Regulation of natural antibiotic expression by inflammatory mediators and mimics of infection in human endometrial epithelial cells

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The natural antibiotic molecules, β-defensins 1 and 2 (HBD1/2) and secretory leukocyte protease inhibitor (SLPI), have an important role in mucosal defence and are present in the uterus. This study details their regulation in primary endometrial epithelial cells and in two endometrial cell lines (MFE/HES). Cells were treated with proinflammatory molecules and mimics of infection [lipopolysaccharide (LPS) and lipoteichoic acid (LTA)]. mRNA for HBD1, HBD2 and SLPI was detected in primary endometrial epithelial cells using real-time quantitative PCR. HBD1 mRNA was present at very low levels preventing conclusive study of its regulation. However, HBD2 mRNA expression was increased by interferon-γ, interleukin (IL)-1β alone and IL-1β+ tumour necrosis factor (TNF)-α. SLPI mRNA was not affected by proinflammatory mediators, although protein levels fell in the presence of IL-1β+c9059 TNFα. LPS had little effect on antimicrobial expression. However, there was a trend towards increased expression with LTA treatment for 4–8 h. Antimicrobial expression in endometrial cell lines was similar to that in primary cells, although SLPI was increased by IL-1β+TNFα treatment. These results suggest that in endometrium some natural antibiotics (e.g. SLPI) may be constitutively expressed providing a basal level of protection, while others (e.g. HBD2) are inducible allowing maximal antimicrobial activity during infection. Natural antimicrobials will have an important role in endometrium in protecting against infection.

Key words: defensins/endometrium/infection/inflammatory mediators/SLPI

Introduction

Successful human implantation and pregnancy are reliant on the prevention of genitral tract infections that can compromise both fertility and the fetus itself. The molecules of the innate immune system, present at mucosal surfaces, are likely to be crucial in the limitation of such infections.

Natural antimicrobial molecules are small, cationic peptides often present at epithelial surfaces (Huttner and Bevins, 1999; Hancock and Diamond, 2000). These molecules are a crucial component of the innate immune system offering a broad range of antibacterial, antiviral and antifungal protection. In the human there are two major groups: the α and β defensins. The α-defensins 1–4 [human neutrophil peptide (HNP) 1–4] are present in neutrophils while α-defensins 5 and 6 [human defensin (HD) 5/6] are located in the Paneth cells of the gut and at other epithelial surfaces. The β-defensins [human beta defensin (HBD) 1–4] are located at epithelial surfaces and are likely to play a major role in mucosal defence. Similarly, secretory leukocyte protease inhibitor (SLPI), a neutrophil elastase inhibitor with antimicrobial activity, is present at mucosal sites and may have an anti-infective role (Franken et al., 1989; Tomee et al., 1998).

Several natural antimicrobials have been detected in the human endometrium. HBD1, HD5 and SLPI have been detected in endometrial epithelium, with both HD5 and SLPI showing maximal expression in the secretory phase of the menstrual cycle (Quayle et al., 1998; Valore et al., 1998; King et al., 2000). SLPI is also present in first trimester and term decidua (Denison et al., 1999; King et al., 2000). HBD2 and 4 have been detected in the uterus (Bals et al., 1998; Garcia et al., 2001), while HBD1 and 3 have been located in placenta and placental membranes respectively (Zhao et al., 1996; Jia et al., 2001). The presence of these molecules in endometrium at the time of implantation and during pregnancy suggests a role in the protection of the fetus from infection.

