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# Regional specialization of the cell membrane-associated, polymorphic mucin (MUC1) in human uterine epithelia

**J.A.DeLoia[1], J.S.Krasnow[2], J.Brekosky[3], A.Babaknia[4], J.Julian[5] and D.D.Carson[5,6]**

Departments of [1]Cell Biology and Physiology and [2]Obsterics/Gynecology and Reproductive Sciences, University of Pittsburgh, Pittsburgh, PA 15213, [3]Magee Women's Research Institute, Pittsburgh, PA 15213, [4]Department of Biochemistry and [5]Molecular Biology, Women's Health Institute of California, Costa Mesa, CA 92626 and Department of Biochemistry and Molecular Biology, M.D.Anderson Cancer Center, Houston, Texas, TX 77030, USA

[6]To whom correspondence should be addressed

**The cell membrane-associated, polymorphic mucin, MUC1, has been proposed to hinder implantation by virtue of its anti-adhesive properties. Consistent with this proposal is the observation of a dramatic decrease in MUC1 protein and mRNA expression in the uterine epithelium of several species at the time of implantation. In contrast, little change in glandular epithelial expression of MUC1 protein or its mRNA during the peri-implantation period has been detected in humans. However, expression in the luminal epithelium, i.e. the epithelium involved in embryo attachment, has not been reported. Using tissue samples with a clearly defined luminal epithelium and antibodies directed against the cytoplasmic domain found in all cell-associated MUC1 species (CT-1) and against two MUC1 ectodomain epitopes, HMFG-1 and HMFG-2, we demonstrate that MUC1 expression in the luminal epithelium is maintained throughout the menstrual cycle. The staining observed with CT-1 correlates with that seen with HMFG-2, but not HMFG-1. HMFG-1 reactivity was high in all regions except basal glands in the mid proliferative endometrium and fell to very low levels throughout the tissue in the mid secretory phase. In all cases, HMFG-1 reactivity could be restored by predigestion with keratanase or neuraminidase which removes keratan sulphates and sialic acids, respectively. These observations suggest that regionally restricted glycosylation generates an altered external structure of MUC1. These alterations appear to decrease accessibility to the MUC1 protein core region and are maximal in luminal epithelium at the receptive phase. Due to their large highly extended structures, MUC1 ectodomains are very likely to be among the first cell surface components encountered during human blastocyst attachment to the luminal epithelium. Thus, MUC1 either must be locally removed during the attachment process or functions actually to promote the initial steps in embryo adhesion to the apical surface of the human uterine epithelium.**

*Key words:* endometrium/human/implantation/mucins

## Introduction

During the process of implantation the embryo must contact the endometrial luminal epithelium, breach the epithelium to enter the endometrial stroma, and subsequently invade the uterine vasculature. Successful implantation requires that the development of the embryo to a state of attachment competence be synchronized with a transition of the uterine luminal epithelia from a functionally non-receptive state to a receptive state that permits embryo attachment. This transition includes changes in the expression of hormonally regulated secreted and membrane-bound glycoproteins (Lessey *et al.*, 1992, 1994; Aplin *et al.*, 1994).

The initial phase of implantation is mediated by interactions between the apical surface of uterine luminal epithelial cells and the trophectoderm of the developing embryo. The apical surfaces of most polarized epithelial cells are protected by a thick glycocalyx composed largely of mucins. Mucins are believed to play a role in protecting the cell surface from pathologic processes (Strous and Dekker, 1992). Anchored to the cell surface by its transmembrane domain, the polymorphic epithelial mucin, MUC1, exerts its anti-adhesive function via a large extracellular domain consisting primarily of identical tandem repeats of 20 amino acids. The number of tandem repeats is variable, resulting in a total length of between 1000 and 2200 amino acids (Gendler *et al.*, 1990). It is estimated that this extensively (>50%) glycosylated protein extends 300–500 nm above the cell membrane, which exceeds the distance spanned by most membrane-associated proteins. This extended structure, coupled with the abundance of MUC1, restricts access to other cell surface components, including cell adhesion-promoting proteins (Hilkens *et al.*, 1992; Kemperman *et al.*, 1994; Wesseling *et al.*, 1995). In mice, MUC1 expression is reduced markedly at the luminal surface of the uterine epithelium at the receptive phase (Braga and Gendler, 1993; Surveyor *et al.*, 1995). A similar decline in luminal MUC1 expression is seen during the receptive phase in baboons (Hild-Petito *et al.*, 1996), pigs (Bowen *et al.*, 1996), rats (DeSouza *et al.*, 1998) and rabbits (Hoffman *et al.*, 1998). Reduction in MUC1 expression is thought to facilitate embryo–epithelial interactions by unmasking cell adhesion molecules on the epithelial cell surface (Surveyor *et al.*, 1995).

