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MUCINS OF THE HUMAN ENDOCERVIX

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1. ABSTRACT

The physical character and amount of mucus secreted by the endocervix changes dramatically at midcycle to facilitate the reproductive process. Mucins expressed by the endocervical epithelium contribute to this all-important physiologic event. This review summarizes work from our laboratory demonstrating the mucin gene expression profile of cervical epithelium and mucin levels in cervical mucus through the menstrual cycle. mRNA levels of the gel-forming mucin MUC5B, the major gel-forming mucin expressed by the endocervical epithelium, peak before midcycle and the amount of MUC5B protein per unit total protein in cervical mucus peaks at midcycle. Message levels for MUC4, a major membrane-spanning mucin of the endocervix, peak at midcycle, but protein levels of MUC4 in human cervical mucus have not been measured. Message for each mucin diminishes dramatically as progesterone levels increase in the blood. These data suggest hormonal regulation of the two mucin genes in the endocervix, but there is no information on their regulation at the biosynthetic level via genomic hormone response elements.

Perhaps, through its hydrophilicity, the MUC5B mucin holds water in place at the endocervical canal surface at midcycle, keeping the canal patent for sperm motility. A second potential role of the increased mucins at midcycle is to protect the cervix and uterus at the time when increased water is secreted into the cervical canal to facilitate sperm penetrance. Pathogens and other seminal fluid components may be excluded from entering the uterus by mucin trapping. Studies to determine the mechanism of hormonal regulation of mucins as well as the function of individual mucins are needed.

2. INTRODUCTION

Mucus plays an important role in reproductive function and defense of the female reproductive tract (1). In the upper portion of the reproductive tract, membrane-associated mucins on the apical membranes of fallopian tube epithelium and endometrial epithelial cells may play a role in implantation and epithelial defense. Reviews and research reports on mucins in the upper reproductive tract in humans and rats have been recently published (2-4). This review will primarily summarize studies from our laboratory focused on the mucins of the lower reproductive tract, particularly those within cervical mucus. The primary source of mucus in the female reproductive tract is the endocervical epithelium. The lumen of the cervix is lined by a columnar epithelium that folds inward to form gland-like, duct-lacking invaginations within the cervical stroma. The cervical epithelial cells in luminal and glandular regions secrete mucus, which provides a barrier to pathogen entrance into the uterus and a protective surface covering for cervical and vaginal epithelia. The characteristics of cervical mucus are important in fertility, because cervical mucus functions to receive, filter, nurture, store and release sperm for successful transport to the egg

Table 1. Characteristics of human epithelial mucins and their distribution/presence in female reproductive tract epithelia

Name	Category	Chromosome Mapping	Amino Acids in Tandem Repeat	Presence in Female Reproductive Tract*	Refs.
MUC1	Membrane-spanning	1q21	20	ft, ut, en, ec, v	78, 79, 37
MUC2	Gel-forming	11p15.5	23	2 of 15 en	80, 37
MUC3A/3B	Membrane-spanning	7q22	17	——	81, 37, 82
MUC4	Membrane-spanning	3q29	16	en, ec, v	43, 37, 34
MUC5AC	Gel-forming	11p15.5	8	en	83, 84, 37
MUC5B	Gel-forming	11p15.5	29	en	85, 37, 86
MUC6	Gel-forming	11p15.5-15.4	169	en	87, 37
MUC7	Soluble/monomer	4q13-q21	23	——	36, 37
MUC8	ND	12q24.3	13/14	ut, en	88-90
MUC9	ND	1p13	15	ft	21
MUC11	ND	7q22	28	ut	22
MUC12	Membrane-spanning	7q22	28	ut (weakly)	22

***:** ft = fallopian tube; ut = uterus; en = endocervix; ec = ectocervix; v = vagina; ov = ovary; ¾ ¾ = negative in all above; ND = not determined

for fertilization (5). It has been shown that cervical mucus increases in quantity, "spinnbarkeit," ferning, pH, hydration, and sperm penetrability, and decreases in viscosity, viscoelasticity and cell content just prior to ovulation; these changes reverse after ovulation (6-8). As alterations in mucus quantity and quality occur with hormone/reproductive status, so do infections and pathology (1,9). The molecular nature of the mucus change during the menstrual cycle has not been well characterized, and there is relatively little information regarding the specifics of hormonal regulation of the mucins in the epithelia of either upper or lower reproductive tracts, particularly in humans.