Several of the natural antibiotics are regulated by inflammatory molecules such as interleukin (IL)-1, tumour necrosis factor (TNF)-α and lipopolysaccharide (LPS) in systems such as lung, gut and gingiva (Sallenave et al., 1994; Diamond et al., 1996; Jin et al., 1998; O’Neil et al., 1999; Harder et al.,
endothelial growth factor (1 ng/ml; Peprotech), basic growth factor (25 ng/ml; Peprotech Ltd, London, UK), vascular endothelial growth factor (VEGF) (2 ng/ml; R&D Systems, Abingdon, Oxon, UK), penicillin (50 μg/ml; Sigma), streptomycin (50 μg/ml; Sigma), gentamycin (5 μg/ml; Sigma), epidermal growth factor (25 ng/ml; Peprotech Ltd, London, UK), vascular endothelial growth factor (1 ng/ml; Peprotech), basic fibroblast growth factor (1 ng/ml; Peprotech), IL-1α (10 ng/ml; Peprotech), IL-1β (10 ng/ml; Peprotech), TNFα (2 ng/ml; Peprotech), IL-1β + TNFα, interferon (IFN)−γ (10 ng/ml; Peprotech), IL-6 (5 ng/ml; Peprotech) and phorbol,1-myristate, 13-acetate (PMA, 10−7 mol/l; Calbiochem, Nottingham, UK). These molecules were investigated because they have been reported to influence natural antibiotic expression in other systems (Sallenave et al., 1994; Harder et al., 2000; Krisanaprakornkit et al., 2000). Dose dependence and kinetics of the response of endometrial epithelial cells to IL-1β and TNFα were determined in the HES endometrial epithelial cell line by measuring SLPI mRNA expression. The response observed at 24 h was greater than at 4 h and concentrations 5-fold higher than those

Materials and methods

Tissue collection

Endometrial biopsies were collected from women undergoing gynaecological procedures for benign conditions. All women reported regular menstrual cycles (25–35 days) and had not received any form of hormonal treatment in the 3 months preceding biopsy. Biopsies were dated from the patient’s last menstrual period (LMP). Histological dating according to published criteria (Noyes et al., 1950) and circulating sex steroid concentrations were consistent with the date of LMP. Written informed consent was obtained from all patients prior to biopsy collection and ethical approval was received from the Lothian Research Ethics Committee.

Tissue samples were collected in RPMI 1640 medium (Sigma, Poole, Dorset, UK) and were subsequently divided into two portions. Endometrium was: (i) fixed in 10% neutral buffered formalin overnight at 4°C, stored in 70% ethanol and then wax embedded; and (ii) separated into glandular and stromal compartments for cell culture.

Separation of endometrial biopsies into glandular and stromal compartments

This method for separation of glandular and stromal compartments of endometrium was adapted from that of Osteen et al. (Osteen et al., 1989). Several modifications were made and the details of the method used in this study are described below. Endometrial biopsies were washed twice in phosphate-buffered saline (PBS; Sigma), sliced into small fragments, immersed in collagenase/DNase (1 and 0.1 mg/ml; both Sigma) and incubated for 80 min at 37°C. After incubation, RPMI 1640 medium was added and tissue was broken up using a syringe. This yielded single cells and larger, glandular fragments. This suspension was centrifuged (450 g, 3 min) and then cells/fragments were resuspended in fresh medium and allowed to separate by density sedimentation. After 5 min the supernatant (stromal compartment) was removed leaving 2 ml of medium. Fresh medium was added and the density sedimentation was repeated. The remaining 2 ml of medium contained glandular fragments that were centrifuged as above. Medium was discarded and the epithelial fragments were incubated with collagenase/DNase for 2 h at 37°C. After incubation, medium was added and the cell suspension was centrifuged (see above). Medium was removed and the epithelial cell pellet was resuspended in 50% Matrigel (BD Biosciences, Bedford, MA, USA).

Cell culture

Primary endometrial epithelial cells

Primary endometrial epithelial cells were grown in Matrigel (see Figure 1 showing cells immunostained for SLPI) in RPMI 1640 medium supplemented with 10% fetal calf serum (Mycoplex; PAA Laboratories, Teddington, UK), penicillin (50 μg/ml; Sigma), streptomycin (50 μg/ml; Sigma), gentamycin (5 μg/ml; Sigma), epidermal growth factor (25 ng/ml; Peprotech Ltd, London, UK), vascular endothelial growth factor (1 ng/ml; Peprotech), basic fibroblast growth factor (5 ng/ml; Peprotech) and estradiol (10−7 mol/l). These growth factors were included in the culture medium as there is evidence that endometrial epithelial cells express their receptors and hence they are likely to be involved in modulation of cell growth (Li et al., 1994; Zhang et al., 1995; Sangha et al., 1997; Meduri et al., 2000).

