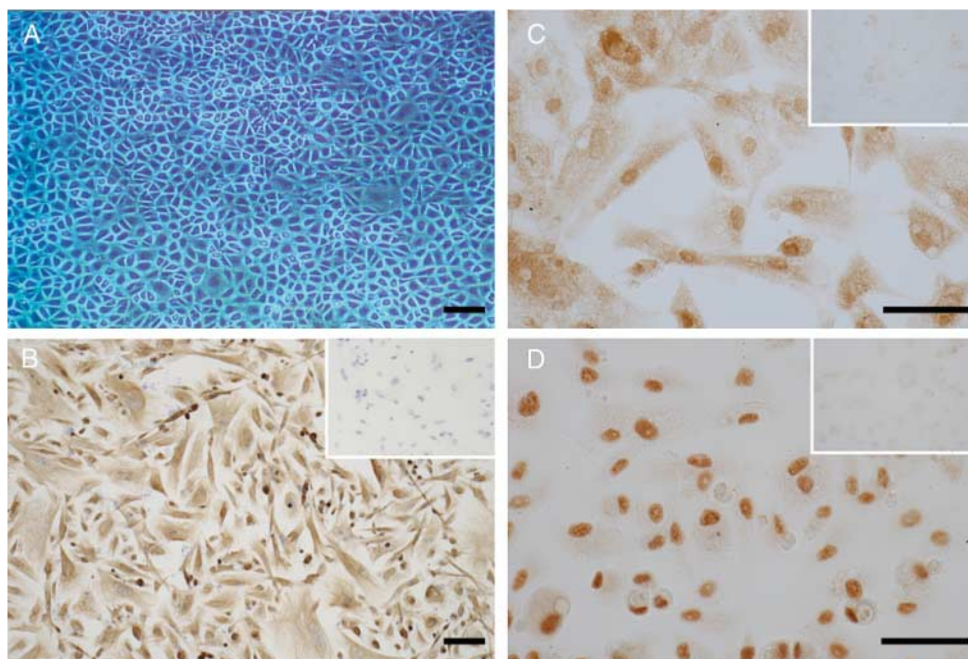

Figure 1 Morphology and immunohistochemical properties of cultured human PSE cells. (A) Phase-contrast microscopy of PSE cell monolayer on day 12 of culture following collection. (B) Cytokeratin immunohistochemistry using antiserum against a panel of low MW cytokeratins confirms epithelial phenotype of cultured PSE cells and purity of cell culture. (C) Glucocorticoid receptor (GR) and (D) progesterone receptor (PR) immunohistochemistry detect each receptor protein in PSE cell nuclei. Insets in B–D show matched non-immune control staining. Bars represent 100 μm.

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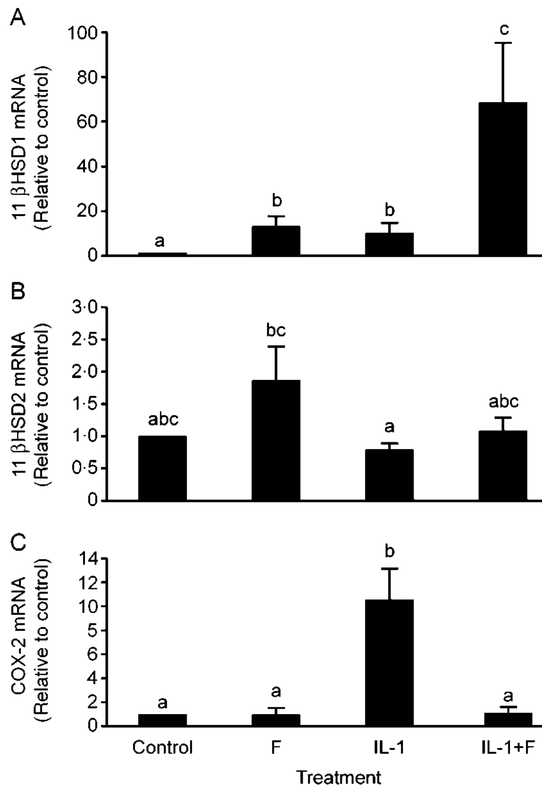

Figure 2 Interaction between interleukin-1α (IL-1α) and cortisol (F) on inflammation-associated gene expression in PSE cells. Quantitative RT-PCR analysis of mRNA for (A) 11βHSD1, (B) 11βHSD2 and (C) COX-2 following treatment with IL-1α (0·5 ng/ml) and/or F (1 μM). Bars represent mean (+S.E.M.) values relative to untreated control for PSE cell cultures from five individual patients. Values with no superscript letter in common are statistically different from each other ($P<0·01$).