3. BIOCHEMICAL AND STRUCTURAL PROPERTIES OF CERVICAL MUCINS

There is a large body of literature on the physical and biochemical properties of human cervical mucus (for reviews see Blandau and Moghissi (10); Hafez (1); Carlstedt et al.(11); Carlstedt and Sheehan (12,13). Cervical mucus is a complex mixture of water (90-98%); low-molecular-weight components, including organic components (amino acids, cholesterol, lipids, glucose, ascorbic acid, polysaccharides) and inorganic ions; and high-molecular-weight components (e.g., enzymes); and bactericidal proteins (secretory IgA, lactoferrin, defensins), plasma proteins, and mucin (1,14-16). Mucins are the major structural protein in mucus, forming an intermolecular gel (17). They are characterized by their huge size, high buoyant density (approximately 1.4 g/ml) and high carbohydrate content. Carbohydrates of these highly glycosylated (50-80% by weight) glycoproteins are almost exclusively O-linked via serine or threonine to the protein backbone. Monosaccharides predominating in cervical mucus are N-acetylgalactosamine, N-acetylglucosamine, galactose, fucose, and sialic acid (18). Eight oligosaccharide structures have been determined. The latter include two neutral, and six sialylated forms with the possibility that some may be sulfated (18,19).

Biochemical characterization of cervical mucus has not been easy (17). Mucins are one of the most notoriously difficult classes of molecules to study; their huge size and high carbohydrate content impede use of conventional biochemical techniques. One can't quantitate them by conventional protein or spectrophotometric assay, and often their carbohydrate moieties mask binding of antibodies to the glycosylated regions of the protein core. Thus, molecular techniques used toward their characterization and study of expression have been welcome tools.

With recent information from molecular cloning, at least twelve distinct human mucins have been reported; they are designated MUCs 1-4, 5AC, 5B (5AC and 5B were once thought to be a single gene product), 6-9 and 11 & 12 in order of discovery (20-22). The apomucins, in addition to a high content of potential O-glycosylation sites, have in common a structural feature consisting of tandemly repeated amino acid sequences. The tandem repeat unit is unique in length and sequence for each mucin species (Table 1) and may be repeated as many as 100 times or more, with the number varying considerably between and within individuals, due to allelic polymorphism (except for MUC5B, which does not show polymorphism) (23-26). These repeats are rich in threonine/serine and proline and constitute a significant percentage of the potential O-linked attachment sites (27).

Based on molecular sequencing it has become evident that there are several distinct classes of human mucin proteins. Three classes have been identified to date; they include the secreted or gel-forming mucins, membrane-spanning mucins, and small soluble mucins (Table 1). Secreted or gel-forming mucins are very large mucins that form a major component of the viscous mucus in mucus secretions. There are four of these mucins, all being coded for within a region of one chromosome, 11p15.5. They are designated MUCs 2, 5AC, 5B, and 6 and in addition to tandem repeats, they have other structural features in common. Each has cysteine-rich domains, homologous to the D domains of von-Willibrand Factor, which are present in the N and C terminal regions (except MUC6, which has only N terminal D domains). The free

cysteines in these domains are believed to form disulfide linkages between monomers to form homomultimers of each secreted type mucin gene product, giving rise to the gel-like or viscous character of mucus. Each of these mucins has a huge central domain of tandem repeats with estimates of the molecular weight of the mature individual gel-forming subunits ranging from 500 to 30,000 kDa, making them some of the largest glycoproteins known (28).

MUC5B is distinct from the other gel-formers in that it has a huge central 10.7 kb exon encoding a 3,570 amino acid region. Within this region, there are four super repeats, each of which contains eleven 29 amino acid repeats, and a unique cysteine-rich region (29). As stated above, unlike other gel-forming mucins, MUC5B does not exhibit genetic polymorphism (26).

Membrane-spanning mucins (MUCs 1, 3, 4, and 12) have in their C-terminal region a membrane-spanning region and a short cytoplasmic domain. Their extracellular domains include the tandem repeat region and between the tandem repeat region and the cell membrane, except for MUC1, EGF-like domains (22,30,31). The extracellular domains of MUC1 and MUC4 have been reported to be cleaved and non-covalently re-associated in the golgi prior to insertion into the apical cell membrane of epithelia (20,32,33). The extracellular domain may be shed from the cell surface to become part of the mucus coat, but details regarding the shedding process are not well documented (34,35). To date only one small soluble mucin has been characterized. This mucin, designated MUC7, is approximately 39 kDa MW and is primarily comprised of a central O-glycosylated tandem repeat protein (36).

