The effects of sex steroid hormones and interleukin-1-beta on MUC1 expression in endometrial epithelial cell lines

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Abstract

Oestrogen, progesterone and paracrine signals from the embryo have been associated with the overall control of implantation. Changes in the expression of the heavily glycosylated transmembrane glycoprotein MUC1 mucin on the endometrial epithelium are also thought to be important for embryo attachment. Increased MUC1 expression has been correlated with elevated progesterone levels in the secretory phase of the menstrual cycle. Embryonic control of endometrial receptivity through changes in MUC1 expression could be achieved through the interleukin-1 system. Four endometrial epithelial cell lines (HEC1A, HEC1B, Ishikawa and RL592) were treated with oestrogen and progesterone (with or without interleukin-1-beta) and were subjected to immunocytochemistry and flow cytometric analysis to determine MUC1 production using MUC1 antibodies. HEC1A (oestrogen receptor (ER) and progesterone receptor (PR) positive) and HEC1B (ER positive and PR negative) were transfected with the MUC1 promoter, underwent similar treatment regimes and the activity of the MUC1 promoter relative to their untreated controls was determined using a chloramphenicol acetyltransferase (CAT) enzyme-linked immunoassay. Using the cell lines, we determined that endometrial MUC1 expression is up-regulated by progesterone, consistent with the in vivo increases in MUC1 related to high progesterone levels. We also revealed that neither oestrogen, nor interleukin-1-beta, appear to modulate MUC1. Progesterone-dependent regulation of MUC1 is likely to be an important factor in determining endometrial receptivity.

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Introduction

The receptive status of the endometrium in embryonic implantation is a balance between the activation of adhesion molecules and the presence of a barrier that the embryo may encounter on the endometrial epithelium (Aplin 1997, Horne et al. 2002a). This process is undoubt-edly influenced by maternal steroid hormones, but may also be affected by local embryonic paracrine signals (Simon & Valbuena 1999, Simon et al. 2000).

Intrinsic heterogeneity in the glycosylation of the heavily glycosylated transmembrane glycoprotein MUC1 mucin on the endometrial epithelium could allow a local mechanism to contribute to the receptivity of the endometrium, because it may be possible that MUC1 glycoforms or the MUC1 protein core could be recognised by the embryo (Hey et al. 1994, DeLoia et al. 1998, Horne et al. 2002b, Brayman et al. 2004). Therefore, a careful evaluation of the regulation of MUC1 at the endometrial surface is necessary. Most of the data on sex steroid control of MUC1 are derived from animal and human models of endometrial MUC1 expression with respect to the menstrual cycle or human cell lines. Human in vivo data, derived from the examination of normal endometrium and endometrial carcinoma, suggest that low levels of MUC1 mRNA correlate with higher levels of plasma oestrogen and that progesterone, preceded by oestrogen priming, increases MUC1 mRNA and MUC1 protein (Hey et al. 1994, DeLoia et al. 1998, Meseguer et al. 2001). There is also evidence that progesterone receptor (PR) negativity in tumour epithelial cells is associated with increased MUC1 protein (Sivridis et al. 2002).

In vitro data from human endometrial, breast and prostate carcinoma cell lines suggest that oestrogen alone has no effect on MUC1 mRNA or MUC1 protein (Gollub et al. 1995, Brotti et al. 1997, McGuckin et al. 1998, Mitchell et al. 2002). The data regarding the effect of progesterone in isolation are conflicting, but progesterone alone (as well as progesterone preceded by oestrogen priming) appears to
increase MUC1 protein expression in some prostatic and breast carcinoma cell lines (Gollub et al. 1995, Botti et al. 1997, McGuckin et al. 1998, Mitchell et al. 2002).