Again, there was no significant difference in the magnitude of response in relation to the site of cell sampling from within the same patient (data not shown). Cortisol alone had no significant effect on COX-2 mRNA expression. However, in combination with IL-1α, cortisol significantly suppressed the IL-1α-induced rise in COX-2 mRNA to near control levels ($P<0·001$).

Anti-inflammatory actions of cortisol and progesterone compared

PSE cell cultures from a further six patients were used to test the relative anti-inflammatory effects of cortisol and progesterone on inflammation-associated gene expression.

Stimulation of 11βHSD1 mRNA by IL-1α alone (average 7·25-fold, $P<0·001$) was confirmed in PSE cell cultures from this second series of patients (Fig. 3a). Cortisol alone was also stimulatory (average 10-fold, $P<0·001$). However, progesterone was inactive (Fig. 3a). Once again, cortisol augmented IL-1α-stimulated 11βHSD1 mRNA expression (to an average fold induction, 28·3; $P<0·01$). Co-treatment with progesterone and IL-1α also consistently increased 11βHSD1 mRNA expression beyond that caused by IL-1α alone (average 11·8-fold) but this effect was not statistically significant.

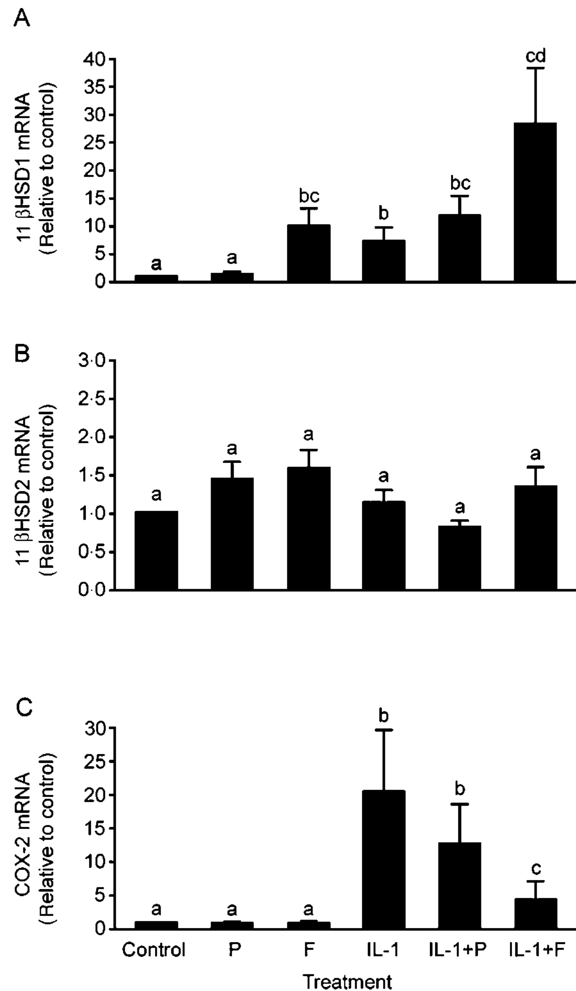

Figure 3 Comparison of the effects of cortisol (F) and progesterone (P) on inflammation-associated gene expression in PSE cells. Quantitative RT-PCR analysis of mRNA for (A) 11βHSD1, (B) 11βHSD2 and (C) COX-2 following treatment with F (1 μM) or P (1 μM) in the presence and absence of interleukin-1α (IL-1α; 0·5 ng/ml). Bars represent mean (+S.E.M.) values relative to untreated control for PSE cell cultures from six individual patients. Values with no superscript letter in common are statistically different from each other ($P<0·01$).

11βHSD2 mRNA expression was unaffected by any of the treatments applied (Fig. 3b).

Stimulation of COX-2 mRNA by IL-1α was strongly suppressed ($P<0·001$) by the presence of cortisol (average fold induction, 4·5) and to a lesser extent ($P>0·05$) progesterone (average fold induction, 12·8; Fig. 3c).

11-Oxoreductase enzymatic activity in PSE cells

The finding that IL-1α stimulates 11βHSD1 mRNA expression in PSE cells predicts the up-regulation of 11-oxoreductase activity, as demonstrated previously in OSE cells (Yong *et al.* 2002). We therefore compared

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11-oxoreductase activity between cell types using PSE cultures from four patients (one of whom also provided an OSE culture for the comparison) and OSE cultures from three others. As shown in Fig. 4, the mean rate of conversion of cortisone to cortisol was significantly less in PSE compared with OSE, under both control (0·14 picomol/culture per h versus 0·93 picomol/culture per h, $P<0·05$) and IL-1α (0·33 picomol/culture per h versus 1·56 picomol/culture per h, $P<0·05$) treatment conditions. Although exposure to IL-1α consistently raised 11-oxoreductase activity in both OSE and PSE cells, only the effect on OSE cells was statistically significant.