4. LOCALIZATION OF MUCIN GENE EXPRESSION IN HUMAN FEMALE REPRODUCTIVE TRACT EPITHELIA

Human female reproductive tract epithelia express members of both the secreted/gel-forming class of mucins and the membrane-spanning class, as well as several mucins that remain unclassified (Table 1). We analyzed mucin gene expression in epithelia from human reproductive tract tissues (fallopian tube, endometrium, endocervix, ectocervix and vagina) obtained at the time of surgery (37). When possible, tissues were divided into parts so that both northern blot analysis and in situ hybridization assays could be used to determine both level and location of mucin mRNA transcripts, and multiple samples were assayed in order to determine if phase of the menstrual cycle altered the localization or level of expression. Phase of menstrual cycle was determined by endometrial histology using criteria of Noyes *et al.*(38). At the time of our study, reproductive tract tissues were assayed for expression of MUCs 1-7. Surveys using probes to more recently described mucins indicate that several additional mucins are expressed in the reproductive tract epithelia. Table 1 summarizes mucins expressed by the female reproductive tract epithelia.

4.1. Secreted Gel-forming Mucins

We found that the endocervical epithelia were the only epithelia of the reproductive tract to express members of the secreted or gel-forming mucin class. Three of these mucins, MUCs 5AC, 5B, and 6 were consistently expressed in all samples assayed using both northern blot and in-situ hybridization. On only two endocervical tissue samples (one by northern blot, the other by in situ hybridization) did we find faint expression of the fourth member of the class, MUC2.

By *in-situ* hybridization, message for MUC5B was found localized uniformly over all endocervical epithelial cells, regardless of glandular or luminal position, and very intense labeling with a 984 bp cRNA antisense probe to the tandem repeat region of this mucin was evident (Figure 1). By comparison, MUC5AC and MUC6 mRNA localization was not as uniformly located over the cervical epithelial cells, nor was the labeling as intense, even with probes to the tandem repeat region that bind to multiple sites and thus have an amplified signal. For MUC5AC luminal epithelia appeared to be more heavily labeled than glandular and MUC6 labeling was patchy and not correlated to luminal or glandular regions. We detected no major variance with cycle for these assays, but neither assay is quantitative, particularly since tandem repeat probes were used. Ovarian cyst mucus has been reported to contain MUC6 (39), but to our knowledge, mucin expression in normal ovarian tissues has not been described.

4.2. Membrane-spanning Mucins

Several membrane-spanning mucins are expressed by reproductive tract epithelia. By northern blot and in situ hybridization, we demonstrated the expression of MUC1 in all human tissues surveyed—fallopian tube, uterus, cervix, ectocervix and vagina; whereas, MUC4 was expressed by the endocervical, ectocervical and vaginal epithelial cells (37). MUC4 was expressed uniformly by the endocervical and ectocervical epithelium and in a patchy distribution by vaginal epithelia. Data from rats demonstrate Muc4 in all epithelia of the female eproductive tract (40). In more recent studies, assays by northern blot analysis demonstrated that uterus/endometria expresses MUC 12 (41).

5. VARIATION OF MUCIN GENE EXPRESSION WITH CYCLE

Having determined the mucin gene expression profile of the human female endocervical epithelia, we sought to determine which of the genes predominate and whether their expression levels vary with the menstrual cycle. RNA was isolated from cytobrush samples taken from the cervix of cycling women at two-week intervals over two menstrual cycles. Blood samples taken at the time of cervical cytobrush sampling were analyzed for estrogen and progesterone levels, and LH surge was detected by urinalysis. Assay of the mRNA levels of the three secreted gel-forming mucins, MUCs 5AC, 5B and 6, and the membrane-spanning mucin MUC4 (each demonstrated to be present by in situ hybridization and northern blot) was done using semiquantitative RT-PCR (42). To do these quantitative assays of the mucins,