Cells were grown to near confluence (7–10 days) in 12-well plates (Nunc, Gibco, Paisley, UK) and then treated with inflammatory mediators or mimics of infection. The inflammatory mediators investigated were as follows: IL-1β (1 ng/ml; Peprotech), TNFα (2 ng/ml; Peprotech), IL-1β + TNFα, interferon (IFN)−γ (10 ng/ml; Peprotech), IL-6 (5 ng/ml; Peprotech) and phorbol,1-myristate, 13-acetate (PMA, 10−7 mol/l; Calbiochem, Nottingham, UK). These molecules were investigated because they have been reported to influence natural antibiotic expression in other systems (Sallenave et al., 1994; Harder et al., 2000; Krisanaprakornkit et al., 2000). Dose dependence and kinetics of the response of endometrial epithelial cells to IL-1β and TNFα were determined in the HES endometrial epithelial cell line by measuring SLPI mRNA expression. The response observed at 24 h was greater than at 4 h and concentrations 5-fold higher than those.
detailed above did not enhance the response. Hence, all subsequent experiments were carried out for 24 h using 1 ng/ml of IL-1β and 2 ng/ml of TNFα. The bacterial products LPS (1–1000 ng/ml; E.coli, Serotype 026:B6; Sigma) and lipoteichoic acid (LTA; 5 µg/ml; Staphylococcus aureus; Sigma) were used as mimics of Gram-negative and Gram-positive bacteria respectively. Time-course experiments were performed with LPS (1 µg/ml) and LTA (5 µg/ml). Both treatments were tested at 4, 8 and 24 h. LPS (1 µg/ml) was also studied in combination with LTA and IL-1. This mimics dual infection with Gram-negative and positive micro-organisms and also infection in combination with the resultant expression of inflammatory mediators.

All treatments contained estradiol (10⁻⁸ mol/l) to maintain cell growth. Controls (estradiol only) were included throughout. Immunohistochemical staining (using mouse anti-human cytokeratin antibody, clone MNF116; Dako Ltd, Cambridge, UK) of representative primary cell cultures for the epithelial marker, cytokeratin, determined that at the time of treatment the purity of epithelial cells was >90%. The majority of contaminating cells were likely to be of stromal origin and morphologically resembled fibroblasts.

**Cell lines**

MFE-296 [(Hackenberg et al., 1994); ECACC, CAMR, Salisbury, UK] and HES [(Desai et al., 1994); a gift from Dr D.Kniss] endometrial epithelial cell lines were seeded at 1×10⁶ cells/flask (25 cm²; Corning Costar, High Wycombe, UK) and allowed to adhere. Cells were then treated with IL-1β (1 ng/ml), TNFα (2 ng/ml), IL-1β + TNFα, IFNγ (10 ng/ml) and PMA (10⁻⁷ mol/l) for 24 h.

**RT and real-time quantitative PCR**

After treatment, cells were harvested in Tri reagent (Sigma) and RNA and subsequently permeabilized with Triton X100 (0.05%; Sigma). Non-specific endogenous peroxidase activity was blocked by treatment with 3% hydrogen peroxidase (BDH Laboratories Supplies, Poole, UK) in distilled water for 10 min at room temperature. Cells then underwent a non-immune block using diluted normal horse serum (Vectastain 4002; Vector Laboratories, Peterborough, UK) for 20 min at room temperature. Cells were incubated overnight at 4°C with 200 µl mouse anti-SLPI antibody (1:200 in diluted horse serum; HyCult Biotechnology, Uden, The Netherlands). In negative control wells, cells were incubated with equimolar concentrations of mouse immunoglobulin (Ig; Sigma). Cells were then incubated with biotinylated horse anti-mouse IgG (Vector Laboratories), followed by an avidin–biotin peroxidase detection system (both for 60 min at room temperature; Elite ABC 6101; Vector Laboratories). Diaminobenzidine (DAB; Vector Laboratories) was used to identify positive staining (Figure 1). Cells were counterstained with Harris’s haematoxylin (Pioneer Research Chemicals Ltd, Colchester, UK) and stored in ethanol.