The molecular mechanism of regulation of MUC1 under these circumstances remains unknown. Analyses of the MUC1 promoter have identified many potential binding sites for transcription regulating factors — including GC boxes, Sp1 sites, and several oestrogen receptor (OR) and PR sites (Lancaster et al. 1990, Abe & Kufe 1993, Kovarik et al. 1993, 1996, Zaretsky et al. 1999, Morris & Taylor-Papadimitriou 2001). Sex steroids could therefore be involved in the regulation of MUC1 transcription either by directly interacting with the MUC1 promoter or indirectly by stimulating or repressing other transcription factors.

MUC1 mRNA and MUC1 protein are up-regulated by the embryo during the apposition phase but locally down-regulated at attachment sites (Meseguer et al. 2001). It is not known precisely how this effect is mediated but it has been postulated that the cytokine, interleukin-1-beta (IL-1β), and its receptor, contribute to the regulation of molecules, such as MUC1, to provide the interface between the embryo and endometrium (Simon et al. 1995, 1998, Simon & Valbuena 1999). IL-1β produced by cultured human embryos and high concentrations of IL-1β (>80 pg/ml) in the conditioned media of cultured embryos have been correlated with successful human implantation after in vitro fertilisation (Zolti et al. 1991, Baranao et al. 1992). IL-1 bioactivity in human serum also reaches maximal levels during the secretory phase (Cannon & Dinarello 1985).

This study was undertaken to investigate the influence of oestrogen and progesterone, in the absence and presence of IL-1β, on MUC1 expression in endometrial epithelial cell lines.

Materials and Methods

Four endometrial epithelial carcinoma cell lines were used: HEC1A, HEC1B, Ishikawa and RL952. The cell lines were treated with a range of sex hormone regimes, with or without IL-1β, and were subjected to immunocytochemistry and flow cytometric analysis to determine MUC1 protein expression using monoclonal antibodies (Mabs) HMFG1, HMFG2, SM3 and CT1. Two of the cell lines, HEC1A (ER and PR positive) and HEC1B (ER positive and PR negative) were transfected with the MUC1 promoter. They underwent similar treatment regimes and the activity of the MUC1 promoter relative to their untreated controls was determined using a chloramphenicol acetyltransferase (CAT) enzyme-linked immunoassay.

Antibodies

The MUC1 Mabs used for this study included HMFG1, HMFG2 and SM3 (all IgG1). HMFG1 and HMFG2 (both from SkyBio, Wyboston, UK) react strongly with malignant tissues, but are not cancer specific and react equally well with normal epithelial tissues (Taylor-Papadimitriou et al. 1981, Hilkens et al. 1984, Xing et al. 1989). The preferred target for these antibodies is located within the extracellular domain of MUC1 which includes one possible O-glycosylation site (Price et al. 1991). The Mabs react differently with MUC1 due to the glycosylation, which can hide peptide epitopes (Burchell & Taylor-Papadimitriou 1993, Ho et al. 1995). CT1 (a kind gift from Dr Joy Burchell, CR UK, London, UK) is the polyclonal antiserum to the cytoplasmic tail of MUC1 (Pemberton et al. 1992). It is one of a few reagents whose reactivity with the MUC1 mucin is independent of the O-glycosylation seen in the extracellular domain. The anti-PR antibody used was a mouse monoclonal (IgG1) which recognises the N-terminal region of both the A and B forms of PR (Novocasta, Peterborough, UK) (Giri et al. 1998, Peterson 2000).

Endometrial epithelial cell lines

The endometrial epithelial carcinoma cell lines HEC1A, HEC1B, Ishikawa and RL952 have previously been extensively characterised (Kuramoto 1972, Kuramoto et al. 1997, Way et al. 1983, Gollub et al. 1993, 1995). All cell lines express ER but HEC1B cells have been shown to be devoid of immunoreactive PR (Gao et al. 1994). Furthermore, an induction in the activity of progesterone-responsive reporter plasmids has only been observed in HEC1B cells in the presence of co-transfected PR-B, and not in untransfected cells (Bamberger et al. 1996). Prior to the following experiments and following treatments, we confirmed that HEC1A cells were PR positive and that HEC1B cells were PR negative (and that PR was not induced by hormonal treatment), using standard Western blotting techniques and the above anti-PR antibody (data not shown).