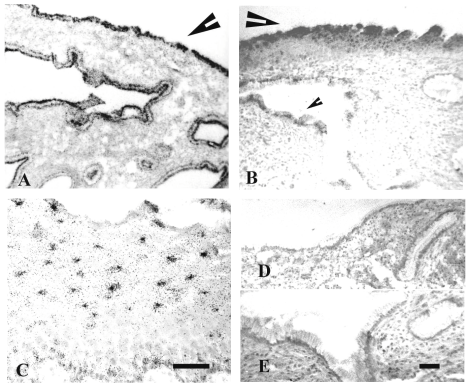

Figure 1. Localization of MUC5B mRNA in human endocervical epithelia (A) and MUC4 mRNA in human endocervical and ectocervical epithelia (B, C) by *in situ* hybridization. An ^{35}S-labeled MUC5B RNA probe and an ^{35}S-labeled oligonucleotide probe for MUC4 tandem repeats were used. Note in A the intense binding of the MUC5B probe to luminal (arrow) as well as glandular epithelia; the section is taken from tissue in the early secretory phase of the menstrual cycle. In the endocervical tissue, at proliferative stage, compared to glandular epithelium (B, small arrow), the MUC4 oligoprobe binding is especially intense at the luminal surface (B, large arrow). Binding of the MUC4 probe to the ectocervical epithelium (C) shows particularly strong binding to apical cells of the stratified epithelium. There is no binding of sense control probes for MUC5B (D) and MUC4 (E) to sections of endocervical tissue. Bars = 100 µm.

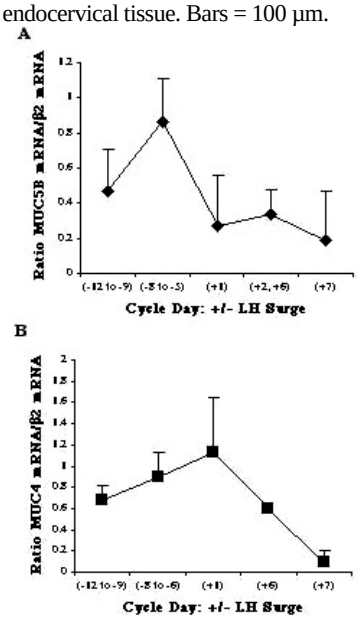

Figure 2. Levels of MUC5B mRNA (A) and MUC4 mRNA (B) relative to that of β^2-microglobulin in endocervical epithelial RNA samples taken through the cycle, as determined by date of LH surge. RNA was isolated from cytobrush samples of human endocervical epithelia. Values represent the mean +/- SEM for 33 samples from 9 individuals (MUC5B, diamonds) and 17 samples from 6 individuals (MUC4, squares). MUC5B mRNA levels peak prior to midcycle, while MUC4 mRNA levels peak at midcycle. Message for both mucins declines late in the cycle when blood progesterone levels rise.

primer sets outside the tandem repeat were used, and relative abundance of each of the mucin gene transcripts was related to β2-microglobulin mRNA levels. In order to assay MUC4, which at the time of the study was relatively uncharacterized—with only the tandem repeat reported (43), we sequenced a 2.7 kb region 5' to the tandem repeat (42). This allowed design of primer sets for the semiquantitative RT-PCR.

MUC5B consistently amplified at fewer cycles than MUC5AC and MUC6 indicating that, of the gel-forming mucins, MUC5B predominates. Of the two membrane-spanning mucins present in the cervical epithelia, MUC4 is expressed at higher levels, and the comparison of relative expression of MUC4 to the gel-forming mucins, demonstrated that, in 15 of 21 samples from six subjects, the ratio of MUC4:β2 was greater than MUC5B:β2. This data demonstrated that MUCs 5B and 4 are the major gel-forming and membrane-spanning mucin species of the endocervix, respectively. Furthermore, the relative levels of m RNA for the two prevalent mucins were correlated to cycle day as well as levels of estrogen and progesterone in the blood (42). Pooling the data for the relative levels of MUC5B mRNA from nine subjects demonstrates that MUC5B mRNA peaks prior to midcycle and drops dramatically after midcycle (Figure 2). Relative levels of MUC4 mRNA from six subjects peaked at midcycle, followed by a drop after midcycle (Figure 2). Although there was no correlation of mucin gene expression to estrogen (E_2) levels, mucin mRNA levels were highest during the stage of the cycle when E_2 was unopposed. There was a consistent inverse relationship between both MUC5B and MUC4 mRNA levels and blood progesterone (P_4) levels (42). Perhaps P_4 antagonizes E_2 upregulation of mucin gene expression.