The relationship of MUC1 to human implantation has been investigated in several laboratories. A panel of monoclonal antibodies generated against the extracellular tandem repeat region of MUC1 has been used to examine expression in uterine epithelium throughout the human menstrual cycle (Rye *et al.*, 1993; Hey *et al.*, 1994). Although the luminal epithelium was not described in these studies, there appeared to be an

increase in glandular MUC1 expression in the mid secretory phase, in contrast to the decline in luminal expression seen in many other species. Treatment of mid and late secretory phase endometrium with neuraminidase increased MUC1 staining in paraffin-embedded specimens. A similar increase in MUC1 protein content following treatment with neuraminidase also was observed by Western blot analysis (Hey *et al.*, 1994), suggesting that antibody reactivity was dependent upon the MUC1 glycosylation in the uterus.

It is possible that retention of MUC1 in human uterine epithelium reflects a species difference in MUC1 function. However, other species, e.g. baboons and rats, demonstrate retention of glandular Muc1 while luminal expression is reduced (Hild-Petito *et al.*, 1996; DeSouza *et al.*, 1998). Therefore, it also is possible that luminal MUC1 expression is distinct from the glandular expression reported to date in humans. In this regard, there is evidence for regional differences in expression of other mucin glycoproteins in the human uterus (Kliman *et al.*, 1995). The present study was undertaken to ascertain the regional expression of MUC1 in human luminal and glandular uterine epithelia using antibodies recognizing all forms of MUC1 as well as particular glycosylation variants (glycoforms).

## Materials and methods

### *Tissue specimens*

Endometrial specimens were obtained from the fundus region of healthy regularly cycling women between the ages of 18 and 45 years during the course of a routine fertility evaluation of women with primary infertility. All samples were dated by an experienced gynaecological pathologist according to standard criteria (Noyes *et al*., 1950). Samples which were out of phase by two or more days were not analysed. The experimental protocol was approved by the institutional review board at Magee Women's Hospital, University of Pittsburgh. Informed consent was obtained from all patients. Two mid luteal biopsies were obtained circumstantially from women that became pregnant in the same cycle. No embryonic tissue was associated with these biopsies. Pregnancy was assessed in both cases by a missed menses and subsequent rise in serum human chorionic gonadotrophin (HCG) concentrations. Pregnancy outcome was not monitored.

Endometrial biopsies were obtained using a Unimar pipelle endometrial suction curette (Prodimed, Neuilly-en-Thelle, France). The sample was divided into two portions. One portion was fixed in 10% (w/v) phosphate-buffered formalin and processed for standard histological dating. The other portion was placed in a cryovial and frozen immediately in liquid nitrogen for subsequent immunohistochemical analysis. Samples collected for double labelling were embedded in OCT cryoprotectant (Baxter, McGraw Park, IL, USA) when they were initially frozen so that the luminal surface was preserved and tissue orientation could be ascertained.

### *Immunohistochemistry*

Frozen samples were embedded in OCT medium. 5 μm sections were mounted onto Superfrost plus slides, fixed for 30 s in acetone and allowed to air-dry overnight before storage at –80°C. Samples were stained either with antibodies to the cytoplasmic tail of MUC1, found in all forms of the intact protein (Pemberton *et al*., 1992) or with ectodomain-directed antibodies (HMFG-1, HMFG-2 or SM3) that recognize particular glycosylation states (glycoforms) of MUC1 (Burchell *et al*., 1987; Ho *et al*., 1995). Prior to staining, the slides were equilibrated to room temperature for 30 min, fixed in acetone for 10 min and air-dried for 1 h. Following two 5-min phosphate-buffered saline (PBS) washes, endogenous peroxidase was quenched with 3% (v/v) hydrogen peroxide for 20 min. Samples were rinsed for 10 min in PBS and incubated for 20 min in Universal Protein Blocking Agent (Shandon Lipshaw, Pittsburgh, PA, USA). Affinity-purified CT-1 antibody at a concentration of 2 μg/ml in PBS was applied to the sections for l h at room temperature, followed by a 10 min wash in PBS. Bound antibody was detected with the Biogenex Super Sensitive detection kit (Biogenex, San Ramon, CA, USA) used according to the manufacturer's directions. Briefly, slides were incubated 20 min with biotinylated secondary antibody, washed 10 min in PBS and incubated 20 min with horseradish peroxidase-conjugated streptavidin. After a 10 min wash in PBS, samples were incubated for 2 min with 3,3′-diaminobenzidine, rinsed in distilled water, and counterstained with Gill's No. 2 haematoxylin. Following graded alcohol dehydration, samples were cleared in xylene and mounted with Accumount (Baxter, McGraw Park, IL, USA).

When samples were to be analysed by fluorescence microscopy, frozen sections (6 μm) were fixed for 10 min at room temperature in acetone:methanol (1:1, v/v) and rehydrated for 15 min in PBS with one change of buffer. Sections then were incubated sequentially at 37°C in undiluted mouse monoclonal antibody to HMFG-1, HMFG-2 or SM3 (1 h), Texas Red-conjugated sheep anti-mouse IgG diluted 1:10 in PBS (40 min), peptide affinity-purified rabbit anti CT-1 diluted to 10 μg/ml in PBS (1 h), and fluorescein isothiocyanate (FITC)-conjugated donkey anti-rabbit IgG (40 min). Sections were rinsed three times for 5 min with PBS between each antibody, and before mounting. Samples were mounted in glycerol:PBS containing 0.01% (w/v) *p*-phenylenediamine to prevent fading and photographed using a double-pass filter set permitting co-visualization of both fluorochromes with a single exposure, or a single filter set permitting visualization of fluorescein only. Serial sections reacted individually with HMFG-1, HMFG-2 or CT-1 displayed patterns of detection identical to those obtained on sections probed as described above.