**SLPI ELISA**

SLPI concentrations in culture supernatants were determined by ELISA. Throughout the ELISA all dilutions were made in ELISA buffer (150 mmol/l NaCl, 100 mmol/l Tris, 0.00015% phenol red solution, 2 mmol/l EDTA, 150 mg/l 2-methylisothiazolone, 150 mg/l bromonitrodioxane, 2 mg/ml bovine serum albumin, 0.05% Tween-20, 0.01% hexadechltrimethylammonium bromide, pH 7.2). Assay plates (96-well; Nunc Maxi-Sorp) were coated overnight at 4°C with 200 µl/well of goat anti-SLPI (2 µg/ml; R&D Systems, Oxford, UK) and then blocked for 30 min with 200 µl/well of blocking/protecting solution (2% polyvinylpyrrolidone, 5 mg/ml bovine serum albumin, preservatives, 5 mmol/l EDTA, 50 mmol/l Tris). Plates were washed with wash buffer (150 mmol/l NaCl, 5 mmol/l Tris, 0.025% Tween-20, pH 7–7.5) and subsequently 200 µl of standard/sample were added to each well and incubated for 2 h at room temperature on a plate shaker. Two non-specific binding wells (200 µl buffer only) were included on each plate. Standards were added in duplicate and their concentration range was 0.024–25 ng/ml (recombinant SLPI; R&D Systems). Plates were washed and then incubated for 1 h with 200 µl/well of biotinylated mouse anti-SLPI (1:10000; Hycult) as above. After further washing, 200 µl/well of streptavidin peroxidase (1:4000; Roche, Lewes, UK) was added and incubation was for 20 min, as above. Plates were washed again and then 200 µl/well of substrate (0.4 g/l urea-hydrogen peroxidase, 0.16 g/l tetramethyl benzidine in 100 mmol/l sodium acetate; pH 6) was added. After 10 min, wells were quenched with 50 µl/well of IM sulphuric acid. Plates were read in a plate reader at 450 nm. Intra- and inter-assay coefficients of variation were 12.80 and 14.76% respectively.

**Statistical analysis**

Significant difference was determined by analysis of variance (ANOVA; Statview 3.0). Fisher’s protected least squares differences test was used to assign individual differences. *P* < 0.05 was considered significant.

No attempt was made to analyse experiments involving epithelial cells derived from proliferative and secretory endometrium separately. However, SLPI mRNA expression in epithelial cells originating from the proliferative and secretory phases was compared using the control samples. There was no significant difference between SLPI levels in cells derived from the proliferative phase and from those in the secretory phase. In *vivo*, SLPI is expressed only in secretory endometrium. This suggests that after 7–10 days in culture, the cells do not exhibit characteristics specific to their original menstrual cycle phase and so it is appropriate to treat them as one group for statistical analysis.
### Results

**Regulation of natural antibiotic expression in primary cultures of endometrial epithelial cells**

The role of inflammatory mediators and mimics of infection in the regulation of HBD1, HBD2, and SLPI in primary endometrial epithelial cells was investigated.

**HBD1**

Primary cultures of endometrial epithelial cells expressed very low levels of HBD1 (data not shown). This low level of expression made investigation of HBD1 regulation inappropriate. Successful detection of HBD1 mRNA in whole endometrial biopsies served as a positive control.

**HBD2**

HBD2 mRNA expression was up-regulated by treatment with several inflammatory mediators. IL-1β alone, TNFα alone and IL-1β + TNFα increased HBD2 levels by 9-, 4- and 8-fold respectively (Figure 2a; \( P < 0.03 \)). Treatment with IFNγ caused mRNA expression to increase by 9.5-fold (\( P < 0.01 \)). IL-6 and PMA treatment had no effect on HBD2 expression.