Having demonstrated that the mRNA of two mucins, the gel-forming MUC5B and membrane-spanning MUC4, are major mucins in human endocervical epithelium and that both appear to be regulated inversely by progesterone secretion, we were then able to proceed to study protein levels of the mucins during the cycle.

6. MUCIN PROTEIN LEVELS IN CERVICAL MUCUS WITH THE CYCLE

Since the physical character and amount of mucin secreted by the endocervix changes dramatically during the menstrual cycle to facilitate sperm migration at the time of midcycle ovulation, and since we identified the gel-forming mucin MUC5B as a major mucin mRNA of the endocervical epithelium, we sought to determine if we could detect and quantify MUC 5B in cervical mucus taken from human donors through the menstrual cycle.

6.1. Specificity of MUC5B Antibody

In order to assay mucin glycoproteins in very small sample sizes, a direct assay that could be used on native mucus was required. We made an antibody to a synthetic peptide, a 19 amino acid segment of a region within the cysteine-rich region of the D4 domain located C terminal to

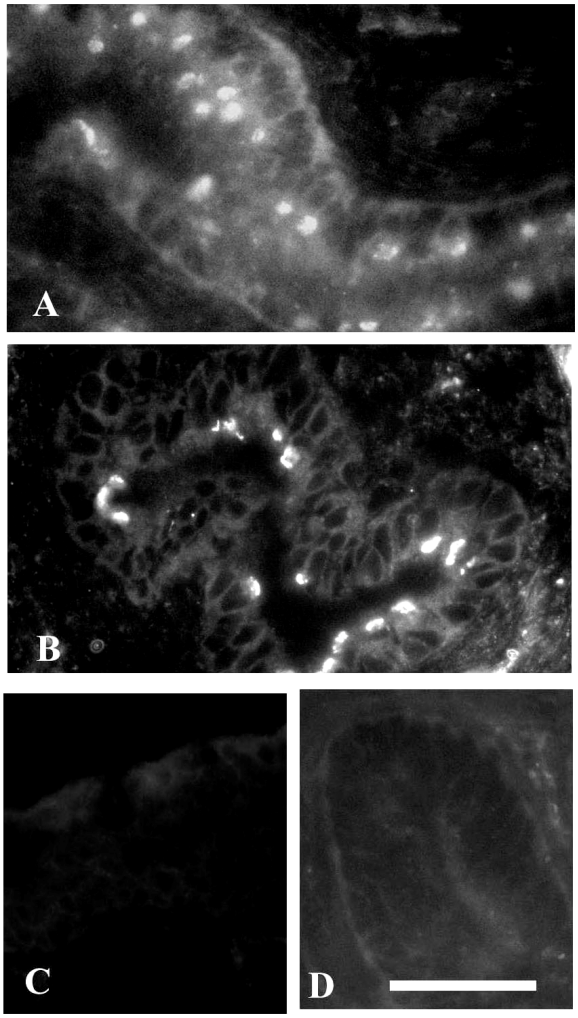

Figure 3. Immunofluorescence microscopy demonstrating binding of MUC5B antibody to MUC5B mucin in endocervix tissue taken at the proliferative stage (A) and the secretory stage (B) of the menstrual cycle. Note the more apical localization of the binding in the secretory sample. The specificity of the binding of the antibody to MUC5B is confirmed by the lack of binding to conjunctival tissue (C), which expresses MUCs 1, 4, and 5AC but not MUC5B. (D) shows the amount of binding to endocervix attributable to background from the secondary antibody. Bar = 10 μm.

the tandem repeat region of the MUC5B glycoprotein. The peptide mimicking the 19 mer in the D4 region is located between cysteines and lacks glycosylation sites. Development of antibodies specific to individual mucin gene products has been difficult and skepticism regarding specificity of mucin antibodies is widespread. Thus, we used several methods to demonstrate the specificity of the Chicken IgY antibody to MUC5B. Firstly, the antibody is preadsorbable with its peptide. Secondly, the antibody binds by immunohisto-chemistry only to mucin-secreting epithelia that express the MUC5B gene (Figure 3). Thirdly, the antibody binds by ELISA only to secretions known to contain MUC5B. By western blot, the antibody binds a very high molecular weight protein.