General maintenance of cell lines

All cell lines were grown in phenol-red-free Dulbecco’s modified Eagle’s medium (DMEM) supplemented with stripped foetal bovine serum (FBS) (both from Gibco) and penicillin-streptomycin-ampthererin B mixture (at a final concentration of 100 U, 100 mg/ml and 0.25 mg/ml respectively) to limit infections. Cells were routinely split at 80% confluency using trypsin-EDTA (0.05% v/v and 0.53 mM respectively; in Hank’s balanced salt solution without calcium and magnesium) (Gibco) or versene (CR UK), routinely sub-cultured at between 1:6 and 1:10 depending upon the cell line, and the medium was routinely changed every 72 h. Prior to immunocytochemistry, the cell lines were grown to 80% confluency, trypsinised and re-suspended in fresh DMEM containing 10% stripped FBS. The cell suspension was further diluted such that 300 μl aliquoted four-well slides produced approximately 30% confluency after 24 h incubation at 37 °C. At this stage, the DMEM was replaced with DMEM containing 2% stripped FBS. After 48 h, incubations with sex steroids and/or IL-1β were started. Prior to flow cytometry, cells

Reproduction (2006) 131 733–742

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were grown in T75 flasks in DMEM containing 10% stripped FBS. When the cells got to 30% confluency, the DMEM was replaced with DMEM containing 2% FBS. After 48 h in 2% DMEM, incubations with sex steroids, with or without IL-1β were started. Culture medium and treatments were changed daily.

Cell treatments
The cell lines were incubated for a range of time periods with different concentrations of sex steroids, with or without IL-1β prior to immunocytochemistry and flow cytometry to examine the effect of the treatments on MUC1 mucin expression, and transfection experiments to measure their effect on MUC1 promoter activity. The sex steroids (ICN Biomedicals, OH, USA) and IL-1β (R&D Systems, Abingdon, UK) were diluted to stock solutions of 1000 x final concentration desired in 100% ethanol and stored at −20°C and −70°C respectively. Each cell line was treated for 48, 72, 96 or 192 h with either 17-beta oestradiol or progesterone alone at either 10^{-10}, 10^{-8} or 10^{-6} M, and also individually treated with a combination of 17-beta oestradiol (10^{-10} M) for 96 h followed by 17-beta oestradiol and progesterone (10^{-7} M) for a further 96 h. The same cells were subjected to the addition of IL-1β at 10 and 100 pg/ml for the last 48 h of their treatments. Mock treatment with ethanol was used as a negative control. Prior to treatment, cells were incubated for 48 h in 2% stripped FBS and were maintained in this concentration of FBS for the duration of the treatment. All concentrations and regimes used are comparable with those established in previous reports and with physiological ranges (Gollub et al. 1995, McGuckin et al. 1998, Mitchell et al. 2002).

Immunocytochemistry
Following hormone treatment, slides were washed with PBS, fixed with −20°C methanol:acetone (1:1) for 10 min and air-dried, then stored at −70°C; prior to use the slides were equilibrated to room temperature for 5 min. The slides were blocked with normal rabbit (goat for CT1) serum (Dako, Cambridge, UK), diluted 1 in 10 in 0.1% v/v bovine albumin in PBS (PBS diluent), for 10 min. HMFG1, HMFG2, SM3 and CT1 (all 1 in 500 dilution in PBS diluent), or PBS diluent alone, were then applied to the sections and left overnight at 4°C. After washing with PBS, a biotinylated rabbit anti-mouse (goat anti-rabbit for CT1) immunoglobulin (Dako) (diluted 1 in 200 in PBS diluent) was applied for 30 min. This was followed by incubation with the avidin–biotin complex (Dako) for 30 min before visualisation with diaminobenzidine (2 mg/ml) in PBS with the addition of hydrogen peroxide (1 in 2000) shortly before use. The reaction was terminated after 10 min by washing in tap water. The slides were counterstained in Mayer’s haematoxylin for 10 s and mounted in Pertex.