### *Enzymatic digestion*

Where indicated, sections were subjected to enzymatic digestion after fixation and rehydration with keratanase (keratan sulphate endo-1,4-β-galactosidase: *Pseudomonas*; 10 U/ml; Seikagaku Corp., Tokyo, Japan) in 0.1 M Tris acetate pH 7.25 or neuraminidase (*Vibrio cholerae*; 3 U/ml; Sigma Chemical Co., St Louis, MO, USA) in 0.1 M NaCl, 4 mM $CaCl_2$ pH 5.5 for 1 h at 37°C and rinsed for 15 min in PBS before application of the initial primary antibody.

### *Confocal analysis*

Samples which had been sequentially probed with HMFG-2 and CT-1 antibodies were subjected to analysis by confocal microscopy in order to obtain a ratio of their respective signal intensities. Using a Molecular Dynamics LSM 2001 (Sunnyvale, CA, USA), paired images, one for each fluorochrome, were recorded at a 0.3 μm stepsize. Four to ten paired images were recorded at each location, with one to five locations selected randomly for each sample of luminal epithelium. Final images were created using ImageSpace software (Molecular Dynamics). The paired images were divided (CT-1 signal/HMFG-2 signal), thus creating a third image in which pixel intensities reflected the ratio of the individual pixel intensities in the original paired images. In the third image, the apical portion of the luminal epithelium was circumscribed and integrated by the software program to yield a mean intensity for all pixels within the circumscribed area. Samples from three to five women each in the mid proliferative or mid secretory phase were examined this way.

### *Antibodies*

The CT-1 antibody was generated in rabbits against a synthetic peptide (CSSLSYTNPAVAATSANL) corresponding to the highly conserved cytoplasmic tail of MUC1. This peptide was synthesized by the Synthetic Antigen Core Facility at the M.D.Anderson Cancer Center. The rabbit immunizations have been previously described (Surveyor *et al.*, 1995). Using an Imject Activated Immunogen Conjugation Kit (Pierce Chemical Co., Rockford, IL, USA), the CT-1 peptide was conjugated to bovine serum albumin (BSA) according to the manufacturer's instructions. The CT-1/BSA conjugate was then reacted with cyanogen bromide-activated Sepharose 4B (Sigma). The resulting affinity resin was used to purify CT-1 antibody. HMFG-1, HMFG-2 and SM3 antibodies were the generous gift of Dr Sandra Gendler (Mayo Clinic, Scottsdale, AZ, USA). Monoclonal antibody 5D4 was purchased from Seikagaku Corp. (Tokyo, Japan). Texas Red-conjugated sheep anti-mouse IgG and FITC-conjugated donkey anti-rabbit IgG were purchased from Amersham (Arlington Heights, IL, USA). The secondary antibodies were species-specific and had been cross-adsorbed by the manufacturer against serum from a variety of species to further reduce cross-reactivity to immunoglobulins of species other than that to be detected. In secondary antibody controls, reactivity was restricted to the respective primary antibody with no reactivity with the alternative primary antibody, the alternative secondary antibody, or endogenous human IgG.

## Results

### *MUC1 is expressed in luminal epithelia throughout the menstrual cycle*

Detection of peptide epitopes located in the MUC1 ectodomain tandem repeats is dependent on the accessibility of peptide epitopes which have the potential to be masked by glycosylation (Burchell *et al.*, 1987; Devine *et al.*, 1990; Hey *et al.*, 1994; Ho *et al.*, 1995). Carbohydrate epitopes associated with ectodomain tandem repeats, although accessible, are variably expressed dependent on the phase of cycle and regional location of the epithelium (Smith *et al.*, 1989). The cytoplasmic tail of MUC1, on the other hand, is highly conserved across species (Pemberton *et al.*, 1992). Furthermore, its detection is not subject to variations due to the state of glycosylation and its presence might be a more reliable indicator of the abundance of cell-associated full-length MUC1 core protein. Reaction of human endometrial sections with a peptide-specific antibody directed to the cytoplasmic domain of MUC1 (CT-1) demonstrated MUC1 presence at the apical surface of luminal and glandular epithelium at all phases of the menstrual cycle (Figure 1a–c, e). Sections incubated with non-immune rabbit IgG (Figure 1d) or CT-1 antibody preabsorbed with an excess of its peptide antigen (data not shown) were negative. There was no apparent loss of MUC1 protein from luminal epithelium during the menstrual cycle, including the mid luteal phase, i.e. the period of endometrial receptivity. Since none of the above samples originated from endometrium in which pregnancy occurred, it could still be argued that in humans, MUC1 expression decreases only in fertile cycles. To address this possibility, luminal cell-associated MUC1 expression was assessed by CT-1 antibody detection during the peri-implantation period (day of LH surge +8 days) during cycles in which conception occurred (Figure 1f; $n = 2$). Staining was comparable with that of luminal epithelium from a similarly phased non-conceptive cycle. Thus, there appeared to be no loss of MUC1 uniquely associated with conceptive cycles.