It is of interest that the MUC5B antibody recognizes both native and denatured MUC5B protein. The binding to native protein may relate not only to lack of glycosylation sites in the antigenic region but also to its position within an intercysteine region of the D4 domain of MUC5B. Intercysteine sequences typically loop out from associated cysteines, thus providing ready access to antibody binding (44). Besides surface exposure, the relative flexibility of the looped sequences may more readily form shapes assumed by small, flexible peptide antigens (45). Examples of looped epitopes among secreted proteins include the gonadotrophins LH/human CG (46,47), FSH (48), and human GH (49). Unique intercysteine sequences from the D domains in other regions of MUC5B, as well as those in the other gel-forming mucins—MUCs 2, 5AC and 6, may also be good candidate sites for developing region- and mucin-specific antibodies.

6.2. MUC5B Protein Levels In Cervical Mucus During The Menstrual Cycle

Using the antibody to MUC5B, we developed a quantitative ELISA using a cervical mucin standard prepared by conventional mucin isolation techniques from mucus obtained from patients at an intrauterine insemination clinic. (Patients with both spontaneous and stimulated cycles were included.) We measured the amount of the mucin per ng total protein in cervical mucus from three subjects sampled each over four hormone cycles (50). LH surge and blood estrogen and progesterone levels were monitored. For each subject, cumulatively, two-to-three samples were taken before LH surge, one around the LH surge, and one-to-two after the LH surge. ELISAs were performed on a range of concentrations of the cervical mucus samples in order to be certain that the full range of detectability was assayed.

At all concentrations of cervical mucus tested, there was an obvious peak in MUC5B antibody binding per unit total protein in the samples from midcycle compared with those from early or late in the cycle. As indicated in Figure 4, which is the average of all the data points from the three subjects, sampled through several cycles, there was a dramatic 3-to-5-fold increase in the amount of MUC5B protein at midcycle. The amount of MUC5B in the cervical mucus dropped precipitously in the luteal phase, as mRNA levels dropped (compare Figure 4 to Figure 2) and blood progesterone levels increased (50).

To date we have not quantified MUC4 in cervical mucus. We have made five synthetic peptide antibodies to non-glycosylated, non-tandem repeat regions of the MUC4 protein, but none of these antibodies have satisfied our specificity criteria.

7. HORMONE REGULATION OF MUCIN GENE EXPRESSION/PROTEIN LEVELS

While there is indication of hormone regulation of levels of mucin mRNA and protein, there is little

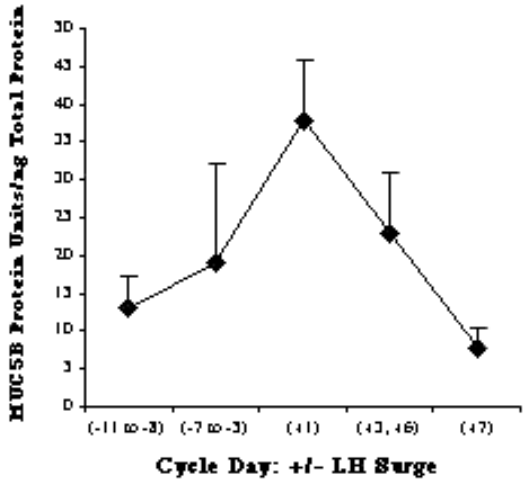

Figure 4. MUC5B mucin protein content in samples of cervical mucus harvested from three individuals at various times in the menstrual cycle, as determined by date of LH surge. Values represent the mean +/- SEM for 25 samples from 3 individuals. The amount of MUC5B protein in cervical mucus peaks at midcycle when the mucus character changes to facilitate sperm penetration.

information demonstrating direct promoter regulation of mucin genes by estrogen (E_2) or progesterone (P_4). As is the case in our studies of MUC5B and MUC4, information regarding regulation of expression of the membrane-spanning mucins MUC1 (51-53), and the rat homologue to MUC4 are limited to correlation of mucin message/protein levels to circulating E_2 or P_4 levels (3,54). In a review of the MUC1 data, DeSouza *et al.*(55) point out that the different regions of the reproductive tract respond differently or distinctly to the steroid hormones. Thus, regulation of MUC1 in the uterus may not relate to either the endocervical or ectocervical/vaginal epithelia, and furthermore, regulatory regions of the different mucins will be unique. Human endometrial cells appear to upregulate MUC1 in receptive compared to unreceptive phases, and *in vitro*, progesterone combined with estradiol priming, induced an upregulation of MUC1 in the receptive endometrium (56).