Discussion

We show that the cells lining the human peritoneum are able to up-regulate 11βHSD1 gene expression in response to the inflammatory cytokine IL-1α *in vitro*. Furthermore, cortisol – the product of 11-oxoreductase activity catalysed by l1βHSD1 – is anti-inflammatory in PSE cells. Basal and IL-1α-stimulated 11-oxoreductase enzymatic activity in PSE cells is, however, substantially weaker than that of closely related OSE cells. Thus, we provide mechanistic insight on anti-inflammatory signalling in human peritoneum and establish differences between OSE and PSE cells that could help explain the relative susceptibility of PSE to adhesion formation following inflammation-associated injury *in vivo*.

Human PSE cells have previously been obtained from resected omentum (Stylianou *et al*. 1990, Pronk *et al*. 1993) or peritoneum (Witz *et al*. 1998) and cultured with varying success. Here, we swept up cells directly from peritoneal sites *in situ*, avoiding enzymatic tissue digestion to obtain pure mesothelial cell cultures. This atraumatic technique of PSE collection allows single-cell-thick sheets of cells to be cultured with the low likelihood of contamination by other cell types. Morphology of the PSE monolayer *in vitro* closely resembles the classic ‘cobblestone’ appearance of OSE cultured under similar conditions. The mesothelial origin of the PSE cultures we studied was also confirmed by the pattern of low-molecular weight cytokeratin expression they showed, which is also similar to OSE (Auersperg *et al*. 2001) and increases confidence that the PSE cultures were not significantly contaminated with any other cell type.

PSE and OSE cells not only look alike but also share a similar biochemical signature in response to inflammation. Along with inflammation-associated COX-2 gene expression in response to IL-1α they show up-regulation of 11βHSD1 mRNA without any change in 11βHSD2. Whether any functional link exists between COX-2 and 11βHSD1mRNA expression in IL-1α-stimulated PSE cells remains to be established. However, this is suggested by the finding that prostaglandin synthesis is required for the stimulation of 11βHSD1 enzyme activity by IL-1β in human granulosa–lutein cell cultures (Jonas *et al*. 2006). PSE also has in common with OSE the feed-forward response of 11βHSD1 when stimulated by IL-1α in the presence of cortisol (Rae *et al*. 2004). This predicts the capacity to mount a compensatory increase in intracellular levels of cortisol in response to inflammatory stimulation *in vivo*.

Owing to the clinical basis of this study, insufficient patient material was available to delineate the post-receptor signalling mechanisms involved in the actions of IL-1α and anti-inflammatory steroids in PSE cells. However, we did document similar levels of mRNA for PR and GR, and clearly detected nuclear PR and GR proteins. In other experimental systems, nuclear GR and PR receptors transduce positive and negative regulation of genes that impact signalling via cell-surface receptors for pro-inflammatory cytokines (Rosen & Milner 2005) and it is reasonable to suppose that these mechanisms operate in PSE cells too. Other caveats to our results include the extent to which pro-/anti-inflammatory properties of PSE and OSE cells propagated *in vitro* might deviate from norm in terms of functionality *in vivo*. Comparisons of 11-oxoreductase activities in PSE and OSE cell cultures from individual patients also proved problematic owing to a variable adaptation of individual cell biopsies to culture. However, we consistently observed a restricted capacity of PSE cells to undertake 11-oxoreductase activity relative to OSE cells. Moreover, for the single patient from whom both PSE and OSE cell cultures were available for direct comparison, the result obtained (embedded within the data set in Fig. 4) faithfully mirrored the aggregate outcome.