Flow cytometry
Following hormone treatment, cells were detached with versene plus gentle agitation and washed twice at 4°C using the washing buffer (PBS containing 1% FBS). Samples were then re-suspended in 75 ml MUC1 antibody (diluted 1 in 500 in washing buffer) and incubated at 4°C for 1 h. One additional sample per cell line was incubated with PBS alone instead of antibody, which served as a negative control. After washing, samples were re-suspended in 300 ml fluorescein isothyanate (FITC)-labelled secondary antibody, and incubated at 4°C for 45 min. Following further washing, samples were re-suspended in 500 ml PBS containing 2% neutral buffered formalin. Flow cytometric analysis was performed on a Coulter Epics XL (Beckman Coulter, High Wycombe, UK). Gating parameters were set to exclude clumped or dead cells, and 20,000 events within the gated area were counted per individual experiment. Each experiment was performed in triplicate.

Transfection of cells with MUC1 promoter
The MUC1 promoter construct (Kovarik et al. 1993) (‘−1401 to +33 bp’ sequence; kind gift from Dr Sandra Gendler, Rochester, NY, USA) was linked to the CAT reporter gene (Alam & Cook 1990). To examine MUC1 promoter activity in HEC1A and HEC1B cells, the cell lines underwent a modified treatment regime using the same range of concentrations of sex steroid hormones as previously (either 17-beta oestradiol or progesterone between 10^{-6} and 10^{-10} M), but for 192 h. Cell lines were also treated sequentially with 17-beta oestradiol for 96 h followed by 17-beta oestradiol and progesterone (10^{-7} M) for a further 96 h. The same cells were subjected to the addition of IL-1β at 10 and 100 pg/ml for the last 48 h of their treatments. All transfections were performed 48 h prior to cell harvesting using the calcium phosphate precipitation method, with reagents from a mammalian transfection kit (Promega). The cells were transfected with the MUC1 promoter construct for 6 h in DMEM containing 5% stripped FBS. The cells were then washed and the medium replaced with fresh DMEM containing 2% stripped FBS with the appropriate treatment. The cells were then cultured until immunocytoassay. Transfections were performed in triplicate. MUC1 promoter activity was measured using a CAT enzyme-linked immunocytoassay (Roche), according to the manufacturer’s instructions. The absorbance of the sample (directly related to the level of CAT present in the medium supernatant) was measured using the OPTImax tunable microplate reader (Molecular Devices, Wokingham, UK) and analysed using SOFTmax PRO computer software (Molecular Devices).

Statistical analysis
Flow cytometric data were analysed as follows. A plot of fluorescent intensity versus frequency was integrated to
obtain a flow cytometric profile corresponding to overall MUC1 expression in each sample. The increases in expression demonstrated with exposure to the different treatment protocols reflect a rightward shift in these curves, i.e. an increase in mean intensity. To allow comparison between each of the treatment protocols in Figs 2 and 3, MUC1 expression (the area under the curve) is expressed as a percentage of MUC1 expression in control medium (which was set at 100%). For statistical analysis, it was found that the variables of interest for particular treatments were more stable when expressed as ratios with the ‘control’ values of the variable. The ANOVA were therefore carried out on those ratios. Consequently, in Tables 1 to 3, the difference between the tabulated figure and 1.0 represents the proportionate increase (or decrease) corresponding to the treatment. The primary purpose of the analysis was to determine whether the differences of the tabulated mean values from 1.0 represent the proportionate changes corresponding to the treatment levels.

Transfection data were analysed as follows. Each cell treatment resulted in a different absorbance reading, which directly related to the CAT level in the cells. Using a range of pre-determined standards, the resulting absorbance for each cell line and each treatment was converted to give a value for the level of CAT activity. As each transfection was performed in duplicate and each CAT assay in triplicate, this resulted in six values for each treatment. The statistical analysis was carried out on those ratios. Consequently, in Tables 1 to 3, the difference between the tabulated figure and 1.0 represents the proportionate increase (or decrease) corresponding to the treatment. The primary purpose of the analysis was to determine whether the differences of the tabulated mean values from 1.0 represent the proportionate changes corresponding to the treatment levels.