### *Regional specialization of MUC1 glycoforms*

The anti-adhesive function of MUC1 is dependent on the glycosylated tandem repeats in the ectodomain (Wesseling *et al.*, 1996). An altered splice variant lacking the tandem repeats is known to exist in other tissues (Zrihan-Licht *et al.*, 1994). In order to determine whether the cytoplasmic tail of MUC1 detected at the apical surface of luminal epithelium co-localized with MUC1 ectodomain, endometrial sections were co-incubated with CT-1 and HMFG-2, an antibody recognizing a peptide epitope in the tandem repeats of the ectodomain (Burchell *et al.*, 1987; Burchell and Taylor-Papadimitriou, 1989). Staining patterns of the luminal epithelium were subjected to confocal analysis. Although the signal intensity for CT-1 was higher than for HMFG-2 during both phases of the cycle, the signal intensities are a function of the ability of individual antibodies to detect their respective epitopes and do not necessarily reflect absolute concentration. A ratio of the individual staining intensities would reflect changes in the stoichiometry of these two epitopes of MUC1. Confocal microscopy revealed a signal ratio of CT-1/HMFG-2 of 51.9 ± 32.9 (SE) in the mid proliferative phase and 54.5 ± 47.3 in the mid secretory phase, using a series of sections from three to five normal women at each stage (Table I). Individual staining intensities for each antibody actually increased in receptive phase luminal epithelium; however, the resulting ratio of intensities remained constant. Thus, both the cytoplasmic tail and ectodomain of MUC1 are present at the apical surface of receptive phase luminal epithelium and do not change relative to each other during the transition to a receptive state.

Detection of epitopes in the MUC1 ectodomain may be affected by glycosylation of the tandem repeats (Burchell *et al.*, 1987; Burchell and Taylor-Papadimitriou, 1989; Devine *et al.*, 1990; Ho *et al.*, 1995). In the present study, no glandular or luminal epithelial reactivity to MUC1 could be detected with SM3 antibody at any phase of the cycle. Examination of human endometrial specimens using HMFG-1, another antibody recognizing a MUC1 ectodomain tandem repeat epitope, demonstrated a graded reactivity with the mid proliferative phase epithelium, progressively decreasing from the luminal surface to the basalis region (Figure 2a–c). In contrast, CT-1 reactivity was consistent throughout these regions (Figure 2a′–c′). Glands of the basalis embedded in the myometrium were HMFG-1-negative. In glands of the supra-basal region, a mixed expression of the two epitopes was detected, with cell-to-cell variations. The glandular epithelium of the functionalis and the luminal epithelium were uniformly positive for both epitopes. In contrast, only a few cells of receptive phase glands or luminal epithelium reacted with HMFG-1 (Figure 2d–f), although strong staining with CT-1 was evident in all epithelial cells (Figure 2d′–f′). Examination of samples from earlier secretory phase endometrium indicated that the strong luminal staining of HMFG-1 in the mid proliferative phase decreased gradually during the advance to mid secretory phase (data not shown).