LPS at several doses had no effect on HBD2 mRNA expression (data not shown). However, treatment with LPS (1000 ng/ml) for 8 h showed a decrease in HBD2 mRNA levels followed by an increase at 24 h (Figure 2b; \( P = 0.055 \)). LTA treatment caused a trend towards increased expression of HBD2 at 4 h, with a subsequent significant decrease in expression at 24 h (Figure 2b; \( P < 0.02 \)). No significant synergistic effect of treatment with LPS in combination with LTA or IL-1β was detected, although increased HBD2 expression was apparent in the presence of both LPS and IL-1β (Figure 2b; \( P < 0.04 \)). However, this can be accounted for by the up-regulation of HBD2 mRNA in the presence of IL-1β alone (see above).

**SLPI**

SLPI mRNA expression was not affected by treatment with any of the inflammatory mediators tested (Figure 3a; not significant).

SLPI mRNA expression was down-regulated by treatment with LPS (1000 ng/ml) for 4 and 24 h (Figure 3b; \( P < 0.05 \)). All other doses and incubation times had no effect on SLPI levels. LTA treatment resulted in a trend towards increased expression of SLPI at 4 h (Figure 3b; not significant). LPS, in combination with LTA or IL-1β, caused a down-regulation of SLPI mRNA expression after 24 h of treatment (Figure 3b; \( P < 0.01 \)). This is consistent with the results for treatment with LPS alone and does not reflect an effect of LTA or IL-1β.

In addition, SLPI protein was localized to the cytoplasm of the primary epithelial cells using immunohistochemistry (Figure 1) and changes to SLPI protein concentrations in cell culture supernatants were determined by ELISA. In the presence of IL-1β + TNFα, SLPI concentrations were reduced (Figure 3c; \( P < 0.04 \)), although treatment with the other inflammatory molecules did not affect SLPI levels. SLPI concentrations were reduced by treatment with LTA for 24 h (Figure 3d; \( P < 0.03 \)). There was also a trend towards increased SLPI release in the presence of LPS + IL-1β.

**Regulation of natural antibiotic expression in endometrial epithelial cell lines**

Expression of HBD1, HBD2, and SLPI was investigated in the MFE and HES endometrial epithelial cell lines. Natural antibiotic expression in these cell lines was similar to that found in primary endometrial epithelial cells. HBD1 mRNA was expressed at very low levels in MFE cells and was undetectable in HES cells (data not shown). Similarly, HBD2 was undetectable in both cell lines under control conditions, although mRNA expression became detectable at very low levels in MFE cells treated with IL-1β + TNFα for 24 h (in two of three experiments; data not shown). SLPI mRNA was detected in both cell lines and was up-regulated by treatment with IL-1β + TNFα (Figure 4a: MFE, \( P < 0.01 \); Figure 4b: HES, \( P = 0.05 \)). SLPI protein release by HES cells into culture supernatants was increased by treatment with TNFα both alone and in combination with IL-1β (Figure 4c; \( P < 0.01 \)). MFE cells released very little SLPI into culture supernatants and so it was not possible to determine the regulation of protein release.

### Discussion

This study details the presence and regulation of natural antibiotic molecules in endometrial epithelial cells. Human β-defensins 1 and 2 and SLPI were detected in primary cultures of endometrial epithelial cells. This is consistent with previous reports detailing the expression of HBD1 and SLPI in the endometrial epithelium and at other mucosal sites (Zhao et al., 1996; Valore et al., 1998; King et al., 2000). HBD2 has also been detected in the uterus (Bals et al., 1998), although this report did not investigate the site of expression within the organ. However, HBD2 is present at other epithelial surfaces (Harder et al., 2000; Krisanaparakornkit et al., 2000).

The regulation of natural antibiotic molecules has not been previously described in endometrial epithelial cells. Our in-vitro study, the first detailing natural antimicrobial regulation...
IL-1 are affected by IL-1 receptor antagonist and IL-1 receptor levels which vary throughout the menstrual cycle (Simon et al., 1993, 1995) and hence, levels of these molecules may influence natural antibiotic expression in vivo. The HBD2 promoter is reported to have several consensus sites for the proinflammatory transcription factors NFκB and AP-1, accounting for its induction by the above mediators (Harder et al., 2000). Additionally, several sites for the transcription factor NF-IL6 have been detected in the HBD2 promoter, although the cytokine IL-6 (which stimulates transcription via NF-IL6) was reported to have no effect on HBD2 expression in lung epithelial cells (Harder et al., 2000). Similarly, IL-6 did not alter HBD2 levels in endometrial epithelial cells. The protein kinase C stimulator, PMA, up-regulates HBD2 in gingival epithelial cells (Krisanaprakornkit et al., 2000), although it had no effect in our system, suggesting that differences in defensin regulation exist between epithelial cells from distinct sites.