The physiological significance of these results is that cytokine-responsive 11βHSD1 could provide a mechanism throughout the peritoneal surface to localise and limit

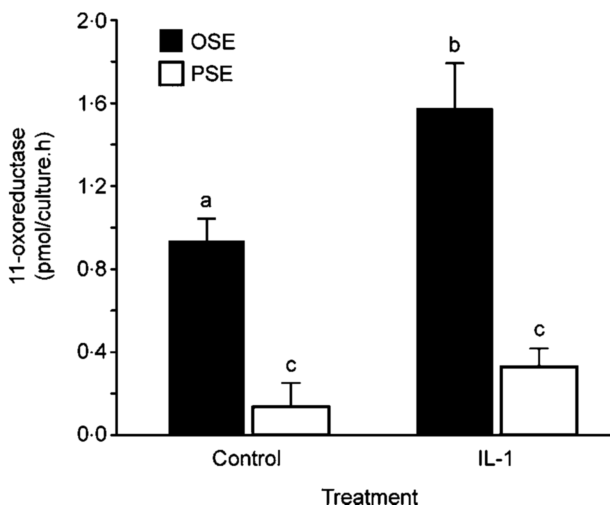

Figure 4 Comparison of 11-oxoreductase activity in PSE and OSE cells. 11-Oxoreductase activity in cultured cells was determined by radiometric assay (see Materials and Methods) following a 48-h incubation with or without interleukin-1α (IL-1α; 0·5 ng/ml). Bars represent mean (+S.E.M.) values relative to untreated control for four patients (cultures), one of whom provided matched PSE and OSE. Values with no superscript letter in common are statistically different from each other ($P<0·05$).

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inflammation-associated injury and promote rapid healing of traumatised tissue through pre-receptor amplification of cortisol formation. However, 11βHSD1 enzyme activity in PSE is lower than that in OSE, indicating that the ovary is better protected by anti-inflammatory mechanisms than the peritoneum. The higher 11-oxoreductase tone of OSE, if reflected in higher local cortisol levels *in vivo*, might be sufficient to minimise fibrosis and thereby reduce the likelihood of postovulatory adhesions. This is borne out by the fact that the ovaries remain mobile and free within the pelvis of most women. On the other hand, the peritoneum is a more stable environment, less prone to natural injury and with a lower 11-oxoreductase tone than the ovarian surface. The sporadic injuries that do occur to PSE tend to be iatrogenic – e.g. during surgery – or involve microbial infection and are inherently more traumatic. This indicates that downstream pro-inflammatory sequelae likely override any compensatory anti-inflammatory benefit arising from 11βHSD1 signalling, permitting fibrosis and adhesion formation to proceed.

Progesterone, present at high concentrations in follicular fluid at the time of ovulation (Andersen & Hornnes 1994), is a candidate anti-inflammatory agent throughout the pelvic cavity. However, progesterone was considerably less potent than cortisol as an anti-inflammatory agent in cultured PSE, matching our previous finding for OSE (Rae *et al.* 2004). Nevertheless, 1 μM progesterone reduced IL-1α-stimulated COX-2 mRNA expression 37%, on average, in 6/6 cases (Fig. 3). Although this effect was statistically non-significant, it may yet be biologically important since progesterone levels in follicular fluid can attain levels up to 30 times higher than those tested here. Accordingly, we do not rule out the possibility that progesterone exerts a physiologically significant action on OSE and/or PSE cells during ovulation *in vivo*. The responsiveness of PSE cells to progesterone also has broader clinical relevance. First, it raises the possibility that the peritoneum may be affected by menstrual fluctuations in ovarian progesterone secretion. Secondly, it is relevant to endometriosis where withdrawal of progesterone prior to menstruation coincides with the most severe symptoms. Thirdly, exogenous progestin is a mainstay treatment for endometriosis.

Finally, our results may have a bearing on the development of epithelial ovarian cancer (EOC). The OSE is widely regarded as the major source of most ovarian cancers (Fleming *et al.* 2006) and EOC frequently involves other peritoneal structures, hence its overall poor outcome (Freedman *et al.* 2004). However, the natural history of EOC is not well defined. This is partly due to the fact that patients most commonly present with advanced stage disease with widespread peritoneal and omental involvement. This in turn makes curative treatment difficult leading to poor survival outcomes of 30% at 5 years. If the disease could be contained within the ovary at FIGO (International Federation of Gynaecology and Obstetrics) stage I, an improvement in prognosis would be expected to follow. Therefore, manipulation of the tumour–peritoneum interaction is a potential therapeutic strategy. Progesterone has been suggested as a potential chemopreventative agent for EOC and lack of progesterone may have pathophysiological roles for both endometriosis and ovarian cancer (Ho 2003, Ness 2003).

In summary, we demonstrate a simple method for the collection and culture of human PSE cells. Using this culture system, we find that PSE cells possess an anti-inflammatory machinery similar to that previously described for OSE cells, including a capacity to up-regulate 11βHSD1 in response to treatment with IL-1α. However, enzymatic activity of 11-oxoreductase is lower in PSE than in OSE. Although the cause for this difference remains unknown, our data provide a possible explanation for the observation that peritoneal healing is often accompanied by adhesion formation, while ovulation-associated ovarian healing is scarless.

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