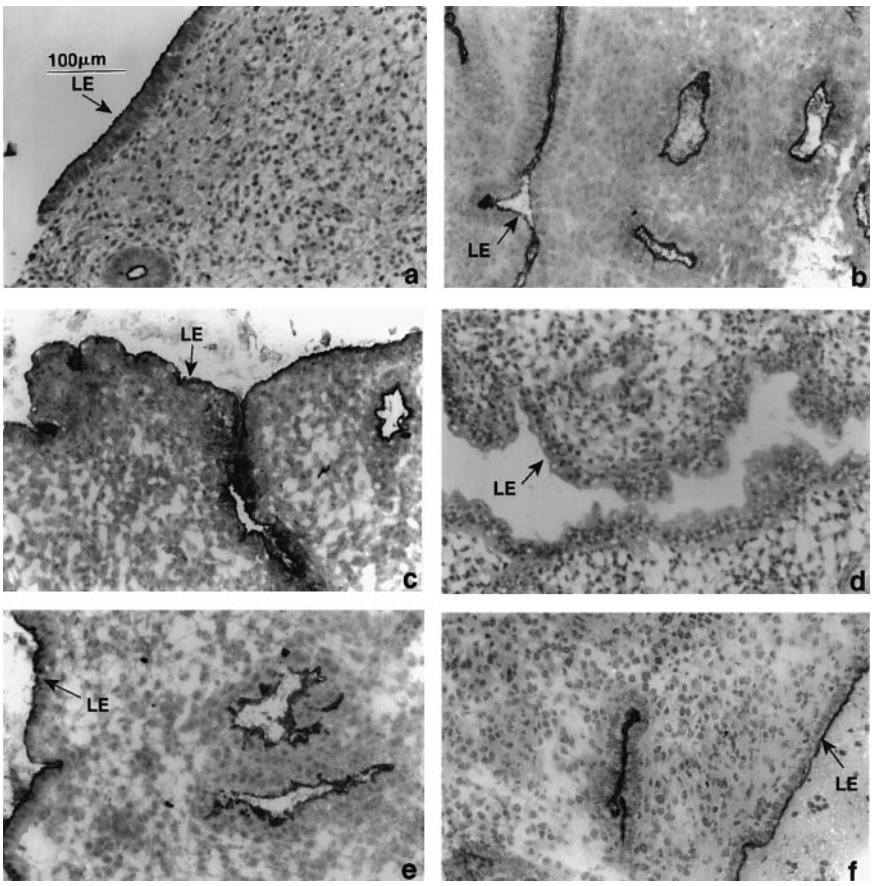


**Figure 1.** MUC1 protein expression persists in luminal epithelium during the menstrual cycle. Frozen sections of human uteri were stained with CT-1 antibody by the peroxidase method and counterstained with haematoxylin as described in Materials and methods. The sections were from the following stages: (**a**) mid proliferative (day 7); (**b**) late proliferative (day 10); (**c**) early luteal (post ovulatory day 3); (**d**) mid luteal (post ovulatory day 7) stained with non-immune rabbit IgG; (**e**) mid luteal (post ovulatory day 7); (**f**) mid luteal (post ovulatory day 8) from a cycle during which pregnancy occurred. Arrows indicate the luminal epithelium (LE). Scale bars (**a**–**f**) = 100 μm.

**Table I.** HMFG-2 and CT-1 staining ratios in luminal epithelia during mid proliferative and mid luteal phases

| | CT-1/HMFG-2[a] |
|---|---|
| Mid proliferative ($n$ = 3) | 51.9 ± 32.9 |
| Mid luteal ($n$ = 5) | 54.5 ± 47.3 |

[a]Double staining of frozen sections with HMFG-2 and CT-1 antibodies and analyses of staining ratios by confocal microscopy were as described in the text. The number of individual patients examined in each case is indicated. Data from each patient represent the average of hundreds of ratios of individual pixel intensities at the luminal epithelial surface and all these values are pooled for the data presented above (mean ± SEM). Variation was observed due to cell-to-cell variations in staining intensity primarily for the HMFG-2 epitope. Nonetheless, the data indicate that staining for HMFG-2, the MUC1 ectodomain-specific antibody, persists in both the proliferative and luteal phases at a similar intensity at the luminal epithelial surface relative to that of CT-1.

Detection of the HMFG-1 epitope in the tandem repeats of the MUC-1 ectodomain is strongly affected by glycosylation (Hey *et al.*, 1994). When either terminal sialic acid residues or keratan sulphate was removed enzymatically prior to staining, the presence of the HMFG-1 ectodomain epitope at the apical surface of luminal epithelium and its co-localization with the cytoplasmic tail was confirmed (Figure 3). Thus, HMFG-1 reactivity appears to be masked by hyperglycosylation of MUC1 in these regions. Keratan sulphate at the apical surface of receptive phase luminal epithelium was further confirmed by direct detection with the keratan sulphate-reactive antibody, 5D4 (Graham *et al.*, 1994; Figure 3e and f). This antibody intensely stained luminal epithelium, but showed little cell-associated reactivity in the glandular epithelium. Nonetheless, 5D4-reactive material was detected in the lumina of glands, indicating that keratan sulphate was associated primarily with secreted products in these regions during the luteal phase. The 5D4 epitope also was detected at the apical surface of luminal epithelium of mid proliferative phase endometrium (data not shown). In proliferative phase endometrium, the 5D4 epitope was absent from basal glands, variable in suprabasal glands, and uniform in functionalis glands and luminal epithelium. Thus, the regional pattern of 5D4 expression in proliferative phase endometrium appeared to be the inverse of that of the keratanase-sensitive glycan masking the HMFG-1 epitope which was restricted to the basal glands. During transition to the receptive state, the 5D4 epitope shifted from the cell surface to secretions in most of the glands, but continued to be associated with the apical cell surface of receptive phase luminal epithelium. Collectively, these results demonstrate a progressive increase in MUC1-associated glycans as the endometrium transits from prolifer-