### Results

**Immunocytochemistry for qualitative analysis of MUC1 production in stimulated endometrial epithelial cell lines**

All cell lines appeared positive for MUC1 showing a cytoplasmic expression pattern with nuclear sparing. It was not possible to differentiate separate membranous expression from the cytoplasmic staining. None of the above cell lines showed any demonstrable changes in MUC1 expression at the light microscope level following any of the sex steroid hormone regimes compared with their controls. The majority of the Ishikawa cells showed an expression pattern of moderate intensity with HMFG1, HMFG2 and CT1. The pattern observed with SM3 was less intense and very heterogeneous. RL952 showed a characteristic ‘patchwork’ effect, with different groups of cells staining with different intensity with all four of the MUC1 Mabs. The expression pattern for the HEC1A cell

### Table 1

Demonstration of the effects of the concentration of oestrogen and progesterone stimulation on MUC1 production in HEC1A and HEC1B cells using flow cytometric analysis and HMFG1 and CT1 (independent of time).

<table>
<thead>
<tr>
<th>HEC1A cell line</th>
<th>Oestrogen</th>
<th>Progesterone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HMFG1</td>
<td>CT1</td>
</tr>
<tr>
<td>Concentration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$10^{-12}$ M</td>
<td>1.250</td>
<td>1.189</td>
</tr>
<tr>
<td>$10^{-9}$ M</td>
<td>1.250</td>
<td>1.191</td>
</tr>
<tr>
<td>$10^{-6}$ M</td>
<td>1.265</td>
<td>1.189</td>
</tr>
<tr>
<td>Standard error</td>
<td>0.024</td>
<td>0.017</td>
</tr>
<tr>
<td>$P$ value</td>
<td>NS</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Note that progesterone has the most pronounced effect. The differences of the tabulated mean values from 1.0 represent the proportionate changes corresponding to the treatment levels.

### Table 2

Demonstration of the effects of the time of exposure to oestrogen and progesterone stimulation on MUC1 production in HEC1A and HEC1B cells using flow cytometric analysis and HMFG1 and CT1 (independent of concentration).

<table>
<thead>
<tr>
<th>HEC1A cell line</th>
<th>Oestrogen</th>
<th>Progesterone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HMFG1</td>
<td>CT1</td>
</tr>
<tr>
<td>Treatment duration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>48 h</td>
<td>1.231</td>
<td>1.175</td>
</tr>
<tr>
<td>72 h</td>
<td>1.194</td>
<td>1.147</td>
</tr>
<tr>
<td>96 h</td>
<td>1.253</td>
<td>1.170</td>
</tr>
<tr>
<td>192 h</td>
<td>1.342</td>
<td>1.268</td>
</tr>
<tr>
<td>Standard error</td>
<td>0.028</td>
<td>0.019</td>
</tr>
<tr>
<td>$P$ value</td>
<td>&lt;0.05</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

The differences of the tabulated mean values from 1.0 represent the proportionate changes corresponding to the treatment levels.

Flow cytometry for quantitative analysis of MUC1 production in stimulated endometrial epithelial cell lines