HBD1 mRNA was found to be expressed at very low levels in both primary endometrial epithelial cells and in endometrial cell lines. As HBD1 has previously been detected in endometrial epithelium in vivo (Valore et al., 1998), our results suggest that a stromal mediator is needed to maintain expression of this defensin in the epithelium. Previous studies have detailed constitutive HBD1 expression in several epithelial cell types and have reported resistance to treatment with proinflammatory cytokines. Our investigation into the regulation of HBD1 expression in endometrium was limited by the low expression of the molecule.

SLPI is also reported to be up-regulated by inflammatory cytokines and LPS (Sallenave et al., 1994; Jin et al., 1998). However, in primary endometrial epithelial cells, SLPI mRNA expression was unaltered by treatment with proinflammatory molecules, while protein concentrations in culture medium fell after treatment with IL-1β+TNFα. In a previous study, we reported that SLPI expression in endometrium is increased during the secretory phase of the menstrual cycle, suggesting regulation by progesterone (King et al., 2000). This, along with our current findings, suggests that in endometrium SLPI may be controlled primarily by the local steroid environment, resulting in peak expression around the time of implantation. However, inflammatory pathways such as the IL-1 system are also involved at this time (Simon et al., 1994) and may be involved in the regulation of SLPI expression under certain circumstances. It should be noted that treatment of both the HES and MFE cell lines with a combination of IL-1β and TNFα resulted in increased SLPI expression. This may represent differences in regulation of SLPI between the primary cells and cell lines, e.g. there may be differences in IL-1 and TNF receptor numbers on the cell surfaces. Also, in the MFE cell line a synergistic action of IL-1β and TNFα was observed. This may be due to the presence of several sites on the SLPI promoter that can respond to these two cytokines, as has previously been reported for the rat nosII gene (Kuemmerle, 1998). For example, the SLPI promoter contains several AP-1 sites and is thought to be responsive to NFκB (Abe et al., 1991; Nguyen et al., 1999).

LPS had little effect on HBD2 expression when dose and
Figure 3. Regulation of SLPI mRNA and protein expression in primary endometrial epithelial cells. Sample numbers are shown below the x-axis. Note that the total number of treatment samples exceeds the number of controls. In most experiments, there were several different treatments relating to one control. Treatment of cells was as described in Figure 2. Due to the distribution of results from primary cell cultures, data were logarithmically transformed prior to statistical analysis by ANOVA. (a) SLPI mRNA—proinflammatory mediators. (b) SLPI mRNA—mimics of Gram-negative, Gram-positive and dual infection. a,b,c,d,e: P < 0.05. (c) SLPI protein—proinflammatory mediators. a: P < 0.04. (d) SLPI protein—mimics of Gram-negative, Gram-positive and dual infection. a: P < 0.03.