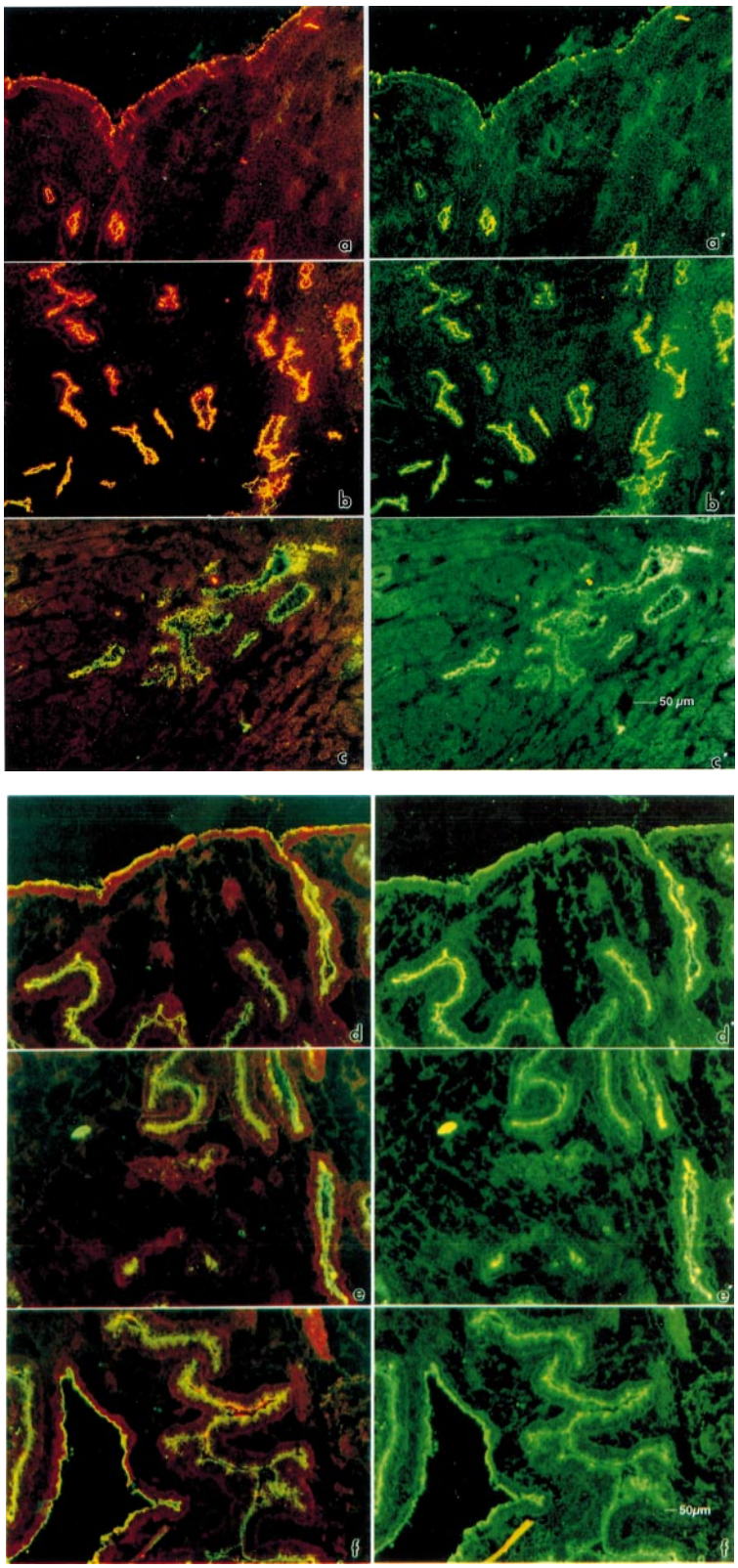


**Figure 2.** Immunofluorescence of human endometrium in the mid proliferative and mid secretory phases. These photographs represent sections from the luminal surface (**a**,**a′**,**d**,**d′**), functionalis (**b**,**b′**,**e**,**e′**,**f**,**f′**), and basalis (**c**,**c′**) regions from the same representative specimens. Panels (**a**–**c**) and (**a′**–**c′**) are from a mid proliferative endometrium while panels (**d**–**f**) and (**d′**–**f′**) are from mid secretory endometrium. In panels (**a′**–**f′**) only cytoplasmic antibody CT-1 is visualized. MUC1 appears green and is present from the lumen to the basalis in both the mid proliferative and mid secretory phases. Panels (**a**–**f**) visualize both the CT-1 antibody and the ectodomain antibody HMFG-1. HMFG-1 staining alone would appear red. The co-recognition of both CT-1 and HMFG-1 results in an orange appearance. HMFG-1 recognition is present in the luminal epithelium and glandular epithelium of the functionalis in the mid proliferative phase (day 9), but is markedly decreased in the mid secretory phase (post ovulatory day 7) luminal and glandular epithelium. Scale bars = 50 μm.