Flow cytometric analysis of MUC1 protein production using HMFG1 and CT1 in HEC1A cells incubated with 17-beta oestradiol showed no evidence of a treatment effect with the range of concentrations used. There was a minimal increase in MUC1 production with increased time of exposure to 17-beta oestradiol, which was strongest after 48 h (reaching a maximum of a 1.342-fold increase at 192 h with HMFG1, \( P < 0.001 \)). Addition of progesterone was associated with an increase in expression of MUC1 in HEC1A cells, as demonstrated by HMFG1 and CT1 (Tables 1 and 2, Fig. 2). A consistent response to progesterone was seen. Although there was some variation in magnitude with each antibody, the increases in MUC1 expression observed using HMFG1 (which detects the glycosylated ectodomain of MUC1) and that observed by CT1 (which detects the cytoplasmic tail), were very similar. The treatment effect was relatively small at 48 h but increased with the passage of time. A 1.922-fold increase was demonstrated with HMFG1 after progesterone exposure of 192 h, independent of treatment concentration (\( P < 0.001 \)). This suggested that the MUC1 core protein detected by CT1, and the MUC1 glycoform detected by HMFG1, were both up-regulated by the addition of progesterone in HEC1A cells.

Analysis of MUC1 protein production using flow cytometry and the same MUC1 Mabs in HEC1B cells produced similar results (Tables 1 and 2, Fig. 3). There was little effect on MUC1 expression with the addition of 17-beta oestradiol, even with increasing concentrations. There was a small but significant effect with time

<table>
<thead>
<tr>
<th>Progesterone concentration</th>
<th>48h</th>
<th>72h</th>
<th>96h</th>
<th>192h</th>
</tr>
</thead>
<tbody>
<tr>
<td>(10^{-10}) M</td>
<td>1.202</td>
<td>1.337</td>
<td>1.460</td>
<td>1.517</td>
</tr>
<tr>
<td>(10^{-9}) M</td>
<td>1.322</td>
<td>1.342</td>
<td>1.745</td>
<td>1.770</td>
</tr>
<tr>
<td>(10^{-8}) M</td>
<td>1.357</td>
<td>1.372</td>
<td>1.707</td>
<td>1.803</td>
</tr>
</tbody>
</table>

Increasing progesterone concentration and treatment time almost doubled the expression of the MUC1 glycoform detected by this Mab (1.803-fold increase with \(10^{-6}\) M progesterone after 192 h, \( P < 0.001 \)). The differences between the tabulated mean values and 1.0 represent the proportionate changes corresponding to the treatment levels.

Table 3 MUC1 (HMFG1) expression in HEC1B cells. Demonstration of the interaction between sex steroid hormone concentration and duration of treatment when HEC1B cells were treated with progesterone and MUC1 expression was examined using HMFG1.
reaching a maximum of a 1.268-fold increase in expression at 192 h, \( P < 0.001 \).

Progesterone again produced a significant increase in MUC1 expression determined with both HMFG1 and CT1 in HEC1B cells. Although little effect was seen until after 48 h, the two factors, time and treatment, independently increased MUC1 production. There was only evidence of an interaction between sex steroid hormone concentration and duration of treatment when the HEC1B cells were treated with progesterone and MUC1 expression was examined using HMFG1 (Table 3). Increasing progesterone concentration and treatment time almost doubled the expression of the MUC1 glycoform detected by this Mab (1.803-fold increase with \( 10^{-6} \)M progesterone after 192 h, \( P < 0.001 \)).

In both cell lines, when progesterone was introduced after 96 h of treatment with 17-beta oestradiol the level of MUC1 expression, as demonstrated by HMFG1 and CT1, showed an equivalent increase to treatment with progesterone alone. Oestrogen priming did not appear to increase the progestogenic effect.

IL-1\( \beta \) appeared to have no effect on MUC1 expression in either cell line treated with this combination of sex steroids at either 10 or 100 pg/ml.

**Analysis of MUC1 promoter activity in stimulated endometrial epithelial cell lines using a CAT enzyme immunoassay**

The effect of the sex steroid hormone regimes on MUC1 promoter activity was analysed using a previously described MUC1 promoter construct linked to the CAT reporter gene (Kovarik et al. 1993). The relative level of CAT activity in treated cells versus an untreated control
was then used to estimate effects on transcriptional activity of the MUC1 gene.