A recent paper from Carraway's group (54) demonstrates that sialomucin complex (SMC) mRNA (the rat MUC4 homologue) is expressed in the uterus of the rat. They studied SMC protein level in ovariectomized rats supplemented with E_2 or P_4 alone, or a combination of the two. They found high levels of SMC protein in E_2-supplemented rats, a diminution in E_2- and P_4-supplemented rats, and no SMC protein with P_4 supplement alone. These data suggest that P_4 downregulated the SMC message. The rat study is in agreement with our data from humans in which we show that MUC4 and MUC5B mRNA are at their maximum during the E_2, follicular phase, and downregulated in the luteal phase (Figure 2) (42). Furthermore, we show that protein levels of MUC5B mucin peak at midcycle and fall as P_4 levels in the blood increase (Figure 4) (50). Taken together, these data indicate there is similarity between human endocervix and rat uterus in that E_2 upregulates mucin gene expression and P_4 downregulates mucin expression.

As reviewed by Cooke *et al.*(57), there is evidence that the estradiol effects on secretory activity of uterine epithelium is a direct rather than indirect effect (via subepithelial cell intermediates) through E_2 receptor-α. Two E_2 receptors have been identified (α and β) and appear to be highly conserved between species (58,59). An E_2 receptor-α knockout mouse (ERKO) (57), which has an intact β receptor, does not respond to E_2 to bring about the uterine and vaginal changes associated with the estrus cycle. Thus, if MUC5B and MUC4 gene expression is directly regulated by E_2, it can be assumed that it is through E_2 receptor-α.

P_4 regulation occurs through P_4 receptors; two forms, α and β, have been identified. They are produced from a single gene by alternative start sites to yield a full-length form (P_4-β, 116 kDa) or an N terminal truncated form (P_4-α, 90 kDa). (For review, see Duffy *et al.*(60); Kastner *et al.*(61); Lydon et al.(62)) Transgenic mice lacking the P_4 receptors develop normally to adulthood, but the adult female has significant defects in all reproductive tissues such that reproduction does not occur, even though basal levels of E_2 and P_4 are produced (62,63).

P_4 antagonism of E_2 action has long been recognized and can be accomplished by several mechanisms (as reviewed by Alexander et al.(64) and Katzenellenbogen (65), and references therein). These included induction of estradiol metabolism, downregulation of E_2 receptors, and competition between steroid hormone receptors for factors that mediate enhancer function. Thus, there are multiple possibilities for direct or indirect mechanisms of regulation of mucin gene expression by E_2 and P_4.

8. ROLE OF MUCINS IN REPRODUCTION

8.1. Secreted Gel-Forming Mucins

The changes that occur in the physical and biochemical nature of cervical mucus during the menstrual cycle have been studied extensively (for reviews see Carlstedt and Sheehan (13), Katz *et al.*(8), Vigil et al.(66)), and the importance of the mucus in mucosal protection and sperm penetrance is widely acknowledged. Until recently, however, there has been little information on the molecular character of the major structural components of cervical mucus—the mucins. As summarized above, data from our studies demonstrate that a major gel-forming mucin of the endocervical epithelium is MUC5B. Of the gel-forming mucins, MUC5B mRNA predominates over that of MUCs 5AC and 6, which are also expressed but at lower levels (42). The amount of MUC5B mRNA is high during preovulatory stages of the menstrual cycle, and there is a dramatic peak in MUC5B glycoprotein/unit total protein in cervical mucus at the time of ovulation. What role does this specific mucin play in reproduction and why, of the four gel-forming mucins, does 5B predominate? At least two roles can be proposed. First-of-all, mucins are extraordinarily hydrophilic, and the ability of their surfaces

to bind water accounts for the mucins' space-filling ability (67). Increased gel-forming mucin in the endocervical canal at ovulation may function to hold water in place at the canal surface, thus, keeping the canal patent for sperm motility. A second potential role is that increased mucin is required for protection of the cervix and uterus at the time when increased water is secreted into the cervical canal to facilitate sperm penetrance. Pathogens and other seminal fluid components may be excluded from entering the uterus by mucin trapping.