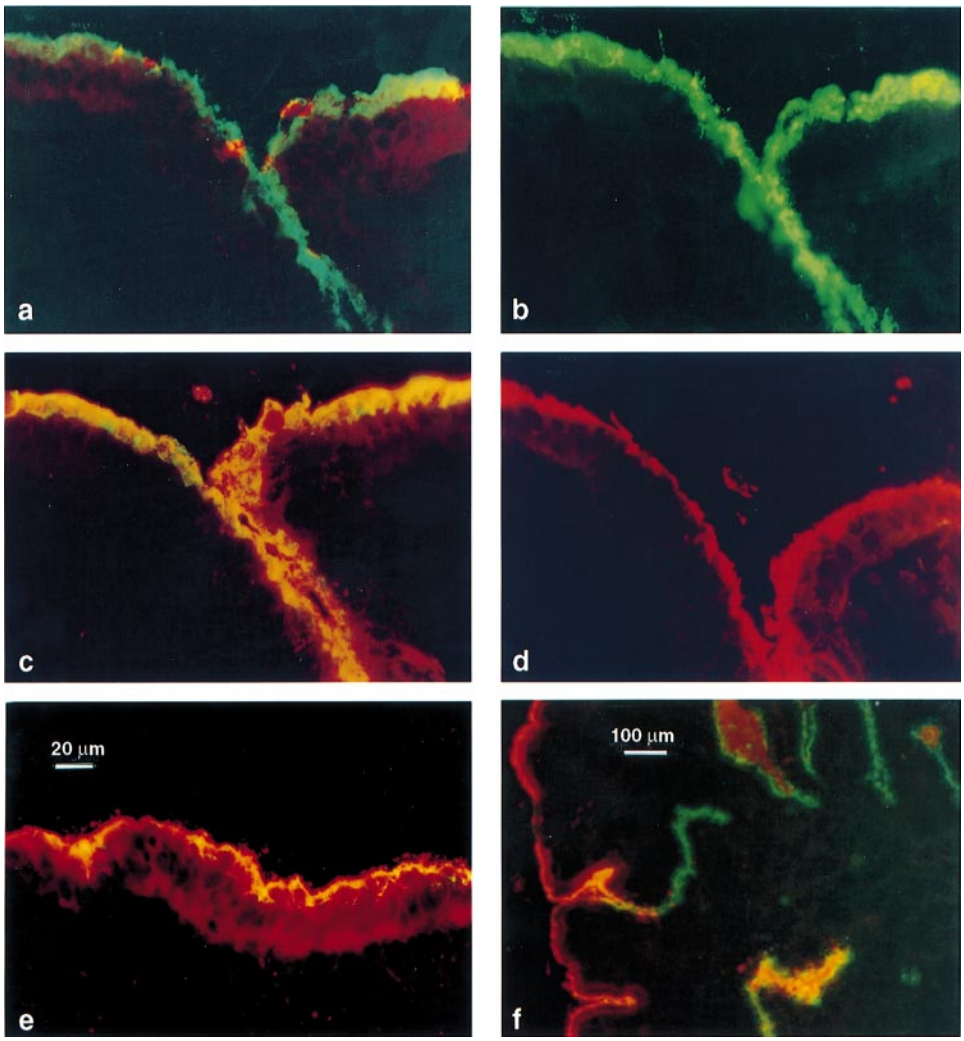


**Figure 3.** HMFG-1 recognition in luminal epithelium is masked by keratan sulphate and sialic acids during the mid secretory phase. Frozen sections of mid secretory tissue (post ovulatory day 7) were doubly stained with HMFG-1 and CT-1 antibodies as described in the legend to Figure 2 (**a**–**d**) or monoclonal antibody 5D4 to keratan sulphate and CT-1 antibody (**e** and **f**) as described in Materials and methods. The luminal epithelium and the neck of a functionalis gland are shown in panels (**a**–**f**). The predominantly green appearance of a section doubly stained with HMFG-1 and CT-1 (**a**) indicates very little staining for HMFG-1 and is similar to that observed when only the CT-1 fluor is visualized (**b**). Predigestion of sections with keratanase (**c**) or neuraminidase (**d**) unmasks HMFG-1 epitopes (reddish-orange appearance). An antibody recognizing a non-sialylated, keratanase-insensitive form of keratan sulphate (5D4) intensely stains luminal epithelium (**e**). Little cell-associated 5D4 staining is apparent in glandular epithelium [green staining in (**f**)]; however, glandular luminal secretions are positive, while the apical surface is recognized only by CT-1. Scale bars: (**a**–**e**) = 20 μm; (**f**) = 100 μm.

ative to secretory phase and show that the glycoform retained at the apical surface of receptive phase luminal epithelium is distinct from that expressed by proliferative phase luminal epithelium.

## Discussion

The present studies demonstrate that both cytoplasmic and ectodomain regions of MUC1 persist in human luminal epithelium during the receptive phase of the menstrual cycle. The intensity of staining with the CT-1 antibody is similar at all stages and in all regions, suggesting that no substantial changes occur in luminal MUC1 expression. The persistent expression of MUC1 in the luminal epithelium in humans is distinct from the pattern observed in most other species examined to date (Surveyor *et al.*, 1995; Bowen *et al*., 1996; Hild-Petito *et al.*, 1996; DeSouza *et al.*, 1998). Following enzymatic treatment to unmask core protein epitopes, the MUC1 ectodomain was uniformly recognized by HMFG-1 at the apical surface of luminal epithelium and co-localized with CT-1 antibody detection of the cytoplasmic tail at all stages of the cycle. The anti-adhesive properties of MUC1 are dependent upon the number of tandem repeat domains of the ectodomain (Wesseling *et al.*, 1996). A form of MUC1 lacking the ectodomain tandem repeats (MUC1/Y) that is expressed in human breast cancer cells has been described (Zrihan-Licht *et al.*, 1994). Expression of MUC1/Y at the luminal surface could theoretically reduce the anti-adhesive properties of MUC1 while preserving immunoreactivity with the CT-1 antibody. However, replacement of full-length MUC1 by the production of significant amounts of MUC1/Y is unlikely since epitopes recognized by antibodies directed at sites in the ectodomain tandem repeats