CAT activity, and thus MUC1 promoter activity, was only very slightly affected by treatment with oestrogen in both HEC1A and HEC1B cells (Fig. 4). Progesterone treatment, however, significantly increased MUC1 promoter activity in these cell lines (1.8-fold ($P = 0.001$) and 2.27-fold ($P < 0.01$) in HEC1A and HEC1B cells respectively) (Fig. 4). This was also reflected in the cells treated with progesterone with prior oestrogen priming (Fig. 4). HEC1B cells showed a 3.35-fold ($P < 0.01$) increase in activity after this combination of treatment. IL-1β appeared to have a small, but contradictory, effect on promoter activity (Fig. 4). There was a slight increase in activity after treatment in HEC1A cells and a slight decrease in HEC1B cells (not significant).

**Discussion**

This study demonstrates that progesterone alone, or when applied after a previous oestrogen stimulus, up-regulates MUC1 protein expression in two human cell lines derived from endometrial epithelial carcinoma cells. It also shows that MUC1 is a hormone responsive, or more specifically progesterone responsive, gene. This correlates strongly with other *in vivo* reports of the normal endometrial epithelium. A recent study in patients undergoing oocyte donation showed that patients treated with 17-beta oestradiol plus progesterone for 6 days exhibited a significantly higher increase in MUC1 mRNA production compared with those treated with 17-beta oestradiol alone (Meseguer *et al.* 2001). Previous observations in natural cycles and in the rabbit confirm this effect (Hey *et al.* 1994, Hoffman *et al.* 1998). Thus, our results suggest that the HEC1A and HEC1B endometrial carcinoma cell lines are useful for studying the hormonal mechanisms controlling transcription and translation of MUC1.

Hormonal regulation of MUC1 has been demonstrated in a number of other organ systems. In human breast carcinoma cell lines, oestrogen and progesterone stimulation is associated with increased MUC1 mucin expression, and a similar effect has been demonstrated in human prostate carcinoma cell lines in response to medroxyprogesterone acetate and androgens (McCuckin *et al.* 1998, Mitchell *et al.* 2002). However, although MUC1 protein is up-regulated by progesterone in breast carcinoma cell lines, MUC1 mRNA levels do not appear to vary following oestrogen or progesterone treatment (either singly or in combination) (Botti *et al.* 1997). It has therefore been postulated that, in breast carcinoma, increased MUC1 protein production is due to a post-transcriptional mechanism. Yet, in the normal endometrium, MUC1 mRNA is increased when the serum level of progesterone is at its highest, in the mid-secretory phase (Hey *et al.* 1994). In our study, MUC1 promoter activity was analysed in endometrial cells, using a previously described MUC1 promoter construct linked to the CAT reporter gene, and was regulated...
by progesterone. Our data suggest that progesterone controls transcription of the MUC1 gene in endometrial epithelial cells, in vitro, consistent with observations in vivo (Kovarik et al. 1993). However, it raises questions regarding the mechanisms by which progesterone regulates MUC1 protein as we have shown that progesterone treatment increased MUC1 transcription in both PR-positive HEC1A cells, and PR-negative HEC1B cells. This finding was somewhat surprising given the well-documented evidence that hormonal steroids modulate transcription by activation of their cognate receptor (Evans 1988, Beato 1989, Mitchell et al. 2002). Certainly, on the basis of results with HEC1B, the regulation of the MUC1 gene by progesterone seems to be independent of PR status.

In most tissues, oestrogen controls the regulation of PR, thereby also controlling sensitivity to progestins (Bouchard 1999). Our results showed that oestrogen priming did not enhance the progesterone effect on MUC1 in the PR-positive cells, suggesting that PR levels were not affected by stimulation with oestrogen. Furthermore, prior oestrogen stimulation did not induce PR in either cell line at the immunohistochemical level. This provides further evidence that the regulation of MUC1 production by progesterone may not conform to the traditional model of steroid hormone action.