Figure 4. Regulation of SLPI mRNA and protein expression in endometrial cell lines. Cells were treated with IL-1β (1 ng/ml), TNFα (2 ng/ml), IL-1β+TNFα, IFNγ (10 ng/ml) or PMA (10⁻⁷ mol/l) for 24 h. The results shown represent the mean of three experiments. (a) SLPI mRNA—MFE cell line. a,b,c: P < 0.01. (b) SLPI mRNA—HES cell line. a: P = 0.05. (c) SLPI protein—HES cell line. *Mean of two samples. a,b: P < 0.01.
time of treatment were varied. Synergistic actions between LPS and other mediators were not observed. Previous studies in other systems have reported contradictory results. Some have shown HBD2 to be up-regulated by LPS (McNamara et al., 1999; Becker et al., 2000). However, others have reported little effect of LPS, but have found increased HBD2 expression upon infection with pathogenic bacteria, suggesting that other components of the bacterial cell wall are involved in stimulating defensin expression (O’Neil et al., 1999; Harder et al., 2000). This also suggests that LPS actions may depend on the cell type investigated. Also, our study used E. coli LPS and it may be that endometrial epithelial cells would respond to LPS from genital tract pathogens (e.g. Chlamydia). SLPI has been reported to be an LPS inducible gene in macrophages (Jin et al., 1997). However, we found SLPI expression to be decreased after treatment for 24 h with 1 μg/ml of LPS. This may indicate that, as expected, innate immune responses to LPS occur over a short time period. Following initiation of the adaptive response, high levels of a leukocyte protease inhibitor may be inappropriate, hence the observed down-regulation. Similarly, at shorter time points LTA caused a trend towards increased expression of HBD2 and SLPI that was not observed after 24 h. Indeed, protein concentrations of SLPI fell after exposure to LTA for 24 h. Responses to PAMPs such as LPS and LTA are thought to be mediated by interactions with cellular or soluble CD14 and subsequent signalling via Toll-like receptors (Chow et al., 1999; Becker et al., 2000). It may be that the poor response to PAMPs by the endometrial epithelial cells was due to low levels of CD14 expression and inadequate soluble CD14 in bovine serum.

The main role for HBD1, HBD2 and SLPI in human endometrium is likely to be in the prevention of uterine infection. Each of these molecules has been shown to have antimicrobial activity, and hence they are likely to be important in the control of sexually transmitted infections. They may serve to limit the spread of disease from the lower to upper genital tract, where infection is associated with infertility (Cates and Wasserheit, 1991; Paavonen, 1993). Also, local defences are crucial to successful pregnancy. A total of 20% of preterm births are associated with uterine infection (Romero et al., 1989c) and increased amounts of HNP1–3 and another antimicrobial compound, lactoferrin, have been detected in these circumstances (Heine et al., 1998; Pacora et al., 2000). This suggests that these natural antimicrobials have a protective effect and HBDs and SLPI are likely to have a similar role.

Constitutively expressed natural antibiotics such as SLPI will provide a basal level of antimicrobial protection in the uterus. This will ensure antimicrobial protection at times when infection may jeopardise endometrial receptivity (e.g. implantation). IL-1β, TNFα and IFNγ are produced by the endometrium under physiological conditions, with TNFα produced by epithelial cells (Tabibzadeh et al., 1995), IL-1β by epithelial and isolated stromal cells (Tabibzadeh and Sun, 1992; Simon et al., 1993) and IFNγ by T cells and polymorphonuclear cells (Tabibzadeh, 1994; Yeaman et al., 1998). These molecules may contribute to regulation of the natural antibiotics during the normal menstrual cycle. In addition, up-regulation of these cytokines occurs during infection and the presence of both IL-1β and TNFα has been reported in amniotic fluid from patients undergoing preterm labour associated with infection (Romero et al., 1989a,b).

Although an antimicrobial role is likely to be the main function of HBDs and SLPI in the endometrium, it should be noted that these molecules have several other actions that may be important. HBD2 chemoattracts immature dendritic cells and memory T cells to sites of inflammation via the CCR6 chemokine receptor (Yang et al., 1999). This will result in activation of the adaptive immune system during infection. HBD2 has also been shown to activate rat mast cells resulting in histamine and prostaglandin D2 release (Niyonsaba et al., 2001). SLPI has generalized anti-inflammatory actions including inhibition of neutrophil elastase and cathepsin G (Thompson and Ohlsson, 1986), down-regulation of matrix metalloproteinase production by monocytes (Zhang et al., 1997) and inhibition of NFκB (Lentsch et al., 1999). Activation of DNA synthesis in porcine endometrial epithelial cells has also been reported (Badinga et al., 1999).

In summary, the natural antimicrobials HBD1, HBD2 and SLPI have been studied in a primary endometrial epithelial cell culture model and in two endometrial epithelial cell lines. Each of these molecules is expressed in the endometrial epithelium and, in other systems, they are differentially regulated, suggesting that they provide both constitutive and inducible antimicrobial protection.

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