(HMFG-2 and unmasked HMFG-1) are detectable throughout the menstrual cycle at a constant level relative to the cytoplasmic tail. The presence of mRNA for an additional variant of MUC1 lacking most of the transmembrane domain and cytoplasmic tail has been reported in human endometrium throughout the cycle (Aplin and Hey, 1995). This form of MUC1 would neither be retained at the apical surface of uterine epithelium nor be distinguished in secretions from ectodomain released from full-length MUC1 which is detached from the cytoplasmic tail in the process of release (Devine *et al.*, 1990; Boshell *et al.*, 1992; Pimental *et al.*, 1996). Expression of this truncated form of MUC1 would potentially be another way of reducing the presence of anti-adhesive MUC1 at the apical surface of receptive phase luminal epithelium. However, the persistent presence of the cytoplasmic tail at the apical surface of luminal epithelium detected by CT-1 argues against the strategy just described. It is possible that both of these alternative forms of MUC1 may be present in human uterine epithelium. If one of these alternative forms accumulated in luminal epithelium during the receptive phase, then the ratio of ectodomain staining to cytoplasmic tail staining should change. The present studies have examined the intensity of two ectodomain-specific antibodies relative to a cytoplasmic domain-specific antibody and show that these relative intensities do not change. Thus, increased expression of alternative forms of MUC1 core protein does not appear to occur to a significant extent during the receptive phase.

Although full-length MUC1 appears to remain at the surface of receptive luminal uterine epithelium, the glycoform expressed differs significantly from that of proliferative phase luminal uterine epithelium. Alterations in the pattern of uterine MUC1 oligosaccharide expression could occur as variations in size, sialylation and/or the number of oligosaccharide chains per core molecule. MUC1 bearing keratanase- and neuraminidase-sensitive keratan sulphate was detected only in the glandular epithelium of the basalis region during the proliferative phase. In the secretory phase, virtually all HMFG-1 epitope was masked by keratanase- and neuraminidase-sensitive keratan sulphate species. Receptive phase luminal uterine epithelium also expressed at its apical surface a non-sialylated, keratanase-insensitive keratan sulphate species recognized by antibody 5D4 (Hoadley *et al.*, 1990). Both of these keratan sulphates have been reported to be associated with the MUC1 ectodomain (Aplin *et al.*, 1994; Aplin and Hey, 1995). Thus, luminal expression of keratan sulphate species appears to be a marker of secretory phase uteri. Furthermore, these studies demonstrate regional specialization of MUC1 glycoform expression in the human uterus.

In the light of the findings that MUC1 expression persists at the apical surface of human luminal epithelium during the receptive phase, it must be considered that MUC1 plays a fundamentally different role in embryo–epithelial interactions in the human than it does in other species. Addition of keratan sulphate to MUC1 during the receptive phase would be expected to further increase non-adhesive functions (Burg and Cole, 1994; Dou and Levine, 1995; Takahashi *et al.*, 1996). Thus, the observed changes in MUC1 glycosylation patterns are difficult to reconcile with a model in which MUC1 facilitates embryo attachment. Under some conditions MUC1 can bear selectin ligands including sialylated Lewis x and Lewis a (Hey and Aplin, 1996). Thus, it is possible that MUC1 might promote embryo attachment if human blastocysts express selectins on their external surfaces. In this regard, evidence has accumulated in various mammalian systems suggesting a role for cell surface carbohydrates during pre- and peri-implantation development; however, gene disruption experiments have not defined the precise roles that glycosyltransferases or mammalian lectins play in early stages of development (Poirier and Kimber, 1998). It is not clear whether MUC1 core proteins are uniformly glycosylated or whether there are functionally different subsets of MUC1 glycoforms. It also is unclear if glycoforms of MUC1 might be rendered selectively adhesive. Alternatively, MUC1 might be locally downregulated at the site of blastocyst attachment as occurs in the rabbit (Hoffman *et al.*, 1998). The nature of the embryonic signals that cause local loss of MUC1 in the rabbit are unclear.

It can be concluded from these and other studies that the regulation of uterine MUC1 expression among mammalian species differs substantially. The continued presence and abundance of full-length MUC1 at the apical surface of receptive phase human luminal epithelium indicates the necessity for functional studies to determine the strategy adopted by the human embryo either to breach the MUC1 barrier or to use MUC1 as an attachment factor.

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