Non-receptor-mediated effects of sex steroids have been demonstrated in a number of studies (Bouchard 1999, Falkenstein & Wehling 2000). A recent report showed that oestrogen-mediated activation of adenyl cyclase and cAMP occurred independently of ERs, via a G-protein-coupled receptor (GPCR), to control the level of extracellular signal-regulated kinases in the epidermal growth factor (EGF) receptor–MAPK pathway (Filardo et al. 2002). This was consistent with reports that have implicated GPCRs in rapid membrane signalling events mediated by oestrogen and progesterone (Machelon et al. 1996, Rosner et al. 1999, Falkenstein & Wehling 2000). Given the importance of the protein kinase A pathway in mediating uterine differentiation of steroid hormone action, this non-classical pathway is worthy of further investigation.

Another possibility is that MUC1 production is mediated by a non-genomic pathway via a membrane receptor. A recent study describes the cloning of a cDNA from spotted seatrat ovaries encoding a protein that satisfies a number of criteria for its designation as a steroid membrane receptor: plausible structure, tissue specificity, cellular distribution, steroid binding, signal transduction, hormonal regulation and biological relevance (Zhu et al. 2003a). Genes homologous to this fish membrane progesterin receptor have subsequently been identified and partially characterised in humans (Zhu et al. 2003b).

Alternatively, it is possible that the progesterone effect is mediated via the genomic pathway, but not via PRA or PRB, rather via another PR isoform (Wei et al. 1997). PR also exists as PRC, a smaller N-terminally truncated progesterin-specific binding protein that would not be detected by the Mab used in our study, and PRC has been shown to be regulated by progestins and anti-progestins (Wei et al. 1997). Furthermore, the PRA and PRB levels in the HEC1B cells may be so low that they cannot be detected using Western blot analysis; detection may require a technique, such as RT-PCR, that is more sensitive to very small amounts of product. Similarly, it is also possible that the progesterone effect is mediated via the androgen receptor (AR). Ligand activation of AR is the mechanism by which androgens up-regulate MUC1 protein (Mitchell et al. 2002). AR has been shown to be regulated by progestins in human breast cancer cells (Bentell et al. 1999, Garcia-Becerra et al. 2004), and AR expression has been demonstrated in HEC1A and HEC1B cells (K Bumhaid, personal communication).

Exposure to the candidate embryonic MUC1 modulator, IL-1β, had no effect on MUC1 at either the protein or the transcriptional level in this study. There are a number of possible explanations for this observation. The levels of and duration of exposure to the cytokine may have not been appropriate. Only two MUC1 Mabs were used and it is possible that IL-1β does affect MUC1 but only at the level of post-translational modification, by affecting the glycoform pattern of MUC1. The IL-1 receptor (type 1) (IL-1R t1) status of either cell line is not known and, if these cell lines are IL-1R t1 negative, an effect might not be seen. This latter explanation, however, seems unlikely as there are a number of reports of proteins being regulated by IL-1β in other endometrial epithelial carcinoma cell lines, such as RL952 and Ishikawa (Mohgul et al. 1994, Makrigiannakis et al. 1999). In addition, there are similar reports of IL-1β regulating gene and protein expression in endometrial stromal cells (Huang et al. 1998, Tsai et al. 2001). It is therefore possible that, in the endometrium, IL-1β could regulate MUC1 by generating a signal from the underlying stromal compartment.

In summary these observations provide evidence for up-regulation of MUC1 protein expression at the transcriptional level in the human endometrial epithelium. Given that over-expression of MUC1 could potentially be associated with infertility and failure of embryo implantation, progesterone-dependent regulation of MUC1 may be important in determining endometrial receptivity and other normal physiological processes.

Acknowledgements

This work was performed at the Institute of Developmental and Reproductive Biology, Imperial College, Hammersmith Hospital, Du Cane Road, London W12 0NN, UK. This work was supported by a grant from the Medical Research Council (G84/5342). Statistical advice was kindly provided by Dr Eurof Walters, Statistical Consultant, Cambridge, UK. The authors would like to thank Professor Hilary Critchley and Dr Anita Abdul-Aziz for their advice and encouragement. The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.
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