The question of why MUC5B predominates is an interesting one. Perhaps MUC5B is more hydrophilic than other mucins; it has an extraordinarily large central hydrophilic domain. Another possibility is the stability of MUC5B. Of the gel-forming mucins, MUC5B is the only one that is not polymorphic (26). If MUC5B is a vital part of the reproductive process, perhaps a consistent size and character is required within the cervical canal to allow sperm to penetrate. Direct proof of the specific function(s) of MUC5B in human reproduction await molecular deletion studies.

8.2. Membrane- Spanning Mucins

8.2.1. MUC1

With regard to reproductive tract epithelia, there has been intense interest in the role of MUC1 in implantation. (For review see DeSouza et al.(55) and Lagow *et al.*(4)). Studies of MUC1 mRNA and protein expression in mice, rat, baboon, and rabbit have led to the hypothesis that reduction of MUC1 expression on the surface epithelium of the uterus is necessary for embryo implantation (55,68-70). Muc1 null mice have been generated but are healthy, normal and fertile, with the only phenotypes described: delayed mammary tumor progression (not metastases) (71), impaired maturation of T-lymphocytes (Gendler, personal communication), and increased ocular surface infection in some but not all strains bearing the null mutation (72,73). The lack of impairment of fertility in MUC1 null mice may indicate that the mucin has no vital role in the reproductive process in mice, but its removal may facilitate or enhance blastocyst adherence. Studies of endometrial samples in mid-proliferative (non-receptive) and mid-luteal (receptive) phases of the menstrual cycle demonstrate that MUC1 expression is maintained in the luminal epithelium throughout the cycle, but that there may be regional specialization in the pattern of expression at the level of carbohydrate structure (74). There is little direct in vivo information on the function of MUC1 in the various regions of epithelia of the human reproductive tract. Recent work with *in vitro* models of human endometrial cells suggests that MUC1 acts as an endometrial anti-adhesive molecule that is locally removed by the human blastocyst at the implantation site (56).

8.2.2. MUC4

MUC4 has also been the subject of recent interest among those studying the reproductive tract. MUC4 has a wide-spread tissue distribution, being present in the simple epithelium of the bronchus, stomach, jejunum, ileum, colon, and prostate (75), endocervix (37), and trachea (76), and the stratified epithelium of the conjunctiva (77), ectocervix and vagina (37).

The role of MUC4 in cervical mucus is unclear. It is not known whether the extracellular domain is shed into the cervical mucus or whether it is retained on the endocervical epithelial cell surface to act as lubricant and anti-adhesive. In rats, rMuc4 protein disappears from uterine epithelium at the time of blastocyst receptivity but does not appear to vary in protein content in cervical tissue (3, 54). Use of human-specific probes/antibodies to MUC4 will facilitate analysis of MUC4 in human uterine and cervical epithelia. In our hands, MUC4 mRNA levels were considerably less in the uterus than in endocervix, at all stages of the menstrual cycle (37). Similarly in rats, cervical and vaginal epithelia express higher levels of rMuc4 protein than does the uterus (3).

9. PERSPECTIVE

Studies of cervical mucin gene and protein expression with the hormone cycle have established the groundwork to determine the structural basis for physical changes in mucins in response to hormone status. The baselines on MUC5B and MUC4 expression will also facilitate future testing of functional roles of each mucin in the reproductive process and allow studies of potential roles of mucin gene expression in unexplained infertility.

Little is known regarding the regulation of mucin genes at the biosynthetic level via genomic hormone response elements. Development and characterization of an appropriate endocervical cell line that expresses MUC5B and MUC4 will be necessary for study of regulation of mucin gene expression by this epithelia. In addition, studies of the effects of hormones on post-translational glycosylation of mucins may yield important information regarding mucin character and function during the cycle.

From a clinical and practical standpoint, availability of cDNA probes and protein core antibodies, as well as carbohydrate epitope probes to cervical mucins, will allow direct assay of alterations in cellular and secreted-mucin content with disease, age, and infertility. Finally, knowledge of mucin character/regulation and functional role may yield clues into more effective methods of vaginal contraception.

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